ICES Journal of Marine Science

ICES Journal of Marine Science (2017), 74(4), 1033–1041. doi:10.1093/icesjms/fsw197

Contribution to Special Issue: 'Towards a Broader Perspective on Ocean Acidification Research Part 2'

Original Article

Survival, growth, and morphology of blue king crabs: effect of ocean acidification decreases with exposure time

William Christopher Long*, Scott B. Van Sant, Katherine M. Swiney, and Robert J. Foy

NOAA, National Marine Fisheries Service, Alaska Fisheries Science Center, Resource Assessment and Conservation Engineering Division, Kodiak Laboratory, 301 Research Ct, Kodiak, AK 99615, USA

**Corresponding author: tel: + 907-481-1715; fax: + 907-481-1701; e-mail: chris.long@noaa.gov.*

Long, W. C., Van Sant, S. B., Swiney, K. M., and Foy, R. J. Survival, growth, and morphology of blue king crabs: effect of ocean acidification decreases with exposure time. – ICES Journal of Marine Science, 74: 1033–1041.

Received 25 May 2016; revised 22 September 2016; accepted 15 October 2016; advance access publication 21 December 2016.

Ocean acidification is an altering marine carbonate chemistry resulting in potential effects to marine life. In this study, we determine the effects of decreased pH on the morphology, growth, and survival of juvenile blue king crab, *Paralithodes platypus*. Crabs were reared at three pH levels: ambient (control, pH ~8.1), pH 7.8, and pH 7.5, for 1 year and monitored for morphological changes, survival, and growth. Exposure to seawater at pH 7.8 had no effect on morphology or mortality and had only a minor effect on growth compared with the ambient treatment. However, exposure to seawater at pH 7.5 substantially increased mortality and decreased growth compared with the ambient treatment. The best fit model of mortality rate at pH 7.5 showed an initially high mortality rate, which dropped to become comparable to the mortality rate in the other treatments. This suggests phenotypic variability or plasticity in juveniles and may indicate acclimation by blue king crab to ocean acidification. As such, blue king crab may have scope for evolutionary adaptation in response to gradually changing pH levels. However, effects on other life-history stages, sub-lethal effects, carryover or transgenerational effects, and interactions with other stressors, such as increased temperature, still need to be investigated.

Keywords: climate change, crustacean, environmental stressor, fishery species, hypercapnia, North Pacific, ocean acidification, phenotypic plasticity.

Introduction

Seawater uptake of anthropogenic CO_2 decreases pH, alters carbonate chemistry (Feely *et al.*, 2004), and is commonly referred to as ocean acidification (OA). This has caused a 0.1 pH reduction in the oceans since the beginning of the industrial revolution and is predicted to reduce the average surface pH of the oceans by about 0.3 pH units by the end of the century (Caldeira and Wickett, 2003; Fabry *et al.*, 2008); however, local changes may be more extreme. In particular, areas in higher latitudes, such as the Bering Sea, are expected to acidify more rapidly than other areas because of colder seawater temperatures, upwelling of CO_2-rich waters, and increased fresh seawater inputs from melting glaciers (Fabry *et al.*, 2009). Already, seasonal benthic respiration can increase pCO_2 to 1600 ppm (approximately four times atmospheric pCO_2) in benthic waters in the Bering Sea (Mathis *et al.*, 2014), and surface waters are expected to become undersaturated with respect to aragonite by 2070 (Mathis *et al.*, 2015). Such changes to the chemistry of the ocean could have a dramatic effect on ecosystems as marine organisms exhibit a wide range of responses (Kroeker *et al.*, 2013).

OA can result in physiological stress to marine organisms, but the results are highly variable (Kroeker *et al.*, 2013). Organisms with calcified exoskeletons, such as crustaceans, are thought to be vulnerable because of increased solubility of calcium carbonate in lower pH seawater (Whiteley, 2011). There is a range of physiological responses to reduced pH among crustaceans. Some are able to buffer their hemolymph via ion-regulation with bicarbonate (Spicer *et al.*, 2007; Knapp *et al.*, 2015), whereas others cannot (Pane and Barry, 2007). Those species that can iono-regulate their hemolymph will probably do so at an energetic cost (Whiteley, 2011), whereas those that cannot may respond by decreasing metabolism and therefore internal CO_2 production (Whiteley, 2011;

Carter *et al.*, 2013). Both increased energetic costs and decreased metabolism can affect a wide range of biological parameters; OA can reduce survival (Kurihara *et al.*, 2008; Baragi and Anil, 2015), growth (Keppel *et al.*, 2012; Long *et al.*, 2013b), calcification (Arnold *et al.*, 2009; Walther *et al.*, 2011), and reproductive output (Swiney *et al.*, 2016), and alter behaviour (Dodd *et al.*, 2015). Other species are relatively robust and do not appear to suffer any effects (Pansch *et al.*, 2013; Schiffer *et al.*, 2013). The response can vary, not only among species but also among life-history stages; for example, *Homarus gammarus* larvae are sensitive to OA at the first and megalops stages but not at the second or third stage (Small *et al.*, 2015). Responses can also be affected by carry-over (i.e. when exposure at one life-history stage affects an individual in a subsequent stage) or transgenerational (i.e. when parental exposure affects the offspring) effects (Schiffer *et al.*, 2014; Long *et al.*, 2016; Swiney *et al.*, 2016).

The blue king crab, *Paralithodes platypus*, is a commercially valuable species in the Bering Sea. There are two stocks that have been harvested in the eastern Bering Sea: the Pribilof Islands stock and the St. Mathew Island stock. Both populations and the fisheries of each stock have fluctuated over the years. At its peak in 1980, the Pribilofs Island harvest was 4.5 million kg, but the stock has crashed and the fishery closed since 1999 (NPFMC, 2015). The St. Mathew harvest peaked at 4.2 million kg in 1983, but it dropped and was closed to fishing from 1999 to 2008 though it recent years it has been reopened to fishing with an exvessel value (Exvessel price reported by Alaska Department of Fish and Game. http://www.adfg.alaska.gov/index.cfm?adfg=CommercialByFisheryshellfish.shellfishcatch_exvessel_crab. Accessed September 13, 2016.) of about $9 million in 2012 (NPFMC, 2015). Like many crab species, it has a complex life-history. Larvae are hatched in the spring after a 1-year brood cycle (Jensen and Armstrong, 1989). The larvae pass through four zoeal stages and one glaucothoe or settling stage (Stevens *et al.*, 2008). The glaucothoe settle in benthic habitat and moult to the first crab stage. Glaucothoe target complex structured habitats for settling (Tapella *et al.*, 2009), such as shell-hash (Armstrong *et al.*, 1985), where their cryptic behaviour reduces predation risk (Daly and Long, 2014a; Lyons *et al.*, 2016). Juveniles grow in the benthic habitat before moulting to adults and mating.

