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Original Article

Effects of ocean acidification on the respiration and feeding of juvenile red and blue king crabs (*Paralithodes camtschaticus* and *P. platypus*)

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Ocean acidification is a decrease in pH resulting from dissolution of anthropogenic CO_2 in the oceans that has physiological effects on many marine organisms. Juvenile red and blue king crabs (*Paralithodes camtschaticus* and *P. platypus*) exhibit both increased mortality and decreased growth in acidified waters. In this study, we determined how ocean acidification affects oxygen consumption, feeding rates, and growth in both species. Juvenile crab were exposed to three pH levels: ambient (pH 8.1), pH 7.8, and pH 7.5 for 3 weeks. Oxygen consumption and feeding ration were determined immediately after exposure to treatment water and after 3 weeks' exposure. Growth was calculated as a change in wet mass. Both species exhibited initially increased oxygen consumption at pH 7.5, but not after 3 weeks. Feeding rations did not vary with pH or exposure time. Red king crabs that moulted grew more in ambient water than in pH 7.5. The initial increase in oxygen consumption at pH 7.5 suggests the crab increased metabolism and expended more energy in osmo-/iono-regulation. Without an increase in feeding ration, it is likely the crab reduced energy expenditure in other areas, explaining the reduced growth and increased mortality observed in this and other studies.

Keywords: blue king crab, crabs, hypercapnia, ocean acidification, red king crab, respiration.

Introduction

The dissolution of anthropogenic CO_2 into the world's oceans is causing a phenomenon called ocean acidification (Feely *et al.*, 2004). The average global pH of surface waters has decreased 0.1 pH units over the past century, and the rate of acidification is projected to accelerate with increasing CO_2 emissions (Caldeira and Wickett, 2003; Fabry *et al.*, 2008). High latitude waters, in which CO_2 is more soluble due to colder temperatures, are likely to acidify faster than the global average (Fabry *et al.*, 2009). As a result, surface waters of the Bering Sea are projected to be undersaturated with respect to aragonite within 60 years (Mathis *et al.*, 2015). Bottom waters in the Bering Sea shelf have already seasonally high pCO_2 levels (1600 µatm) due to increases in respiration (Mathis *et al.*, 2014). These projected changes in water chemistry can have physiological effects on marine organisms, although the effects are highly variable, both within and among taxa (Kroeker *et al.*, 2010).

Crustaceans comprise a diverse group of calcifying marine organisms that can be vulnerable to ocean acidification. The crab exoskeleton comprised calcium carbonate (primarily calcite and amorphous calcium carbonate) embedded in a chitin matrix (Raabe *et al.*, 2006; Boßelmann *et al.*, 2007; Chen *et al.*, 2008).

Although their internal calcification process and organic-layer shielded calcified structures may protect them against direct dissolution (Whiteley, 2011), ocean acidification can still cause physiological stress, affecting macro-scale responses including growth and survival (Keppel *et al.*, 2012; Baragi and Anil, 2015). Most decapod crustaceans buffer their haemolymph in response to ocean acidification (Meseck *et al.*, 2016) primarily via active bicarbonate transport at the gills (Appelhans *et al.*, 2012). This can prevent or reverse acidosis, but it is not always fully effective (Zittier *et al.*, 2013), and some crustaceans may not use this mechanism at all (Pane and Barry, 2007). In addition, this mechanism is energetically expensive, and may divert energy away from other vital biological processes, including growth and reproduction (Whiteley, 2011; Swiney *et al.*, 2016). As many crustaceans are important commercial species, there are concerns that ocean acidification could result in reduced populations and fishery yields (Punt *et al.*, 2014, 2016).

Red and blue king crabs, *Paralithodes camtschaticus* and *P. platypus*, are two important fisheries species in Alaska waters which have a similar life-history and overlapping range, though blue king crab may be more cold-tolerant than red king crab (Somerton, 1985). Both species release larvae in the late spring and early summer after brooding the embryos for about a year (Jensen and Armstrong, 1989; Stevens and Swiney, 2007). The larvae are planktonic in the water column so that their pH exposure can vary diurnally (due to diel vertical migration) and seasonally (Wainwright *et al.*, 1991; Cross *et al.*, 2013). The larvae pass through four zoeal stages before moulting to the glaucothoe or settling stage (Shirley and Shirley, 1989; Stevens *et al.*, 2008). The glaucothoe settle in complex habitats (Stevens, 2003; Tapella *et al.*, 2009), usually in nearshore areas (McMurray *et al.*, 1984; Armstrong *et al.*, 1987), where ocean pH is more stable than the upper water column or deeper benthic areas but may be affected by seasonal upwelling, where they use cryptic behaviour in the available complex habitat (Daly and Long, 2014b) to avoid predation (Daly *et al.*, 2012) and cannibalism (Long and Whitefleet-Smith, 2013; Daly and Long, 2014a). For both species, distribution generally shifts to deeper, soft-bottomed habitats, where pH can be seasonally undersaturated with respect to calcite, as crab grow large enough to deter most predators (Armstrong *et al.*, 1987; Dew, 1990).

