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ORIGINAL PAPER

Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (*Cancer magister*)

Jason J. Miller[1] · Michael Maher[1] · Erin Bohaboy[1] · Carolyn S. Friedman[2] · Paul McElhany[1]

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Abstract The Dungeness crab, *Cancer magister*, is an important resource species, and in Puget Sound, USA, where the adults occur in inshore waters that have summer pH as low as 7.6, future levels are predicted as low as 7.1. Using eggs and larvae from females captured in Puget Sound in late 2012, this laboratory study examined hatching success, larval survival, and larval development rate at target pH of 8.0, 7.5, and 7.1, which represent present open ocean, present coastal upwelling, and projected upwelling conditions. Toward the end of their development, the eggs of one *C. magister* were exposed to the three treatments and they began to hatch after 22 days. Hatching probability was unaffected by lower pH, but hatching was delayed at pH 7.1. In a second experiment, significantly more *C. magister* larvae survived after 45 days at pH 8.0 than at the two lower pH: 58, 14, and 21 %. The sizes of the zoeae were unaffected by treatment, but larvae in the low-pH treatments progressed through larval stages more slowly. This study shows that low-pH seawater slows embryonic and early larval development and causes appreciable larval mortality. It suggests that ocean acidification could have a measurable impact on the population dynamics of *C. magister*.

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✉ Jason J. Miller
jason.miller@noaa.gov; seabaldy@gmail.com

[1] Conservation Biology Division, Northwest Fisheries Science Center, 2725 Montlake Blvd. E, Seattle, WA 98112, USA

[2] School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St., Seattle, WA 98105, USA

Introduction

Ocean acidification (OA), the decrease in seawater pH due to an increase in dissolved CO_2, has the potential to substantially change the abundance and distribution of individual marine species and alter entire ecosystems (Fabry et al. 2008). Laboratory experiments rearing organisms in seawater with control and low pH are important first steps for estimating how individual species may respond to ocean acidification. These experiments have shown a variety of positive, negative, and no-effect responses (Kroeker et al. 2013). The response to low pH can be species-specific within a genus (Dupont and Thorndyke 2009) and can vary among populations or strains within a single species (Dupont et al. 2010). This level of variability presents a real challenge to understanding the ecological and economic consequences of OA because it is difficult to extrapolate results from experiments on one species to other species. To understand potential OA effects on a species of importance on the North East Pacific Coast, we conducted a series of pH laboratory experiments on eggs and larvae of Dungeness crab, *Cancer magister*.[1]

Cancer magister is an ecologically, economically, and culturally important marine species. A mature female

[1] Schweitzer and Feldmann (2010) proposed elevating the *Cancer* subgenus, *metacarcinus*, outlined by Nations (1975) to the generic level re-classifying the Dungeness crab as *Metacarcinus magister* based solely on the shape of carapace teeth. Due to a lack of molecular evidence (Harrison and Crespi 1999) to support Nations' subdivisions, the lead author elected to maintain the use of *Cancer magister*.

crab can produce over two million offspring (Pauley et al. 1986); these offspring provide valuable prey items for forage fish and economically important species like salmon, rockfish, and herring (Reilly 1983; Bollens et al. 2010; Kemp et al. 2013). Adult *C. magister* males are heavily harvested and culturally important throughout their coastal range from south central California to the Gulf of Alaska (Jensen 2014; Online Resource 1). The annual combined commercial and tribal landings averaged over 2003–2012 were 35.7 thousand tonnes (FAO 2016) with ex-vessel commercial and tribal landings reaching over $176 million in 2012.

Female *C. magister* extrude and brood eggs beginning in September at the southern end of the range and as late as November at the northern end of the range (Stone and O'Clair 2001). Although water temperature influences hatch timing and larval duration (Shirley et al. 1987; Sulkin and Mckeen 1989), the effect of pH on these parameters is unknown. Eggs hatch 90–160 days post-extrusion (Rasmuson 2013). Immediately after hatching the zoeae rise in the water column where they catch tidal currents moving them offshore. Larvae progress through five zoeal stages and the transitional megalops stage (Online Resource 1).

Cancer magister larvae spend 2–5 months in the water column before settling into the nearshore benthos as juveniles (Moloney et al. 1994; Park and Shirley 2005). While adults inhabit depths from the intertidal nearshore to 230 m (Jensen 2014), planktonic zoeae perform diel vertical migrations away from surface waters during daylight typically reaching depths of 20–30 m with the ability to perform deeper migrations as they progress through later stages (Sulkin and McKeen 1989; Hobbs and Botsford 1992). Megalopae perform diel vertical migrations to depths of 60–70 m (Hobbs et al. 1992; Shanks 2013) and have been recorded to exceed 160 m depth in the Strait of Georgia, British Columbia (Jamieson and Phillips 1993). Due to their diverse geographical, meroplanktonic, and benthic life history, *C. magister* are likely exposed to a variety of pH levels during development.