Nothing is known about blue king crab sensitivity to OA; however, the closely related red king crab (*Paralithodes camtschaticus*), which has a similar distribution (Somerton, 1985), has been more extensively studied. OA affects red king crab at embryo, larval, juvenile, and adult stages, increasing mortality, decreasing growth, decreasing condition, and altering morphology (Long *et al.*, 2013a, b). Additionally, increased temperature can increase the sensitivity of juvenile red king crab to OA (Swiney *et al.*, in review). These effects are predicted to cause a substantial decrease in population abundance and subsequently, the fishery for Bering Sea red king crab in the near future (Punt *et al.*, 2014). Therefore, it is critical for fishery managers to understand how OA will affect blue king crab populations. In this article, we report on a year-long laboratory experiment to assess the effects of OA on growth, morphology, and survival of juvenile blue king crab.

Material and methods

Animals

Blue king crab juveniles were reared in the laboratory, as described in Long (2016). Broodstock were captured in commercial fishing pots in the Bering Sea near St. Matthew Island in the winter of 2010. Animals were held at the Alaska Fisheries Science Center's Kodiak Laboratory seawater facility in flow-through ambient seawater and fed a diet of mixed herring and squid. Larvae were collected at hatching from six females and reared in a 2000-l tank stocked at 30 larvae L^{-1}. Larvae were fed newly hatched *Artemia nauplii* enriched with DC DHA Selco (INVE Aquaculture, UT, USA) except during the glaucothoe stage, which is a non-feeding stage. Glaucothoe were provided with artificial macroalgae to settle on prior to moulting to the first crab stage.

Experimental design and setup

Three pH treatments were used in this study based on projected future ocean conditions based on the Intergovernmental Panel on Climate Change's IS92a scenario (IPCC, 2001; Caldeira and Wickett, 2003): ambient (pH ~8.1), pH 7.8 (predicted mean surface ocean pH by ~2100), and pH 7.5 (predicted by ~2200). The ambient treatment was our control treatment, which we will refer to as 'ambient' to emphasize the pH treatment level the crabs were exposed to. Seawater for this project was acidified with CO_2 as described by Long *et al.* (2013a). Briefly, seawater was acidified to pH ~5.5 using bubbled CO_2 and mixed with ambient seawater using a peristaltic pump controlled by a Durafet III pH probe which kept the pH constant in a head tank. Seawater from the head tanks was used in experimental tanks. Temperatures were allowed to vary naturally but were chilled when necessary to keep the seawater under 10°C, which is well within the range of thermal tolerance for blue king crab (Stoner *et al.*, 2013). Thirty juvenile blue king crab at the first crab stage were randomly assigned to each treatment (90 total in all three treatments). Animals were held in individual cells made of sections of PVC pipe with mesh-covered bottoms to allow for water outflow. Their diameters were 5.1 cm, which is large enough not to affect the growth or survival of juvenile red king crab (Swiney *et al.*, 2013). Cells were placed inside three experimental tanks, which received flow-through seawater at the one of the three pH treatments. Seawater was circulated within each tank into each of the individual containers. Crab were fed to excess three times a week on a diet of 'Gelly Belly' (Florida Aqua Farms, Inc., Dade City, FL, USA) mixed with Cyclop-eeze powder (Argent Laboratories, Redmond, WA, USA) and pollock bone powder (United States Department of Agriculture, Agricultural Research Service, Kodiak, AL, USA). The experiment was conducted from 17 June 2011 to 14 June 2012.

Water chemistry

The temperature and pH of five randomly selected individual containers within each treatment were measured daily using a Durafet III pH probe. Weekly, 300-ml seawater from each treatment head tank was collected, poisoned with mercuric chloride to a final concentration of 55 μM, and sent to the AFSC's Auke Bay Laboratories for analysis of dissolved inorganic carbon (DIC) and total alkalinity (TA). DIC was determined using a CM5014 Coulometer with a CM5130 Acidification Module (UIC Inc., Joliet, IL, USA) using certified reference material from the Dickson Laboratory (Scripps Institution, San Diego, CA, USA), TA was measured via open cell titration; both procedures followed the methods in Dickson *et al.* (2007). The seacarb package in R (V2.14.0, Vienna, Austria) was used to calculate the other

Table 1. Components of the carbonate system in experimental tubs.

Treatment	pH_F	pCO_2 µatm	HCO_3^- mmol/kg	CO_3^{-2} mmol/kg	DIC mmol/kg	Alkalinity mmol/kg	$\Omega_{Aragonite}$	$\Omega_{Calcite}$
Ambient	8.07 ± 0.07	390.64 ± 54.27	1.89 ± 0.05	0.09 ± 0.01	2.00 ± 0.04	2.13 ± 0.07	1.42 ± 0.19	2.26 ± 0.30
pH 7.8	7.80 ± 0.03	766.59 ± 44.95	1.98 ± 0.04	0.05 ± 0.00	2.07 ± 0.04	2.13 ± 0.08	0.78 ± 0.06	1.25 ± 0.10
pH 7.5	7.49 ± 0.03	1627.00 ± 83.53	2.03 ± 0.04	0.03 ± 0.00	2.14 ± 0.04	2.13 ± 0.06	0.39 ± 0.03	0.62 ± 0.04

Values are means ± *SD*. pH was measured daily on the free scale in individual cells, dissolved inorganic carbon (DIC) and alkalinity were measured weekly in seawater from the head tanks, and all other parameters were calculated. $\Omega_{Aragonite}$ and $\Omega_{Calcite}$ indicate the saturation states of aragonite and calcite.

parameters of the carbonate system, including the pCO_2, the concentrations of bicarbonate and carbonate and the saturation states of calcite and aragonite, from the measured pH, temperature, and TA using the default reaction rates (Table 1; Lavigne and Gattuse, 2012).

Crab growth, survival, and morphometrics

Each juvenile crab was checked daily for moulting or mortality. When a crab moulted, the exuvia was carefully removed and photographed under an Olympus SZ60 stereo-microscope using a Jenoptik ProgRes SpeedXT core 5 digital camera. Dead crabs were also removed and similarly photographed. Morphometric measurements, including carapace length and width, rostrum base width, orbital spine width, and the first spine length (Figure 1) (Long *et al.*, 2013b), were made to the nearest 0.01 mm from the images using Image-Pro Plus v. 6.00.260 (Media Cybernetics, Inc., Bethesda, MD, USA). The microscope was calibrated using a micrometer each time pictures were taken. At the beginning of the experiment, and 7 days after moulting event, crabs were carefully removed from the seawater, blotted dry, massed, and returned to the seawater. On completion of the experiment, crabs that had moulted within 7 days were kept in their treatment tanks to allow a final mass to be measured, and all crabs were photographed for morphometric analysis as above.