Both species are sensitive to ocean acidification. When exposed to acidified waters, red king crab embryos show altered development (Long, Swiney, and Foy, 2013). Exposure at both the embryonic stage (carryover effects) and larval stage reduces the time to larval starvation in the laboratory (Long, Swiney, and Foy, 2013). Juveniles suffer increased mortality and decreased growth in acidified water (Long, Swiney, Harris, *et al.*, 2013), and there is a synergistic negative effect on survival when acidification is combined with increased temperatures (Swiney *et al.*, 2017). Blue king crab are less well-studied but, similarly to red king crab, the juveniles have decreased survival and growth in acidified water (Long *et al.*, 2017). Red king crab appear to be more sensitive; juvenile red king crab are affected at a pH of 7.8, whereas blue king crab are not affected at pHs above 7.8. Although the calcium content is not affected, the juveniles of both species have reduced microhardness in the chela (though not in the carapace) when held at a pH of 7.5 (Coffey *et al.*, 2017). Given these results, it is projected that the level of ocean acidification projected for the next 50–100 years will have a substantial negative effect on red king crab populations and fisheries in the absence of any acclimation (Punt *et al.*, 2014).

These studies set the stage for our beginning to understand the energetic and physiological responses of these species to ocean acidification. Understanding how metabolic demand changes during exposure to ocean acidification is critical to interpreting the whole-organism responses found in these initial studies. In addition, many species are able to balance the increased energetic demands of ocean acidification on organismal physiology, provided sufficient food is available to meet the demand (Hettinger, Sanford, Hill, Hosfelt, *et al.*, 2013; Pansch *et al.*, 2014). Consequently, understanding how feeding changes in light of metabolic demand is also important. In this study, we determine how respiration, feeding ration, and growth in juvenile red and blue king crabs are affected by ocean acidification.

Methods

Juvenile king crab for this experiment were reared by the Alutiiq Pride Shellfish Hatchery using standard published protocols (Swingle *et al.*, 2013; Long, 2016) from red king crab broodstock obtained from Alitak Bay in 2013 and blue king crab broodstock obtained from the St. Matthew stock in 2012. All broodstock were caught in crab pots. Year-0 red king crab, ~3 weeks from settlement, and year-1 blue king crab, ~1 year from settlement, were used for this study. Juvenile crab were shipped to the Kodiak Laboratory in insulated containers, and were held in tanks with flowing, unfiltered seawater at ambient pH and temperature. Juvenile crab were fed a diet of frozen *Artemia* (Brine Shrimp Direct, Ogden, UT, USA), frozen bloodworms (Brine Shrimp Direct), frozen Cyclop-eeze (Argent Laboratories, Redmond, WA, USA), Cyclop-eeze flakes, and Gelly Belly (Florida Aqua Farms, Dade City, FL, USA) mixed with Cyclop-eeze powder, and walleye pollock (*Gadus chalcogrammus*) bone powder (US Department of Agriculture, Agricultural Research Service, Kodiak, AK, USA) three times per week to excess prior to the experiment. The average mass of red king crab at the beginning of the experiment was 0.0111 ± 0.0032 (*SD*) g and crabs were at the first to second crab stage. Blue king crab were 0.0486 ± 0.0156 (*SD*) g and were probably at the fifth to sixth crab stage.

During these experiments, crab were held in individual cells made out of PVC pipe (52 mm diameter, 5 cm tall) with mesh bottoms, which were placed in three larger experimental tubs [53 (L) × 38 (W) × 23 (H) cm], water volume 24 l. Although there was no replication at the tub level (meaning that treatment differences cannot be fully disentangled from tub) given the crabs were isolated from each other, the flow rate and temperatures were checked daily, the high exchange rate of water (1 tank exchange every 24 min), and the very low biomass (~370 mg crab per tank or ~15 mg/l) the effect of tub can be reasonably assumed to be negligible. Cells received flow-through water at 100 ml/min and were large enough to avoid causing stress to the animals (Swiney *et al.*, 2013). Crab were fed Gelly Belly (above) three times a week to excess during the experiment. Water temperature was maintained at 5°C, using a recirculating chiller, which is well within the natural range experienced by both species in the wild (incoming ambient seawater in our laboratory ranges from ~0 to 12°C annually and Kodiak is within the geographic range of both species) and within the thermal tolerance range for both species (Long and Daly, 2017). Each of the tubs was fed with flow-through seawater at 1 l/min at one of three experimental pH levels. Water flow, into both the tubs and the individual inserts, was checked daily and adjusted if necessary. Seawater was acidified as