Laboratory pH treatments should be established based on the carbonate chemistry environment experienced in the natural habitat (McElhany and Busch 2013; Reum et al. 2014). It is important to choose treatment levels that encompass current conditions and future scenarios to get an idea of worst case impacts on *C. magister* populations and to understand their physiological tolerance limits. Puget Sound (Washington, USA), where the adult crabs for this study were collected, is characterized by relatively low-pH and highly variable conditions (Feely et al. 2010; Reum et al. 2014). An analysis of carbonate chemistry data in this region shows summer pH in the upper 50 m of the water column presently average 8.1–7.6 as a function of temperature (360–1270 µatm pCO_2, Reum et al. 2014). Future pCO_2 levels in the same region are predicted to average pH ~7.8 to ~7.3 (760–2150 µatm pCO_2, Reum et al. 2014), and in some parts of Puget Sound, pCO_2 is expected to occasionally exceed 4000 µatm (~pH 7.1) by 2100.

Low-pH (elevated CO_2) exposure studies with other decapod crustaceans have shown a variety of effects ranging from reduced survival (e.g., Red King crab, *Paralithodes camtschaticus*, Long et al. 2013b; intertidal Porcelain crab, *Petrolisthes cinctipes*, juveniles, Ceballos-Osuna et al. 2013) to sublethal, physiological impacts (e.g., slowed metabolic rate in *P. cinctipes* embryos, Carter et al. 2013), to no noticeable effects (e.g., *P. cinctipes* larvae, Ceballos-Osuna et al. 2013; Northern shrimp, *Pandalus borealis*, Arnberg et al. 2013). Similar mixed effects are observed in other crustacean (non-decapod) species (summarized by Kroeker et al. 2013; Wittmann and Pörtner 2013). In general, crustaceans are considered less susceptible to the effects of increased CO_2 than extensively calcified organisms like corals and bivalves (Wittmann and Pörtner 2013). However, both groups can show high variability in response to some species of Crustacea showing greater sensitivity than some species of strong calcifiers (e.g., Long et al. 2013b). Although the exact mechanism by which increased CO_2 affects crustaceans is not entirely clear, it likely varies among individual species and life stages (Whiteley 2011). Given that crustacean hard structures are composed primarily of chitin and protein, it is likely that CO_2-sensitive crustaceans respond to the effects of seawater pH through the internal acid–base balance rather than the effect of calcium carbonate saturation state on calcification (Whiteley 2011). Given the high variability in response to low pH among decapod crustacean species, species-specific experimentation under ecologically relevant conditions remains necessary.

In this laboratory study we exposed *C. magister* eggs and newly hatched zoeae to pH 8.0, 7.5, and 7.1 (corresponding to CO_2 concentrations of ~466, 1781, and 3920 µatm, respectively) to encompass the wide range of pH levels currently experienced by *C. magister* larvae and to predicted future decreases (Miller 2015). The pH 8.0 treatment represents the present pH and serves as a control, the pH 7.5 treatment is observed occasionally in the region, but is expected to be relatively common in the future, and the pH 7.1 treatment is predicted for 2100. Although, as daily vertical migrators, *C. magister* larvae experience highly variable pH conditions as they move through the water column, pH values in this initial experiment to test sensitivity were held constant. We hypothesized that metrics of hatching success (whether an egg hatched or not), time to hatching, survival, growth, and rate of development would decrease relative to the reference condition pH 8.0 with each incremental pH reduction tested.

Materials and methods

Crab collection

For the hatching success experiment, a *C. magister* female already brooding eggs was collected by divers from Puget Sound on December 20, 2012. For the zoeal exposure experiment, *C. magister* females that had not yet extruded eggs were collected from the same site on several dates in September and October 2012 (Washington Department of Fish and Wildlife permit #13-204) and held in individual aquaria at NOAA's Mukilteo Research Station in Mukilteo, Washington, at ambient temperature (~9 °C). Aquaria were filled with sand (~10 cm depth) found on site to provide burrowing habitat and help with formation of the egg mass during egg extrusion. Carapace width for the adult crabs was 13–17 cm. *Cancer magister* females were fed live mussels (*Mytilus* sp.) collected off nearby piers. Eggs and freshly hatched zoeae were collected from gravid females in February and March of 2013 as described in the following sections.

Laboratory system

All experiments were conducted at NOAA's NWFSC ocean acidification laboratory in Seattle, Washington. The laboratory housed a 20,000-L recirculating seawater system consisting of six individually controlled units that delivered filtered (1 μm), UV-sterilized (Emperor Aquatics, Pottstown, Pennsylvania), and degassed (membrane contactors, Liqui-Cel, Charlotte, North Carolina) seawater to experiments at target temperature and pH levels. An automated feedback loop algorithm using LabVIEW software (National Instruments, Austin, Texas) maintained target pH levels with inputs from pH probes (Honeywell Durafet III) and computer-controlled gas solenoid valves bubbling on-site generated CO_2-free air (Twin Tower Engineering, Broomfield, Colorado) and CO_2 gas. The pH probes, calibrated at 12 °C with pH-certified Tris buffer (Dickson Laboratory, Scripps Institution of Oceanography, San Diego, California), continuously measured system pH and temperature. By regular bubbling of air and CO_2-free air, dissolved oxygen remained saturated in all treatments (checked periodically with Honeywell DL5000 sensor). Temperature was maintained at 12 °C using LabVIEW software to control a feedback loop with temperature sensors, heat pumps, and immersion heaters. Deionized water was added continuously to the system by peristaltic pump to reduce the effects of evaporation at a rate manually adjusted based on daily salinity observations. Salinity was measured from treatment tank conductivity probes (Honeywell model 4905) as well as from discrete water samples (ThermoScientific Orion Star A322).