Statistical analyses

Morphometric measurements were analysed using principle component analysis (PCA) in Primer 6.1.13 (Plymouth, UK). In essence, PCA takes multivariate data sets, rotates, and reprojects them (as one might rotate a '3D' plot on a 2D computer screen) to maximize the amount of variance shown. Each axes, or PC, in the new projection can be ranked by its eigenvalue (which is directly correlated to the proportion of the data it explains) and is a linear combination of all the variables, as expressed by the eigenvector. PCs can therefore be interpreted by examining their eigenvector. The goal is to reduce a multivariate dataset down to one or more PCs that contain most of the variance in the data in order to simplify visualization, analysis, and interpretation. It is especially useful when variables are correlated with each other, as is usually the case in morphometric data (e.g. a crab with a larger carapace width will also tend to have a larger carapace length). Measurements were normalized (i.e. expressed in terms of their z-score) prior to analysis to ensure that all features would be equally weighted in the analysis. We retained all PCs for further analysis that explained a cumulative 90% of the data. Differences in retained PCs (i.e. those components that contained sufficient amounts of the data to warrant further examination) were analysed with a fully-crossed, two-way analysis of variance (ANOVA) with pH treatment fully crossed with moult stage and crab number, nested within treatment, as factors. For this, and for all ANOVA-type analyses, the assumption of homogeneity of variance was checked with Levene's test.

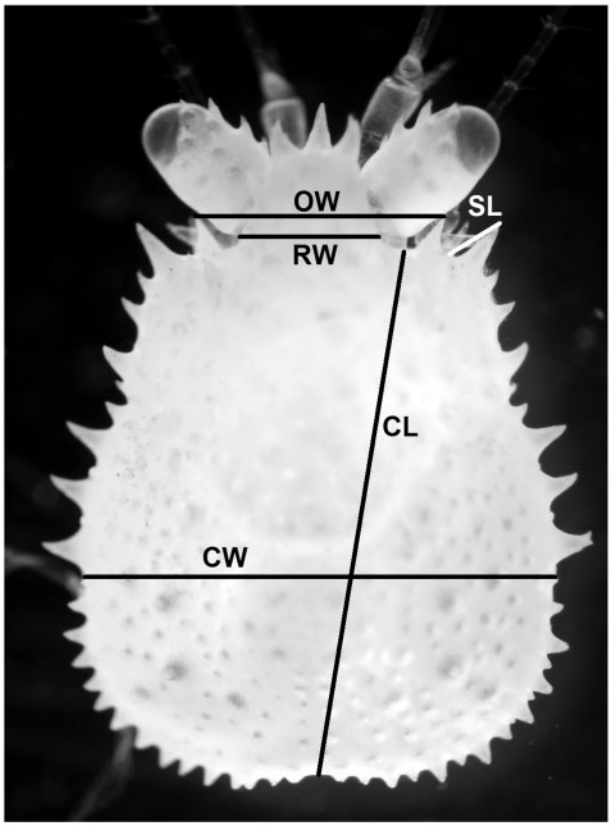

Figure 1. Photograph showing details of morphometric measurements made on blue king crab, which included carapace width (CW), carapace length (CL), rostrum base width (RW), orbital spine width (OW), and the first spine length (SL).

Growth data in terms of carapace length were analysed using an analysis of covariance (ANCOVA) with treatment fully crossed with time and crab number (nested within treatment) as factors. For all growth analysis, time was expressed in degree-days (i.e. the sum of the average daily temperature in degrees Celsius) to account for the changing temperature over the course of the experiment (Long *et al.*, 2013b). Data on wet mass were fitted to a series of exponential models such that $M = ae^{bt}$, where M is the wet mass, t is the time, a is a parameter indicating the initial size, and b is the growth rate. In the different models, the parameters a and b were allowed to vary with treatment. Models were fitted in R 3.1.2 (Vienna, Austria) assuming a normal distribution of errors using the maximum likelihood *mle* function (R Core Team

and contributors worldwide, 2013) and the AIC_c (Akaike's Information Criterion corrected for sample size) was calculated for each model with the most parsimonious model selected. Models whose AIC_cs differed by <2 were considered to explain the data equally well (Burnham and Anderson, 2002).

Survival was initially modelled with a binomial distribution of errors as a difference equation such that: $p_{M(t)} = m_t p_{M(t-1)}$, where $p_{M(t)}$ is the probability of mortality at time t, m_t is the mortality rate at time t, and t is the time in days. Initially, the number of crab alive in each treatment on each day was fitted to a series of models in which m was constant over time and was a linear function of pH treatment. Post hoc we noted that the mortality rate apparently decreased over the course of the experiment. We therefore included models where m decreased either linearly or exponentially to a minimum with time and either did or did not differ among pH treatments. Models were fitted using maximum likelihood as described above and the AIC_c was calculated for each model, with the best model selected as described above.

Results

Carbonate chemistry

Temperatures in experimental containers varied seasonally from highs of around 9.5°C in the summer of 2011 to lows of about 1°C in the winter of 2012 (Figure 2), while salinity was relatively constant averaging 31.3 ± 0.3 (*SD*). The average pH in the ambient treatment was about 8.1 and the average pH in experimental tubs was within 0.01 units of the nominal value (Table 1). Unsurprisingly, the pCO_2 was increased in the lower pH treatments where CO_2 was added to the water. As expected, the TA did not vary among treatments, while the DIC increased with the amount of CO_2 added. With the increase in DIC and decrease in pH, the concentration of carbonate decreased with pH but the concentration of bicarbonate increased with DIC. This led to an increase in the solubility of both forms of calcium carbonate with decreasing pH. Although the saturation state of calcium carbonate varied with the temperature throughout the experiment, the pH 7.8 treatment was always undersaturated with respect to aragonite and the pH 7.5 treatment was always undersaturated with respect to both aragonite and calcite (Table 1, Figure 2).

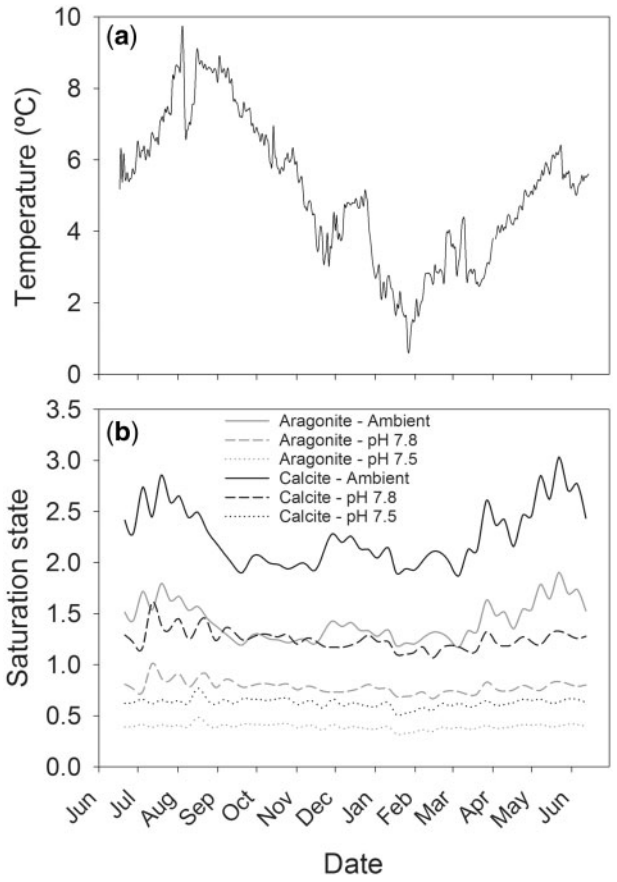

Figure 2. Average temperatures (a) in the three experimental tubs holding blue king crab measured daily from June 2011 to June 2012, and the saturation state of calcite and aragonite (b) estimated weekly from DIC and pH measurements. Average daily *SD* in temperature was 0.1 °C.