described by Long, Swiney, and Foy (2013). In brief, CO_2 was bubbled into seawater to reduce the pH to 5.5. This low pH water was mixed with ambient filtered seawater into treatment head tanks. The flow rate of pH 5.5 water was controlled via feedback from pH probes in the head tanks that adjusted the speed of peristaltic pumps. Three pH treatments were used: ambient (pH ~8.1), pH 7.8 (pH expected in global surface waters in ~2100), and pH 7.5 (~2200) (Caldeira and Wickett, 2003). These pH treatments were, in part, use to allow direct comparisons between this experiment and previous experiments performed on the same species and life-history stages (Long, Swiney, Harris, *et al.*, 2013; Long *et al.*, 2017). The pH and temperature were measured in a randomly selected cell in each treatment once a day using a Durafet III pH probe which was calibrated daily using TRIS buffer made according to Millero (1986). Previous experiments using the same experimental setup have demonstrated that the variability in the pH and temperature measurements among cells in the same tank is less than the nominal precision of the probe, and typically 0 (Long *et al.*, 2017). Weekly water samples were taken from each treatment, poisoned with 0.02% of total sample volume of saturated mercuric chloride solution according to the best practice (Dickson *et al.*, 2007), and sent to an analytic laboratory for dissolved inorganic carbon (DIC) and total alkalinity (TA) analysis. DIC and TA were determined using a VINDTA 3 C (Marianda, Kiel, Germany) coupled to a 5012 Coulometer (UIC, Inc., Joliet, IL, USA), using Certified Reference Material from the Dickson Laboratory (Scripps Institution, San Diego, CA, USA) and the procedures in DOE (1994). Other components of the carbonate system were calculated from the measured pH and DIC using seacarb package in R (V2.2.3, Vienna, Austria, Lavigne and Gattuse, 2011). As an internal check of our measurements we also calculated the pH of the seawater from the measured DIC and Alkalinity using seacarb and compared it to the measured pH. The average difference between the calculated and measured pH was -0.01 ± 0.018 (s.e.) pH units indicating a good level of agreement.

In this experiment, we measured respiration and feeding ration twice for each crab once immediately after exposure to treatment water and once after a 3-week acclimation period in treatment water. A 3-week exposure period was selected, in part based on previous experiments where juvenile red king crab mortality rate in acidified conditions increased substantially after the 3-week mark (Long, Swiney, Harris, *et al.*, 2013). Thus, 3 weeks represent the maximum exposure time possible to ensure a sufficient sample size at the end of the experiment in the lowest pH treatment while getting measurements over the longest time frame possible. In addition, keeping mortality to a minimum avoided complications in interpretation inherent if differential mortality among the treatments weeded out the individuals most sensitive to low pH. Sample size was 6 crab per species per treatment, except for red king crab at pH 7.5, which we increased to 10 crab in anticipation of a higher mortality rate at that treatment. Total stocking density was 12–16 crabs/tub. As no more than five respirometry measurements could be made per day, trials for individual crab were staggered, and crab were started in a random order, with each crab assigned to its treatment randomly. Partway through the initial sets of measurements for the experiment, an equipment failure caused mass mortality in the pH 7.5 treatment. All crabs (14 out of 16 total for that treatment) present in the tub at the time were removed regardless of whether they had died, and the initial respiration/feeding trials were re-run using new crabs according to the same protocol. Each crab was starved for 1 day prior to measuring the respiration and feeding ration to standardize hunger levels.