To validate the readings from the system pH probes, discrete water samples were drawn weekly from each of the six treatment tanks and analyzed for dissolved inorganic carbon (DIC) and total alkalinity (TA) using the methods outlined in the Guide to Best Practices for Ocean CO_2 Measurements (Dickson et al. 2007). In addition to continuous readings from pH probes and discrete water samples, spectrophotometric pH (spec-pH) measurements (Yamazaki et al. 1992, Ocean Optics USB 230 2000+ Fiber Optic Spectrometer) were taken from each system at least twice a week. Spec-pH and DIC were then used to calculate TA and pCO_2 using the Seacarb package (Lavigne and Gattuso 2013) in R (R Core Team 2013), with K1 and K2 constants from Lueker et al. (2000) and B_T constant from Lee et al. (2010). All pH values were reported on the total pH scale. The three target pH levels were randomly assigned to the six tanks providing a replicate for each treatment.

Test subjects were held in customized 250-mL polypropylene jars designed for equal distribution of treatment water (Fig. 1). Flow was gravity controlled at a rate of approximately 40 mL min^{-1}. To help maintain treatment temperature, the jars were placed in a system delivered water bath. On each sampling day, spec-pH measurements were collected from two randomly selected 250-mL jars in each of the six treatment tanks and compared with pre-jar system-treatment water to ascertain whether the pH of the seawater in the jar was representative of the treatment. Water bath tanks were enclosed in blackout curtains to isolate eggs and zoeae from light within the laboratory. This mimicked natural light exposure because brooding eggs are buried with the female parent in sediment and zoeae undergo vertical migration to near or below the photic zone during the day.

Chemistry

The similarity of within jar and treatment tank spec-pH samples verified that the target pH was maintained inside the 250-mL jars and carbonate chemistry was distinct among treatments (Table 1). Measurements are presented ±SD. The overall mean of measured TA was 2220 ± 86 μmol kg^{-1}, $n = 54$. The SD of measured TA across all 6 treatment tanks on any one of the 11 individual sample days was <8 μmol kg^{-1}. Calculated TA averaged 2191 ± 73 μmol kg^{-1}. Salinity averaged 31.2 ± 1.1. Evaporation exceeded freshwater replacement over the course of the experiment; TA, DIC, salinity, and Ω_a all followed a similar temporal trend (Fig. 2). Average temperatures in the treatment tanks were 12.05–12.13 °C with a mean of 12.11 ± 0.03 °C. Additional tables on treatment chemistry are provided (Online Resources 2 and 3).

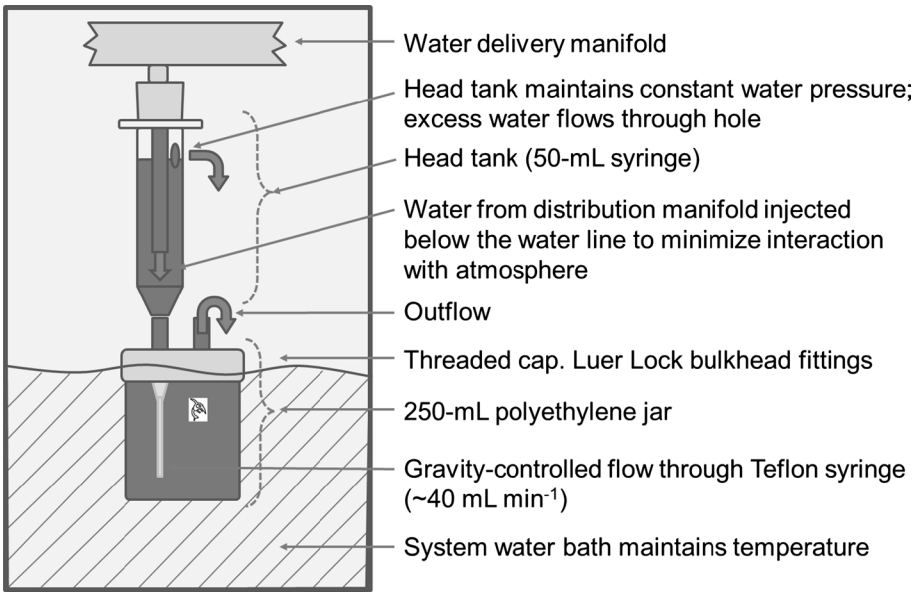

Fig. 1 Diagram of 250-mL jar for rearing small marine organisms (e.g., crab eggs and zoea) under constant, low flow conditions. Design credit Paul McElhany

Table 1 Summary of treatment carbonate chemistry (mean ± SD)

Treatment	DIC (μmol kg^{-1})	pCO_2 (μatm)	Ω_{ar}	Tank	Mean system Durafet-pH	Mean system spec-pH	Mean jar spec-pH
pH 8.0	2058 ± 77	466 ± 24	1.74 ± 0.11	1	8.02 ± 0.01	8.00 ± 0.01	7.99 ± 0.02
				2	8.02 ± 0.01	7.97 ± 0.02	7.96 ± 0.04
pH 7.5	2208 ± 86	1781 ± 106	0.54 ± 0.04	3	7.47 ± 0.01	7.44 ± 0.03	7.44 ± 0.02
				4	7.47 ± 0.01	7.44 ± 0.02	7.43 ± 0.04
pH 7.1	2306 ± 87	3920 ± 306	0.25 ± 0.03	5	7.18 ± 0.01	7.12 ± 0.04	7.13 ± 0.04
				6	7.18 ± 0.01	7.08 ± 0.04	7.09 ± 0.04