Morphology

Crabs moulted up to six times during the experiment; however, as only five moulted a sixth time, we excluded these from the morphometric analysis because of the low sample size. The PCA of crab morphometrics resulted in two retained PCs that together explained virtually all (95.3%) of the variance. The first PC explained 89% of the variance and was negatively associated with all measurements approximately equally (Table 2). It was interpreted as being negatively correlated with crab size because as you move along that PC (axis) all the measured parameters decrease proportionally indicating a change in size but not in shape. The second PC explained 6.3% of the variance and was positively associated with the rostrum base width and negatively associated with the first spine length. The first PC varied with moult stage, treatment, and their interaction (Table 2). At the beginning of the experiment, there was no difference among the treatments, but after the second moult, crabs in the pH 7.5 treatment were significantly smaller than those in the ambient treatment, and after the third moult, they were smaller than both the pH 7.8 and the ambient treatments and this difference continued for every subsequent moult stage (Figure 3). The crabs also grew as they passed through subsequent moult stages. The second PC differed among moult stages, but not treatments, and was not further analysed because it explained such a small proportion of the variance and was not affected by treatment and is therefore simply indicative of a small change in carapace shape that occurred in the crabs as they passed through moult stages (Table 2).

Growth

Carapace length varied with the interaction between treatment and time (ANCOVA, $F_{(2,74)} = 4.865$, $P = 0.008$), indicating a violation of the assumption of homogeneity of slopes and therefore that the growth rate (the slope) differed significantly among the treatments. Therefore, each treatment was analysed separately using linear regression. Crabs in ambient seawater grew in terms of carapace length faster than those in both pH 7.8 and pH 7.5 seawater (Figure 4). The difference between ambient and pH 7.8 was slight, with crabs being only 2% larger in the ambient seawater at the end of the experiment, but the difference between ambient and pH 7.5 was much greater: 10% at the end. In the best fit model of growth in terms of wet mass, the initial size (a) did not vary among treatments, but the growth rate (b) did (Table 3, Figure 4). Crabs in ambient seawater grew faster than

Table 2. PCA of blue king crab morphometrics for crab held at three different pH treatments.

PC	Eigenvalues	%Variation	Cum.%Variation
1	4.45	89.0	89.0
2	0.31	6.2	95.3

	Eigenvectors	
Variable	**PC1**	**PC2**
Carapace width	−0.466	0.08
Carapace length	−0.467	0.032
Rostrum base width	−0.434	0.554
Orbital spine width	−0.451	0.108
1st spine length	−0.416	−0.821

ANOVA

Variable	Factor	F	*P*
PC1	Treatment	12.569	<0.0005
	Stage	357.559	<0.0005
	T*S	1.956	0.038
	Crab(Treatment)	3.831	<0.0005
PC2	Treatment	0.67	0.513
	Stage	34.892	<0.0005
	T*S	0.368	0.959
	Crab(Treatment)	1.702	0.001

The first two principle components (PC) representing 95% of the variance are retained. Treatment (T) represents the pH treatment and Stage (S) the moult stage.

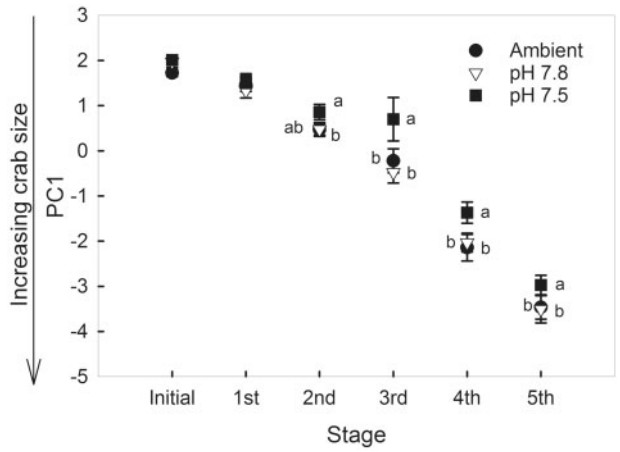

Figure 3. Average principle component 1 scores from blue king crab morphometric analysis in the three treatments. Error bars are SE. Differences among treatments within each moult stage are indicated with different letters.

those in pH 7.8, which grew faster than those in pH 7.5 (Figure 4).

Survival

In the best-fitted model of crab survival, the mortality rate differed among all three treatments and decreased exponentially to a minimum over time; no other model had any weight (and therefore support) at all and the presented model is unequivocally the best model of all those fitted (Table 4). The estimated mortality rate for crabs in ambient seawater was higher than that for crabs in pH 7.8 seawater at the beginning of the experiment; however, the rate in ambient seawater decreased quickly such that,

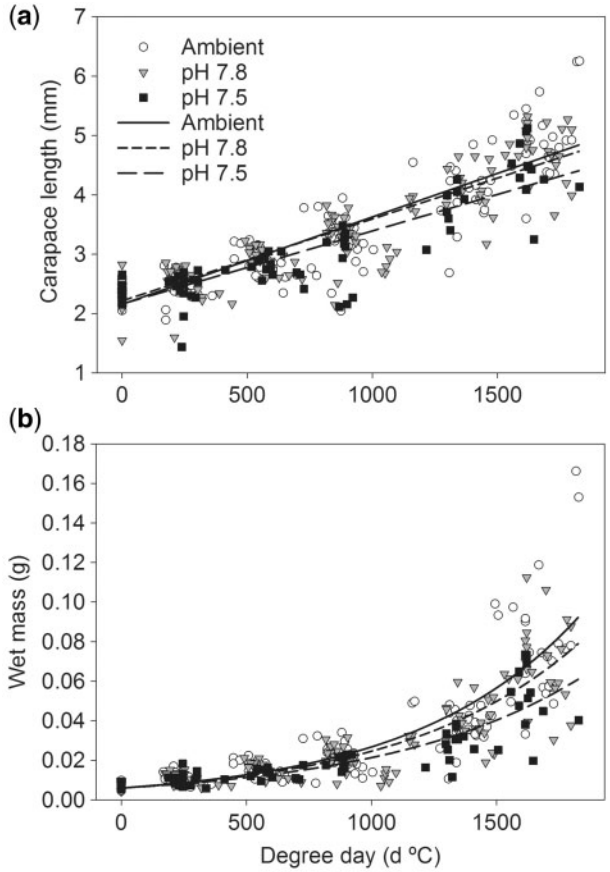

Figure 4. Effect of pH on growth in blue king crabs in terms of (a) carapace length (CL) and (b) wet mass (WM). Points indicate individual measurements and lines are best fit models (see Methods for description). Time is expressed in degree-days (i.e. the sum of the average daily temperature in degrees Celsius). Equations for the models are: ambient-CL = 2.16 + 0.00147*t*, WM = $0.00599e^{0.00150t}$; pH 7.8- CL = 2.12 + 0.00138*t*, WM = $0.00599e^{0.00141t}$; pH 7.5- CL = 2.16 + 0.00123*t*, WM = $0.00599e^{0.00127t}$, where *t* is the time in degree days.

Table 3. Model selection table for models of blue king crab growth in terms of wet mass using Akaike's information criterion corrected for sample size (AIC_c).

Model	K	AIC_c	ΔAIC_c	Likelihood	AIC_c weight
a, b	3	−2326.93	35.71	0.00	0.00
a(T), b	5	−2356.69	5.95	0.05	0.04
a, b(T)	5	−2362.64	0.00	1.00	0.79
a(T), b(T)	7	−2359.51	3.12	0.21	0.17

Model indicates which parameters were included (see text for details). Where *T* is included parenthetically it indicates the parameter was modelled as a linear function of pH treatment. K indicated the number of parameters for each model.

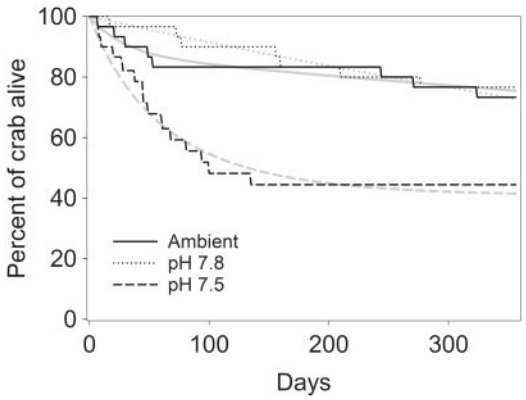

Figure 5. Effect of pH on survival of juvenile blue king crabs. Black lines show the observed daily survival in each treatment and the grey lines plot the best-fit model. Equations for models of best fit mortality rate for each of the three treatments are ambient $= 0.00041 + 0.0043e^{-0.032t}$, pH 7.8 $= 0.00088 + 0.0000044^{-0.000000011t}$, pH 7.5 $= 0.0000000045 + 0.010e^{-0.011t}$, where t is the time in days.

after about day 100, the rate was lower than in pH 7.8. This difference in mortality rate was of little consequence as the difference in overall survival between the ambient and pH 7.8 treatment was never >12% and at the end of the experiment it was only 3% or one crab (Figure 5). In contrast, the mortality rate in pH 7.5 treatment at the beginning of the experiment was more than double that in the ambient treatment; however, it dropped quickly such that it was about the same as the rates in the ambient and pH 7.8 treatment by day 200 (Figure 5). Because of the initially high mortality rate, by the end of the experiment, overall survival in the pH 7.5 treatment was 30% lower than in the ambient and pH 7.8.

The raw data associated with this study and the associated metadata have been included as a supplement to this article (Supplementary Material S1).

Discussion

Decreased seawater pH resulted in increased mortality and decreased growth in juvenile blue king crabs in this long-term exposure experiment. Subtle effects, a small decrease in growth rates, occurred at pHs expected in surface waters within the next 80 years (pH 7.8), with effect sizes on growth and mortality becoming much larger at a pH expected by the 22nd century (pH 7.5) (Caldeira and Wickett, 2003). Although such changes in growth and survival could have a substantial negative effect on the population biology and fishery stocks, the decrease in mortality rate at the lowest pH suggests that phenotypic plasticity (altering physiology to adapt to different conditions) or phenolic variability could substantially reduce or eliminate any potential large-scale population effects. Because the pHs at which blue king crab is affected will not be reached for many decades, there is ample time for evolutionary adaptation to work on phenotypic variability in the population. Thus, blue king crab may prove resilient to changes in ocean carbonate chemistry over the next two centuries; however, more research is needed to confirm this.

Overall mortality during the first year of life of blue king crabs was substantially higher at a pH of 7.5 but not at a pH of 7.8 compared with ambient (pH 8.1). A similar response occurred for red king crabs, *P. camtschaticus*, and southern Tanner crabs, *Chionoecetes bairdi*, both of which also experienced reduced survival under acidified conditions (Long *et al.*, 2013b). However, blue king crab had a lower effect size and lower pH threshold in their response; both red king crabs and southern Tanner crabs had increased mortality at pH 7.8, and red king crabs exhibited 100% mortality after <100 days at pH 7.5. Other juvenile crustacean species have an even greater tolerance to OA; neither European lobsters, *H. gammarus* (Agnalt *et al.*, 2013), nor blue crabs, *Callinectes sapidus* (Ries *et al.*, 2009), had decreased survival rates under acidified conditions. The overall mortality at pH 7.5 would likely lead to a decrease in the population abundance and fisheries yield for blue king crab, similar to what is predicted for red king crab and southern Tanner crab (Punt *et al.*, 2014, 2016); however, the overall mortality is not the whole story.

Table 4. Model selection table for models of blue king crab mortality using Akaike's information criterion corrected for sample size (AIC_c).

Model	K	AICc	ΔAICc	Likelihood	AICc weight
m	1	7913.69	4289.52	0.00	0.00
m(T)	3	4404.67	780.49	0.00	0.00
Linear m(Time)	2	7258.91	3634.73	0.00	0.00
Exponential m(Time)	2	7221.23	3597.06	0.00	0.00
Linear m(T, Time)	6	3682.57	58.39	0.00	0.00
Exponential m(T, Time)	6	3624.18	0.00	1.00	1.00

Model indicates how *m* the mortality parameter was modelled (see Methods for details). Where *T* is included parenthetically it indicates the parameter was modelled as a linear function of pH treatment. *Linear* and *exponential* indicate that m was allowed to decrease over time linearly or exponentially respectively. K indicates the number of parameters for each model.