DIC is a measured value ($n = 18$), and pCO_2 and Ω_{ar} are calculated. Accuracy and precision for DIC measurements were 1.74 and 3.39 μmol kg^{-1} (=0.084 and 0.167 %), respectively. DIC, pCO2, and Ω_{ar} are system averages; pH values are shown by tank

Egg exposure experiment

Multiple egg strands were removed with forceps from random sites across the egg mass of a single, live, gravid *C. magister* female. A single female was used to reduce sources of variability given the sample size constraints imposed by limited aquarium capacity in the experimental system. Because the female was already brooding eggs at the time of capture from Puget Sound, the exact age of the egg mass and the stage of embryonic development within eggs were unknown. Wet weights of the egg strands were recorded in an effort to place equal numbers of eggs into each jar. Egg strands were divided among 18 jars filled with pH 8.0 treatment water. Three jars were placed in each of the six tanks with water in the jars transitioning from the initial pH 8.0 condition to treatment conditions over approximately 10 min. Eggs from each 250-mL jar were inspected daily for atypical coloration, fungal outbreaks, and hatching before being transferred to a clean jar. No fungicides or antibiotics were used. Hatched larvae were enumerated, assessed for survival, and removed from the jar. Unhatched eggs remaining at the end of the experiment were counted under a dissecting microscope. Counts of hatched larvae were added to the unhatched egg counts to obtain the initial number of eggs jar^{-1}. The exposure study lasted 34 days and ended when hatching had ceased in all jars.

Zoea exposure experiment

Eggs from each of three adult *C. magister* females that extruded on the same day hatched 118 days post-egg deposition. Approximately 500 newly hatched zoeae (<12 h after hatch) were collected from each of the three broods and transported to the laboratory research facility at NWFSC, Seattle, Washington. Three broods were used in an effort to measure maternal effects on pH sensitivity. After a 1-h acclimation to the system water temperature of 12 °C, zoeae were individually placed into jars, one zoea per 250-mL jar, and fed *Artemia salina* (San Francisco

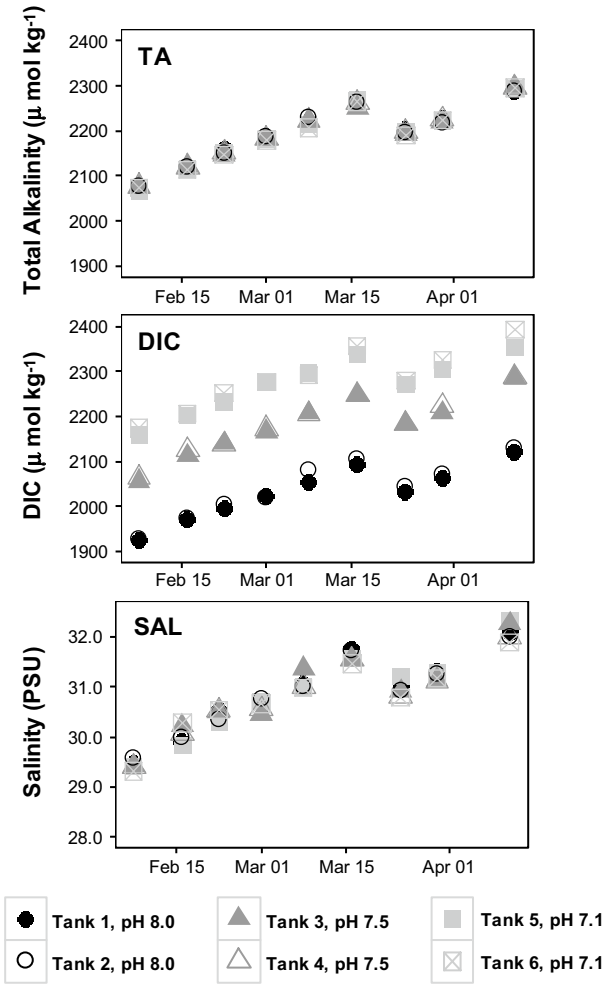

Fig. 2 *TA* total alkalinity, *DIC* dissolved inorganic carbon, *SAL* salinity measured from discrete system water samples. Changes over time were caused by evaporation outpacing addition of deionized water. Three treatments, pH 8.0, pH 7.5, and pH 7.1, are each represented by two tanks in each plot. On March 19, ~8000 L of seawater was added to system resulting in a drop in TA, SAL, and consequently DIC

brand) nauplii at a target concentration of 1 nauplius mL^{-1} of seawater (rearing conditions consistent with Sulkin and McKeen 1989). Seven zoeae from each brood were held in each of the six treatment tanks ($n = 21$ jars per tank). Jars were then allowed to reach target pH as treatment water flowed into the jars at ~40 mL min^{-1}. Every third day each zoea was assessed for survival, placed into a clean jar and fed fresh *A. salina* nauplii. *Artemia salina* were hatched in pH 8.0 seawater and used within a day of hatching. Dead zoeae were preserved individually in 95 % EtOH. A single zoea was placed in each jar with the intent of tracking the individual timing of each molting event by observing discarded carapace molts. However, it was not possible to consistently observe the discarded molt material and life stage could only be determined by microscopic examination of preserved zoea at the completion of the experiment. The experiment was terminated after 45 days when survival in the highest mortality treatment approached 10 %. This termination criterion was a compromise between running the experiment as long as possible and having enough individuals in all treatments for an adequate comparison of final life stage. Zoeae surviving to the final day were euthanized and preserved in 95 % EtOH. Of the 126 zoeae in the experiment, 13 were lost during the sampling process leaving 113 for the survival analysis. Individuals preserved in 95 % EtOH were digitally photographed and then assessed for zoeal stage (1–5) according to documented morphological traits (Poole 1966; Lough 1974). Damaged or unmeasurable zoeae were removed from subsequent size analysis.