The mortality rate at pH 7.5 started off much higher than in other treatments at the beginning of the experiment; however, there is strong evidence that the mortality rate did not remain constant but rather declined quickly over the first couple of months such that, by the end of the experiment, it was similar to or even lower than the rate in the other treatments. Indeed, there was not a single mortality in the pH 7.5 treatment after day 135 through the end of the experiment on day 356. The mortality rate at pH 7.8 was low and fairly constant and while the mortality rate at ambient pH decreased over time, the reduction was smaller than at pH 7.5 and was driven by a few mortality events at the beginning of the experiment. The difference in mortality rate between the ambient and the pH 7.8 treatments is driven by the timing of the mortality events (not the number of mortalities) and therefore may have no significance. It is possible the reduction in mortality rate at pH 7.5 is linked to temperatures, which was highest at the beginning of the experiment; however, this is unlikely because if this were the case a second increases in mortality should have occurred at the end of the experiments when temperatures were the same as at the beginning. The decrease in mortality could also reflect ontogenetic changes in the ability of the crabs to osmoregulate. More likely, the steep reduction in the mortality rate at pH 7.5 suggests a possible level of phenotypic plasticity and potential capacity in blue king crab to acclimate to acidified conditions, a level of phenotypic variability within the population with some individuals able to survive and grow under acidified conditions, or a combination of the two. However, in either case, more research is needed to elucidate the mechanism.

Acclimation or individual resistance to OA occurs in other species, can occur on multiple scales (Sunday *et al.*, 2014), and is likely because of altered or different physiology and energy allocation (Thor and Dupont, 2015). The green sea urchin, *Strongylocentrotus droebachiensis*, exhibited reduced fecundity after 4-month exposure to acidified water, but no reduction after 16-month exposure (Dupont *et al.*, 2013). Sometimes, a carryover effect such as exposure to acidified seawater during one life history stage alters the response of subsequent life history stages (e.g. transgenerational effects where exposure of the parents alters the response of their offspring). This can have positive consequences; parental exposure of both the green urchin and the oyster, *Saccostrea glomerata*, ameliorates effects of acidification on larval and juvenile stages (Parker *et al.*, 2012; Dupont *et al.*, 2013; Parker *et al.*, 2015). However, for some crab species the effect is negative. Red king crab larvae died more quickly under acidified conditions if exposed as embryos (Long *et al.*, 2013a), and a similar pattern occurs in *Hyas araneus* (Schiffer *et al.*, 2014). Negative transgenerational responses also occur in southern Tanner crab between the embryo and larval stages (Long *et al.*, 2016; Swiney *et al.*, 2016). Given the range of responses, it will be important to examine carryover and transgenerational effects in blue king crab as these could potentially either ameliorate or exacerbate the initial high mortality at the juvenile phase.

Sub-lethal effects of OA on blue king crab were also observed. Growth was significantly reduced by a slight 2% at pH 7.8. However, at pH 7.5, the differences in growth among treatments were more substantial and, unlike the mortality response, the growth data do not suggest that the effect of pH decreased with exposure time. This is likely because of increased energetic costs associated with the physiological processes involved in acclimating to a low pH environment (Spicer *et al.*, 2007; Whiteley, 2011; Salaberria *et al.*, 2012). Increased energy expenditure in acclimating to reduced pH could therefore reduce energy availability for other processes such as growth (Pedersen *et al.*, 2014), as seen in other decapod species (Keppel *et al.*, 2012; Long *et al.*, 2013a). This energy reallocation could also lead to other sub-lethal effects such as reduced fecundity (Kurihara *et al.*, 2008) or reduced immunological function (Wood *et al.*, 2014; Meseck *et al.*, 2016), which are areas for future investigations in blue king crab.

The mechanism(s) by which increased CO_2 affects crabs are still not clear because of the many ways in which CO_2 alters seawater chemistry. In this experiment, as expected, added CO_2 lowered the pH, increased the DIC and concentration of bicarbonate, and lowered the concentration of carbonate. The decreased carbonate concentration led to decreased saturation states for the various forms of calcium carbonate. Under hypercapnia, CO_2 concentrations increase within animals and crustaceans which are capable of responding buffer their hemolymph with bicarbonate to prevent (or reverse) a decrease in pH (Pane and Barry, 2007; Spicer *et al.*, 2007). This suggests that the CO_2 and the resulting pH changes are directly responsible for any effects on crabs either directly (if they cannot buffer their hemolymph) or indirectly through increased energy expenditure in ion transport (Whiteley, 2011). The saturation state of calcium carbonate, independent of pH, seems to be the primary cause of effects in some species like bivalves (Waldbusser *et al.*, 2015). However, as the calcified portion of the crab exoskeleton is protected by an epicuticle (Roer and Dillaman, 1984), unlike the shells of most bivalves that are directly exposed to seawater, the saturation state of seawater may not be as important to crustacean physiology. Indeed, the increased bicarbonate concentrations in the hemolymph may actually aid calcification; other crustaceans including red king crab can maintain or even increase calcification under acidified conditions (Ries *et al.*, 2009; Long *et al.*, 2013a,b).

Like red king crab and southern Tanner crab (Long *et al.*, 2013b), OA did not affect blue king crab morphology except that crabs exposed to pH 7.5 seawater were smaller than in other treatments at a given moult stage. In other species, such as the European lobster or the shrimp, *Palaemon pacificus*, exposure can cause deformities (Kurihara *et al.*, 2008; Agnalt *et al.*, 2013), but that is not the case here. Despite no visible effects, OA can reduce exoskeleton strength in blue king crabs (Coffey *et al.*, in review), which could affect feeding or defence; a decrease in chela strength could reduce a crab's ability to open hard shelled prey such as clams and a decrease in carapace strength could conversely make it easier for predators to penetrate the blue king crab's defences

In summary, exposure to pH 7.8 seawater did not affect mortality and had only minimal effects on growth, indicating that blue king crab will likely be unaffected to pH changes projected for this century OA, at least at the juvenile stage. In addition, although the initial mortality rate was high at pH 7.5, the rate decreased rapidly, suggesting that phenotypic plasticity could compensate for decreased pH over the long term or that there are individuals within the current population that are resistant to decreased pH. Either possibility suggests that there is wide scope for evolutionary adaptation. A study examining changes in physiology or protein expression during exposure to low pH waters could help confirm this and identify the mechanisms involved. In addition, the population-level response could be affected indirectly through the effects of OA on other species; since red king crab, a competitor/predator species (Daly and Long, 2014b; Long *et al.*, 2015), are more strongly affected than blue king crab, potentially giving blue king crabs an advantage. However, other life-history stages could respond differently and the response could be exacerbated (or further ameliorated) by carryover or transgenerational effect. Further research is needed to quantify these responses. Additionally, synergistic interactions with other stressors could potentially alter the response of blue king crab to OA (Breitburg *et al.*, 2015). In particular, increased temperature, which causes synergistic effects in red king crab (Swiney *et al.*, in review) and is of concern in the Bering Sea, warrants study.

Funding

Funding for this project was provided by the National Oceanic and Atmospheric Administration's Ocean Acidification Program. (http://www.oar.noaa.gov/oceans/ocean-acidification/index.html). We thank A. Emily, S. Dresdow, and the staff of the Kodiak Laboratory for help with conducting this experiment. Comments from S. Kotwicki, J. Napp, J. Long, and three anonymous reviewers improved earlier versions of this paper. Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA. The findings and conclusions in the article are those of the authors and do not necessarily represent the views of the National Marine Fisheries Service, NOAA.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

References

Agnalt, A. L., Grefsrud, E. S., Farestveit, E., Larsen, M., and Keulder, F. 2013. Deformities in larvae and juvenile European lobster (*Homarus gammarus*) exposed to lower pH at two different temperatures. Biogeosciences, 10: 7883–7895.

Armstrong, D. A., Armstrong, J. L., Palacios, R., Williams, G., Jensen, G. C., and Pearson, W. 1985. Early life history of juvenile blue king crab, *Paralithodes platypus*, around the Pribilof Islands. *In* Proceedings of the International King Crab Symposium, pp. 211–229. Ed. by S. K. Davis, F. Gaffney, J. McCrary, A. J. Paul, and R. S. Otto. Alaska Sea Grant College Program, University of Alaska Fairbanks, Anchorage, AK.

Arnold, K. E., Findlay, H. S., Spicer, J. I., Daniels, C. L., and Boothroyd, D. 2009. Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). Biogeosciences, 6: 1747–1754.

Baragi, L. V., and Anil, A. C. 2015. Interactive effect of elevated pCO_2 and temperature on the larval development of an inter-tidal organism, *Balanus amphitrite* Darwin (Cirripedia: Thoracica). Journal of Experimental Marine Biology and Ecology, 471: 48–57.

Breitburg, D. L., Salisbury, J., Bernhard, J. M., Cai, W. J., Dupont, S., Doney, S. C., Kroeker, K. J., et al. 2015. And on top of all that... Coping with ocean acidification in the midst of many stressors. Oceanography, 28: 48–61.

Burnham, K. P., and Anderson, D. R. 2002. Model selection and multimodel inference: A practical information-theoretic approach, Springer Science + Business Media, New York.

Caldeira, K., and Wickett, M. E. 2003. Anthropogenic carbon and ocean pH. Nature, 425: 365-365.

Carter, H. A., Ceballos-Osuna, L., Miller, N. A., and Stillman, J. H. 2013. Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes*. The Journal of Experimental Biology, 216: 1412–1422.

Coffey, W. D., Yarram, A., Matoke, B., Long, W. C., Swiney, K. M., Foy, R., and Dickenson, G. in review. Ocean acidification leads to altered micromechanical properties of the mineralized cuticle of juvenile red and blue king crabs. The Journal of Experimental Biology.

Daly, B., and Long, W. C. 2014a. Inter-cohort cannibalism of early benthic phase blue king crabs (*Paralithodes platypus*): Alternate foraging strategies in different habitats lead to different functional responses. PloS One, 9: e88694.

Daly, B., and Long, W. C. 2014b. Intra-guild predation among early benthic phase red and blue king crabs: Evidence for a habitat-mediated competitive advantage. Journal of Experimental Marine Biology and Ecology, 451: 98–104.

Dickson, A. G., Sabine, C. L., and Christian, J. R. 2007. Guide to best practices for ocean CO2 measurements. PICES special publication 3. PICES special publication 3. 191 pp.

Dodd, L. F., Grabowski, J. H., Piehler, M. F., Westfield, I., and Ries, J. B. 2015. Ocean acidification impairs crab foraging behaviour. Proceedings of the Royal Society B-Biological Sciences, 282: 9.

Dupont, S., Dorey, N., Stumpp, M., Melzner, F., and Thorndyke, M. 2013. Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. Marine Biology, 160: 1835–1843.

Fabry, V. J., McClintock, J. B., Mathis, J. T., and Grebmeier, J. M. 2009. Ocean Acidification at High Latitudes: The Bellweather. Oceanography, 22: 160–171.

Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal of Marine Science 414–432.

Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science, 305: 362–366.

IPCC 2001. Climate Change 2001: The scientific basis. Intergovernmental Panel on Climate Change. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Ed. by Houghton J., Ding Y., Griggs D., Noguer M., Van der Linden P., Dai X., Maskell K., and Johnson C. Cambridge University Press, Cambridge and New York.

Jensen, G. C., and Armstrong, D. A. 1989. Biennial reproductive cycle of blue king crab, *Paralithodes platypus*, at the Pribilof Islands, Alaska and comparison to a congener, *P. camtschatica*. Canadian Journal of Fisheries and Aquatic Sciences, 46: 932–940.

Keppel, E. A., Scrosati, R. A., and Courtenay, S. C. 2012. Ocean acidification decreases growth and development in American lobster (*Homarus americanus*) larvae. Journal of Northwest Atlantic Fishery Science, 44: 61–66.

Knapp, J. L., Bridges, C. R., Krohn, J., Hoffman, L. C., and Auerswald, L. 2015. Acid-base balance and changes in haemolymph properties of the South African rock lobsters, *Jasus lalandii*, a palinurid decapod, during chronic hypercapnia. Biochemical and Biophysical Research Communications, 461: 475–480.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., et al. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology, 19: 1884–1896.

Kurihara, H., Matsui, M., Furukawa, H., Hayashi, M., and Ishimatsu, A. 2008. Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. Journal of Experimental Marine Biology and Ecology, 367: 41–46.

Lavigne, H., and Gattuse, J. 2012. seacarb: seawater carbonate chemistry with R. http://CRAN.R-project.org/package=seacarb. R package version 2.4.6 edn.

Long, W. C. 2016. A new quantitative model of multiple transitions between discrete stages, applied to the development of crustacean larvae. Fishery Bulletin, 114: 58–66.

Long, W. C., Swiney, K. M., and Foy, R. J. 2013a. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus*. Marine Pollution Bulletin, 69: 38–47.

Long, W. C., Swiney, K. M., and Foy, R. J. 2016. Effects of high pCO_2 on Tanner crab reproduction and early life history, Part II: carryover effects on larvae from oogenesis and embryogenesis are stronger than direct effects. ICES Journal of Marine Science, 73: 836–848.

Long, W. C., Swiney, K. M., Harris, C., Page, H. N., and Foy, R. J. 2013b. Effects of ocean acidification on juvenile red king crab (*Paralithodes camtschaticus*) and Tanner crab (*Chionoecetes bairdi*) growth, condition, calcification, and survival. PloS One, 8: e60959.

Long, W. C., Van Sant, S. B., and Haaga, J. A. 2015. Habitat, predation, growth, and coexistence: Could interactions between juvenile red and blue king crabs limit blue king crab productivity? Journal of Experimental Marine Biology and Ecology, 464: 58–67.

Lyons, C., Eckert, G., and Stoner, A. W. 2016. Influence of temperature and congener presence on blue (*Paralithodes platypus* Brandt, 1850) and red (*Paralithodes camtschaticus* Tilesius, 1815) king crabs habitat preference and fish predation. Journal of Crustacean Biology, 36: 12–22.

Mathis, J. T., Cooley, S. R., Lucey, N., Colt, S., Ekstrom, J., Hurst, T., Hauri, C., et al. 2015. Ocean acidification risk assessment for Alaska's fishery sector. Progress in Oceanography, 136: 71–91.