Egg experiment analysis

All analyses were run in the statistical program R, ver. 2.13.1 (R Dev. Core Team 2013) unless otherwise noted. An egg hatch ratio was calculated for each jar by dividing the total number of individuals hatched by the total number of eggs. To test for differences among treatments for probability of hatching, or 'hatching success,' a generalized linear mixed model was run with a binomial distribution using the 'Lme4' package (Bates et al. 2013) where each egg was assessed as either hatched (success) or not hatched (failure) with 'tank' and 'jar' as random effects. To test for differences in 'time to hatch' among treatments, an accelerated failure time (AFT) model was constructed with the Weibull distribution (Kleinbaum and Klein 2005, chapter 7) and run with the 'Surv' and 'survreg' functions in the 'survival' package (Therneau 2013) to produce an 'acceleration factor.' The ATF model is an alternative to the more common Cox proportional model for analyzing survival data that provides a more intuitive output metric and is more robust to missing covariates. The acceleration factor describes the rate at which one treatment 'ages' relative to another. Hatch timing or 'time to hatch' was defined as the number days that an egg hatched after a reference date, which was selected as the day the first egg hatched in the experiment. Daily counts of hatched eggs were divided by the total number of hatched eggs to produce hatch proportions within each individual jar. These daily proportions entered the AFT model as the dependent variable with 'jar' and 'tank' as random variables (referred to as 'frailty terms' in the AFT literature). The basic ATF model is $\log(T_i) = \mu + \beta_1 x_1 \cdots + \beta_k x_k + \varepsilon_i$, where T_i is the 'failure time' (e.g., hatching) of individual i, μ is the intercept, β_1–β_k are the regression parameters of interest, x_1–x_k are the predictor variables (e.g., pH treatment), and ε_i is the error. In a frailty model, assumptions about the correlations among the errors caused by the random variables (e.g., tank and jar) modify the regression parameters. The selection

of the Weibull distribution for $\log(T_i)$ was determined by visual assessment of the transformed Kaplan–Meier estimate against log time plot (Kleinbaum and Klein 2005, chapter 7).

Zoea experiment analysis

To test for differences in survival probability among tanks, broods, and treatments, semiparametric Kaplan–Meier (KM) survival analyses were employed using the log-rank Chi-square test statistic in the 'survival' package (Therneau 2013). Multiple pairwise comparisons among KM plots were made using the Holm–Sidak method (Sigma Plot, Systat Software, Inc). An AFT model was used to estimate the mortality acceleration factor, the rate at which mortality occurs in one treatment relative to another. The AFT was run within the 'survival' package (Therneau 2013) using the logistic distribution and incorporated the potential variation from the random effect of 'tank' or 'brood' with the 'time to mortality' set as the dependent variable. The selection of the logistic distribution was determined using the method of Kleinbaum and Klein (2005, p 279).

In order to assess any differences in 'size at stage' among treatments, preserved zoeae were measured for carapace length (CL) following the method from Hirota and Fukuda (1985) using digital images and imaging software (Nikon NIS-Elements). Lengths were assessed for normality using the Shapiro–Wilk's test and homogeneity of variance using the Fligner–Killeen test.

To assess any impacts of treatment on the zoeal stage reached by the end of the experiment (day 45), a priori contrasts were set up (Crawley 2007) within a generalized linear mixed model (Lme4 package, Bates et al. 2013) to test for a difference between the stage reached by zoeae in the control treatment (pH 8.0) versus the low-pH treatments (pH 7.5, 7.1). This was a logistic model probability of reaching zoeal stage 3 or 4 by the end of the experiment with 'tank' as a random factor.

Results

Egg exposure study

The mean ± SD number of eggs jar^{-1} was 593 ± 98. Total eggs in the pH 8.0, 7.5, and 7.1 treatments were 3671, 3220, and 3794, respectively. Eggs were incubated in treatment water for 22 days before hatching commenced, and the experiment lasted for 34 days. Given that the average development period from extrusion to hatching for other crabs reared in the laboratory during this same season was 118 days, the eggs were exposed for approximately the last 20–30 % of development time. Eggs within each treatment started hatching on the same day and daily counts of hatch numbers peaked for all jars 3 or 4 days after hatching commenced, though the pH 7.1 treatment eggs showed a secondary peak 6 days post-hatching that was not observed at pH 8.0 and 7.5 (Fig. 3a). The proportion of eggs hatching in pH 8.0, 7.5, and 7.1 was 0.77 (95 % CI 0.62–0.97), 0.59 (95 % CI 0.26–0.80), and 0.72 (95 % CI 0.48–0.90), respectively, and did not differ among treatments ($P > 0.05$ for all comparisons). The acceleration factor from the AFT model for 'time to hatch' for eggs in the pH 8.0 treatment compared to the pH 7.5 treatment was 0.93 (95 % CI 0.91–0.94; Fig. 3b), indicating that the probability of hatching at time t for eggs in the pH 8.0 treatment was the same as the probability of hatching at time $0.93t$ in the pH 7.5 treatment (pH 8.0 eggs hatched more slowly). The acceleration factor for the pH 8.0 treatment compared to the pH 7.1 treatment was 1.24 (95 % CI 1.22–1.26), indicating that the pH 8.0 eggs hatched sooner than the pH 7.1 eggs. The 95 % confidence intervals for the acceleration factors did not include '1,' indicating that the effect was statistically significant. As an example of the acceleration factor, if it takes 84 h for 50 % of the eggs to hatch at pH 8.0, it is expected to take 78.1 h for 50 % of the eggs to hatch at pH 7.5 and 104.2 h for 50 % of the eggs to hatch at pH 7.1. No fungus or developmental abnormalities were observed during the experiment.