Mathis, J. T., Cross, J. N., Monacci, N., Feely, R. A., and Stabeno, P. 2014. Evidence of prolonged aragonite undersaturations in the bottom waters of the southern Bering Sea shelf from autonomous sensors. Deep-Sea Research Part II-Topical Studies in Oceanography, 109: 125–133.

Meseck, S. L., Alix, J. H., Swiney, K. M., Long, W. C., Wikfors, G. H., and Foy, R. J. 2016. Ocean acidification affects hemocyte physiology in the Tanner crab (*Chionoecetes bairdi*). PloS One, 11: e0148477.

NPFMC. 2015. 2015 Stock assessment and fishery evaluation report for the king and Tanner crab fisheries of the Bering Sea and Aleutian Islands regions. 950 pp.

Pane, E. F., and Barry, J. P. 2007. Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. Marine Ecology Progress Series, 334: 1–9.

Pansch, C., Nasrolahi, A., Appelhans, Y. S., and Wahl, M. 2013. Tolerance of juvenile barnacles (*Amphibalanus improvisus*) to warming and elevated pCO_2. Marine Biology, 160: 2023–2035.

Parker, L. M., O'Connor, W. A., Raftos, D. A., Pörtner, H. O., and Ross, P. M. 2015. Persistence of positive carryover effects in the oyster, *Saccostrea glomerata*, following transgenerational exposure to ocean acidification. PloS One, 10: e0132276.

Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A., and Pörtner, H. O. 2012. Adult exposure influences offspring response to ocean acidification in oysters. Global Change Biology, 18: 82–92.

Pedersen, S. A., Hakedal, O. J., Salaberria, I., Tagliati, A., Gustavson, L. M., Jenssen, B. M., Olsen, A. J., et al. 2014. Multigenerational exposure to ocean acidification during food limitation reveals consequences for copepod scope for growth and vital rates. Environmental Science & Technology, 48: 12275–12284.

Punt, A. E., Foy, R. J., Dalton, M. G., Long, W. C., and Swiney, K. M. 2016. Effects of long term exposure to ocean acidification on future southern Tanner crab (*Chionoecetes bairdi*) fisheries management. ICES Journal of Marine Science, 73: 849–864.

Punt, A. E., Poljak, D., Dalton, M. G., and Foy, R. J. 2014. Evaluating the impact of ocean acidification on fishery yields and profits: The example of red king crab in Bristol Bay. Ecological Modelling, 285: 39–53.

R Core Team and contributors worldwide 2013. stats4-package. R package version 3.1.0 edn.

Ries, J. B., Cohen, A. L., and McCorkle, D. C. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology, 37: 1131–1134.

Roer, R., and Dillaman, R. 1984. The structure and calcification of the crustacean cuticle. American Zoologist, 24: 893–909.

Salaberria, I., Tagliati, A., Gustavson, L. M., Hakedal, O. J., Hansen, B. H., Altin, D., Olsen, A. J., et al. 2012. Developmental and reproductive adaptation to CO_2-induced ocean acidification scenarios: A multi-generational study using the marine copepod *Calanus finmarchicus*. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology, 163: S6-S6.

Schiffer, M., Harms, L., Portner, H., Lucassen, M., Mark, F. C., and Storch, D. 2013. Tolerance of *Hyas araneus* zoea I larvae to elevated seawater PCO_2 despite elevated metabolic costs. Marine Biology, 160: 1943–1953.

Schiffer, M., Harms, L., Pörtner, H., Mark, F., and Storch, D. 2014. Pre-hatching seawater pCO_2 affects development and survival of zoea stages of Arctic spider crab *Hyas araneus*. Marine Ecology Progress Series, 501: 127–139.

Small, D. P., Calosi, P., Boothroyd, D., Widdicombe, S., and Spicer, J. I. 2015. Stage-specific changes in physiological and life-history responses to elevated temperature and Pco_2 during the larval development of the European lobster *Homarus gammarus* (L.). Physiological and Biochemical Zoology, 88: 494–507.

Somerton, D. A. 1985. The disjunct distribution of blue king crab, Paralithodes platypus, in Alaska: some hypotheses. *In* Proceedings of the international king crab symposium, pp. 13-21. Ed. by B. Melteff. University of Alaska Sea Grant Program, Anchorage, AK, USA.

Spicer, J. I., Raffo, A., and Widdicombe, S. 2007. Influence of CO_2-related seawater acidification on extracellular acid-base balance in the velvet swimming crab *Necora puber*. Marine Biology, 151: 1117–1125.

Stevens, B. G., Persselin, S., and Matweyou, J. 2008. Survival of blue king crab *Paralithodes platypus* Brandt, 1850, larvae in cultivation: effects of diet, temperature and rearing density. Aquaculture Research 390–397.

Stoner, A. W., Copeman, L. A., and Ottmar, M. L. 2013. Molting, growth, and energetics of newly-settled blue king crab: Effects of temperature and comparisons with red king crab. Journal of Experimental Marine Biology and Ecology, 442: 10–21.

Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H., and Reusch, T. B. H. 2014. Evolution in an acidifying ocean. Trends in Ecology & Evolution, 29: 117–125.

Swiney, K. M., Long, W. C., and Foy, R. in review. How do ocean acidification and increased temperatures affect young-of-the-year red king crab (*Paralithodes camtschaticus*) survival, growth and morphology? ICES Journal of Marine Science

Swiney, K. M., Long, W. C., and Foy, R. J. 2016. Effects of high pCO_2 on Tanner crab reproduction and early life history, Part I: long-term exposure reduces hatching success and female calcification, and alters embryonic development. ICES Journal of Marine Science, 73: 825–835.

Swiney, K. M., Long, W. C., and Persselin, S. L. 2013. The effects of holding space on juvenile red king crab (*Paralithodes camtschaticus)* growth and survival. Aquaculture Research, 44: 1007–1016.

Tapella, F., Romero, M. C., Stevens, B. G., and Buck, C. L. 2009. Substrate preferences and redistribution of blue king crab *Paralithodes platypus* glaucothoe and first crab on natural substrates in the laboratory. Journal of Experimental Marine Biology and Ecology, 372: 31–35.

Thor, P., and Dupont, S. 2015. Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. Global Change Biology, 21: 2261–2271.

Waldbusser, G. G., Hales, B., Langdon, C. J., Haley, B. A., Schrader, P., Brunner, E. L., Gray, M. W., et al. 2015. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. Nature Climate Change, 5: 273–280.

Walther, K., Sartoris, F. J., and Portner, H. 2011. Impacts of temperature and acidification on larval calcium incorporation of the spider crab *Hyas araneus* from different latitudes (54 degrees vs. 79 degrees N). Marine Biology, 158: 2043–2053.

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. Marine Ecology Progress Series, 430: 257–271.

Wood, H. L., Skold, H. N., and Eriksson, S. P. 2014. Health and population-dependent effects of ocean acidification on the marine isopod *Idotea balthica*. Marine Biology, 161: 2423–2431.

Handling editor: David M. Fields