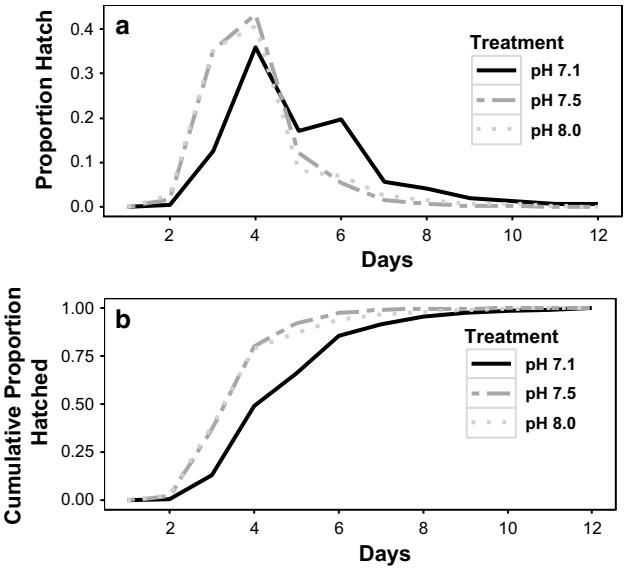

Fig. 3 **a** Proportion of eggs hatched each day. Peak hatching occurred 3 days following first hatched egg. **b** Cumulative proportion of eggs hatched each day

Zoea exposure study

Survival was highest in the pH 8.0 treatment with an average survival by the last day of the experiment of 57.9 %;

Table 2 Numbers of zoeae entering survival analysis and percent survival on final day for each tank within each treatment and associated two-proportion *z*-test

Treatment	Tank	$N_{t=0\ \text{days}}$	$N_{t=45\ \text{days}}$	% Survival	*z*	*P*
pH 8.0	1	20	10	50.0	1.041	0.300
	2	18	12	66.7		
pH 7.5	3	19	2	10.5	0.551	0.582
	4	18	3	16.7		
pH 7.1	5	19	4	21.1	0.000	1.000
	6	19	4	21.1		

Table 3 KM survival analysis pairwise comparisons among treatments using Holm–Sidak method

Comparison	χ^2	*P* value
pH 8.0 versus pH 7.5	12.13	$5.0\ e^{-4}$
pH 8.0 versus pH 7.1	$5.8\ e^{-3}$	$5.8\ e^{-3}$
pH 7.5 versus pH 7.1	$2.2\ e^{-2}$	0.973

average survival in pH 7.5 and 7.1 was 13.5 and 21.1 %, respectively (Table 2). Survivorship did not vary between experimental tanks within a single treatment, nor were there significant differences in survival probabilities among the broods used within each treatment (Online Resource 4). Therefore, all tanks and broods within each treatment were pooled; the initial number of zoeae used in the analyses for treatment pH 8.0, 7.5, and 7.1 was 38, 37, and 38, respectively. Survivorship differed among treatments (Table 3; Fig. 4, $\chi^2 = 13.8$, 2 *df*, $P = 0.001$). Survival for zoeae held at pH 8.0 was significantly higher than those held at pH 7.5 and 7.1, which were similar to one another (Table 3). The acceleration factors of mortality (AFT model) with 'tank' as random variable for zoeae held in the reference condition of pH 8.0 relative to the pH 7.5 and 7.1 treatments were 0.544 (95 % CI 0.349, 0.879) and 0.628 (95 % CI 0.394, 0.999), respectively. With 'brood' as a random variable the resulting acceleration factors of mortality (AFT model) were 0.554 (95 % CI 0.356, 0.863) and 0.628 (95 % CI 0.403, 0.978) for pH 7.5 and 7.1, relative to pH 8.0. Both models were similar and indicated that zoeae in the low-pH treatments died more quickly than in the control pH. For example, a zoea held in the pH 7.5 treatment had approximately the same probability of living past 11 days as a zoea held in the pH 8.0 treatment had of living past 20 days. We did not observe any zoea that died mid-molt (i.e., still attached to partially discarded carapace) and could not evaluate the role of molting in pH sensitivity.

Carapace degradation or breakage from handling reduced the numbers of preserved zoeae for zoeal stage identification or CL measurements. Of the 113 zoeae entering survival analysis, 91 were identified to zoeal stage and 61 were measurable for CL, which resulted in a small sample size for some stage/treatment combinations (Online Resource 5).

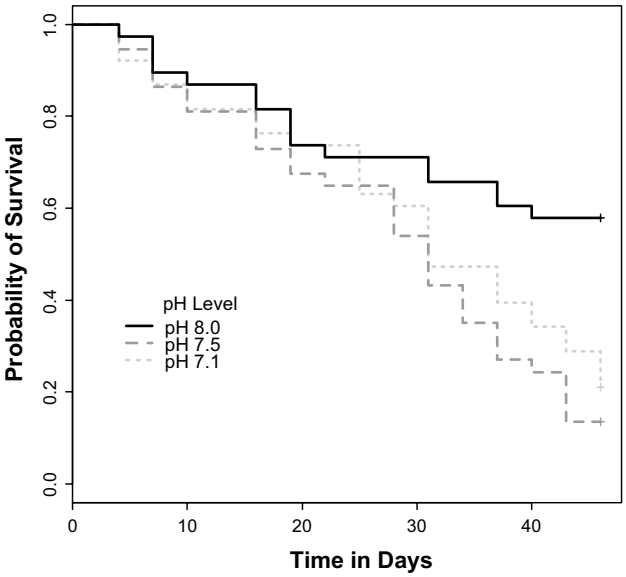

Fig. 4 KM survival curves for the three treatment levels showing probability of survival. Initial numbers of zoeae for pH 8.0, pH 7.5, and pH 7.1 were 38, 37, and 38, respectively (three broods and two replicates per treatment were pooled). Zoeae were checked for mortality every third day

The CL data were consistent with ANOVA assumptions of normality and homoscedasticity. Based on AIC comparison, the ANOVA model incorporated tank as a random effect but not brood. Using a Bonferroni correction for multiple tests, the ANOVA indicated no significant differences in CL among treatments for zoeal stages 2, 3, and 4.

The highest zoeal stage reached by any zoea was stage 4. A greater proportion of larvae progressed to zoeal stage 4 in the control (68 %) versus the two low-pH treatments combined (25 %) such that individuals were 1.77 times more likely to reach stage 4 in the control treatments versus the low-pH treatments (GLM with 'tank' as a random factor, $\chi^2 = 7.00$, $df = 2$, $P = 0.03$, odds ratio 1.77:1).

Discussion

While hatching success, i.e., the probability of hatching, was similar in all three pH treatments, hatching at pH 7.1

was delayed relative to pH 7.5 and 8.0. For zoeae, survival was reduced and development delayed in the two low-pH treatments relative to pH 8.0. These results indicate that low-pH seawater does affect *C. magister* early life history stages and suggest that OA could have deleterious impacts on the population.

Contrary to our hypothesis of decreased hatching success in low-pH seawater, hatching success was not significantly affected by low pH. Our nonsignificant result is similar to a low-pH exposure study where the hatching success of the intertidal crab, *P. cinctipes*, was not different between pH 7.6 and 7.9 (Ceballos-Osuna et al. 2013). Ceballos-Osuna et al. (2013) used multiple broods and posited that hatching success could be brood specific relative to low pH; some broods had greater and some lower success in pH 7.6 relative to pH 7.9. Our study used a single brood, and the hatching success varied widely among replicate treatments (Online Resource 6). Multiple male parentage can occur in a single *C. magister* brood; 40 % of *C. magister* broods in a parentage assignment study had multiple male parents (Jensen and Bentzen 2012). If the effect of low pH on hatching success is brood specific in *C. magister* as it may be in *P. cinctipes*, the potential multiple male parentage within the single brood in this study may account for some of the variation within jars in a single tank. It will be important to include multiple broods in future studies.

It is possible that *C. magister* eggs may be well adapted to low pH as adult females brood while buried in the bottom sediment for weeks at a time (Jensen 2014) where interstitial pore waters could have a relatively low pH (Murray and Gill 1978). In this case, a more suitable 'control' pH treatment for eggs might be <pH 8.0. It is also possible that the 22- to 34-day treatment period might have been too brief, or too late in development, to see an effect.

While hatching success was not affected by low pH, it is interesting that pH had a significant effect on the time to hatch. Eggs required 24 % longer to hatch in the pH 7.1 treatment than those held at pH 8.0. In contrast, eggs held at pH 7.5 hatched 7 % faster than at pH 8.0. This small increase in time to hatch may not be biologically relevant or may indicate a benefit of pH 7.5 (e.g., hormesis, Miller et al. 2013). The data support a threshold for negative effects in time to hatch between pH 7.5 and 7.1. Eggs from *P. camtchaticus*, a deep water crab, experienced a 33 % longer hatch duration, the period of time when hatching commenced to when hatching ended, at pH 7.7 versus an ambient pH 8.0 (Long et al. 2013a). Embryos of *P. cinctipes* exposed for 7–10 days to pH 7.6 had an 11 % lower metabolism than those at pH 7.9 (Carter et al. 2013). The exposure of *C. magister* eggs to low pH in this study may have reduced embryonic metabolic rate, which could decrease developmental rates causing a longer hatch duration.

The changes in hatch duration we observed were relatively small, with mean hatching time ~1 day later at pH 7.1 compared to pH 8.0. It is not clear that this change in duration would have any population-level effects. However, prolonged hatch duration may affect dispersal patterns since dispersal is dependent on when zoeae enter the water column (Shanks 2013). Likewise, hatch duration could alter the chance for trophic mismatch (Edwards and Richardson 2004; Byrne 2011), though the effect of pH is likely subtle compared to other drivers such as temperature (Hays et al. 2005; Schindler et al. 2005).

Zoeal survival was significantly reduced upon exposure to low pH demonstrating potential negative effects of ocean acidification on crab larvae. Three to four times more zoeae survived in the pH 8.0 treatment (55–67 %) than at pH 7.1 (21 %) and 7.5 (14 %). Survival of pH 8.0 larvae was similar to previous studies in which 50–76 % of larvae survived through zoeal stage 4, the latest stage reached by zoeae in the present study (Gaumer 1973; Sulkin and McKeen 1989). A pH of 7.5 was recently recorded in the surface 50 m of Puget Sound (Feely et al. 2010; Reum et al. 2014), and pH 7.1 is 0.2 pH units lower than the seasonal pH in deep waters of the Hood Canal, Washington (Feely et al. 2010), indicating that present and near-future ocean chemistry may impact *C. magister* life history. Additional research is needed to discern whether the observed effects carry over into subsequent life stages and generations and whether the observed effects are due to direct, indirect, or combined mechanisms.

In separate experiments conducted concurrently with this study, water from the experimental system had deleterious effects on herring (*Clupea pallasii*), copepod (*Calanus pacificus*), and krill (*Euphausia pacifica*) larvae. Subsequent investigation suggested that exposure to acrylonitrile butadiene (Buna N) and ethylene propylene diene monomer (EPDM), rubber compounds commonly used on valves and pipe fittings in aquaculture facilities, could cause high mortality in larvae of all three species. The system water produced no noticeable toxic effects in survival and growth of *C. magister* or in other concurrent studies on oysters (*Crassostrea gigas*), geoducks (*Panopea generosa*), and pygmy rock crabs (*Cancer oregonensis*) or in a short-term study on pteropods (Busch et al. 2014). However, it is possible that some compound in the system water provided an additional stressor on crab larvae in all treatments during the experiment. Had we not been trying to rear larval stages of chemically sensitive species in the same experimental system we would not have been aware of this as a potential issue. This experience suggests that any CO_2 sensitivity experiment has the potential to contain hidden stressors (e.g., aquarium material, handling, diet, etc.) with potential unrecognized interaction effects.

It is important to recognize that a shorter experiment might not have captured the significant differences in survival among treatments. It took 27 days of continuous zoea exposure to low pH before a divergence among treatments was apparent. A delay in seeing reduced survival in low pH relative to controls has been seen in other decapod species (Kurihara et al. 2008; Long et al. 2013b) and could be a reflection of their ability to regulate their acid–base equilibrium (Henry and Wheatly 1992). Adult *C. magister* have the ability to recover from a 24-h exposure to pH 7.1 (Pane and Barry 2007). Regardless of the ability of *C. magister* larvae to maintain internal acid–base equilibrium, continued regulation likely comes with increased energetic costs and less energy prioritized for other physiological functions (Whiteley 2011).

Although we found no significant differences in zoea CL at stage among the treatments, development was delayed at low pH. Once a zoea reached each stage, its size was indistinguishable from those in the control (pH 8.0) treatment. This developmental delay from low-pH exposure has occurred in other species at the larval stage as well: Northern shrimp, *Pandalus borealis*, at pH 7.6 relative to those in pH 8.1 (Arnberg et al. 2013); sea urchin, *Strongylocentrotus purpuratus*, at pH 7.7 relative to those in pH 8.1 (Stumpp et al. 2011); and spider crab, *Hyas araneus*, at pH 7.3 relative to those in pH 8.1 (Walther et al. 2010). The mechanism for this delay could be a decrease in metabolism. Exposure to low-pH seawater can create changes in the extra and intracellular acid–base balance in crustaceans leading to lower overall metabolic rates, though, as noted above, there is at the same time an increase in energy used to maintain acid–base balance (Whiteley 2011). If *C. magister* metabolism is being affected by low pH as observed in the embryonic stages of the intertidal porcelain crab (Carter et al. 2013), the resultant developmental delay at the larval stage could have negative implications for *C. magister* at the population level. An increase in the duration of larval stages could increase the risk of predation in the water column and/or put settling larvae out of synchronization with other food or habitat resources (Dupont and Thorndyke 2009).

As surface waters continue to acidify from absorption of rising levels of atmospheric CO_2, the likelihood of long-term, low-pH exposure of *C. magister* larvae increases. Areas of greatest vulnerability will likely be where deep waters, naturally low in pH, meet acidified surface waters. This exchange often occurs in areas of coastal upwelling as well as estuarine systems (Hoffman et al. 2011). Annual average surface pH in the eastern Pacific along the continental USA is predicted to reach 7.8 by 2050, with potentially even lower pH during coastal upwelling (Gruber et al. 2012). Estuarine systems such as Hood Canal, Washington, have already had pH as low as 7.6 in surface waters (Feely et al. 2010). These values and predicted low-pH stress the importance of monitoring coastal pH levels and research on the physiological effects of pH 8.0–7.5.

This study shows that early life stages of *C. magister* are susceptible to low pH relevant to those predicted for the effects of OA. Lower survival combined with slower development would likely have population scale impacts. By increasing predation, reducing survival, or by a mismatch with tidal and ocean currents that disperse larvae, long-term exposure to low pH could reduce the number of *C. magister* recruits and cause a decline in the fishery.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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