Respiration was measured in a static, adjustable volume (max 5 ml) Plexiglas cell with an integrated Clark electrode oxygen sensor which continuously recorded the concentration of dissolved O_2 in mg/l to the nearest 0.01 mg/l. This sensor was calibrated daily using a two-point calibration procedure at 0 and 100% saturation. The cell was jacketed in a secondary chamber that allowed flow-through water to maintain the cell at a constant temperature (but this water flowed around the cell and did not flow into the cell), and the whole apparatus was placed inside a temperature-controlled room at 5°C. To measure respiration rates, crabs were taken out of the ambient pH water in their holding tank and placed into the cell with a known volume of water (2 ml for red king crab and 3 ml for blue king crab) at the appropriate treatment pH. A vented plunger was depressed into the cell to purge all air in the cell. The water within the cell was continuously mixed with a magnetic stir bar and was not exchanged during the trial. A small piece of fibreglass mesh inserted into the cell was used to keep the crab away from the stir bar. Trials were run for ~1.0 to 1.5 h. Immediately after the trial, the crab were removed from the chamber and blotted dry, and the individual wet mass was determined. The rate of oxygen consumption in the cell was calculated by determining the slope of the change in oxygen concentration over time once the trend became linear, usually within ~15 min, and was normalized to the wet mass of each crab.

After the initial respiration trials, the crab were placed in their holding cells within the experimental holding system (see above). Feeding ration was determined the same day as respiration measurements were taken. A pre-massed piece of squid mantle (blotted dry) ~50% of the mass of the given individual crab was placed into each cell. Crab were allowed to feed for 24 h, after which the remaining food was collected, blotted dry, and massed. As the red king crab were smaller than the blue king crab, the mass of food given to each species differed accordingly. Control trials without crab were performed in each pH treatment for each species (to account for any potential difference in the initial mass of the food samples), with three replicates of each pH/species combination. On average, the mass of squid increased by $0.8\% \pm 7.8$ (s.e.) and did not differ among pH treatments (two-way ANOVA, $F_{2,13} = 0.184$, $p = 0.834$) or species (two-way ANOVA, $F_{1,13} = 1.318$, $p = 0.272$), so the overall mean was used when calculating the feeding ration. The mass of food consumed was determined, and the feeding ration was calculated as the percent of the crab's mass consumed corrected for mass change in control trials. The crab were held in their treatment water for ~21 days (range: 20–24 days), and checked daily for moults or mortalities. After the ~3 weeks, the respiration and feeding ration for each crab was then determined a second time following the above procedures.

Respiration and feeding ration were analysed via repeated-measures ANOVAs, with time (initial and final measurements) fully crossed with pH treatment, species, and crab number (nested within pH treatment and species) as factors and with crab mass as a covariate. In all analyses, treatment could not be unambiguously disentangled from any potential tub effects because of a lack of replication at the tub level. Homogeneity of variance was verified with Levene's test and *post hoc* comparisons were tested using Fishers least significant difference tests in all ANOVA analyses. Growth was calculated as the percent change in wet weight

Table 1. Water chemistry parameters in the three treatment tanks during experimental exposure of red and blue king crabs to varying levels of ocean acidification.

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	8.14 ± 0.03	331.83 ± 28.95	1.86 ± 0.01	0.11 ± 0.01	1.98 ± 0.01	2.12 ± 0.01	1.63 ± 0.11	2.60 ± 0.18
pH 7.8	7.80 ± 0.03	767.92 ± 53.53	1.98 ± 0.01	0.05 ± 0.00	2.07 ± 0.01	2.10 ± 0.02	0.80 ± 0.06	1.27 ± 0.10
pH 7.5	7.50 ± 0.03	1555.49 ± 44.22	2.04 ± 0.00	0.03 ± 0.00	2.15 ± 0.00	2.11 ± 0.01	0.41 ± 0.01	0.66 ± 0.02

The pH (free scale) was measured daily, the DIC and alkalinity were measured weekly ($N = 5$), and the other parameters were calculated. Values are means ± 1 *SD*. $\Omega_{Aragonite}$ and $\Omega_{Calcite}$ are the saturation states of aragonite and calcite, respectively.

between the initial and final measurements. As few blue king crab moulted (only 2 in the pH 7.5 treatments), there was insufficient data for analysis. Growth in red king crab, where three crabs moulted in each treatment, was analysed with a fully crossed two-way ANOVA, with pH treatment and moult status (whether they moulted or not) as factors.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

The pH in the ambient tank averaged 8.14 ± 0.03 (*SD*) while the pHs in the two treatment tanks averaged 7.80 ± 0.03 and 7.50 ± 0.03 (Table 1). As expected, TA did not vary among the treatments. The water in the pH 7.8 treatment was undersaturated with respect to aragonite, and the water in the pH 7.5 treatment was undersaturated with respect to both calcite and aragonite (Table 1).

There were no mortalities noted during the ∼3 weeks exposure for either species although two red king crabs, one in the ambient and one in the pH 7.8 treatment, died after the second set of respiration and feeding ration measurements. In respiration trials, initial crab respiration was typically high during the first 5–15 min of the trial, likely due to handling and air exposure (Figure 1). Linear fits used to determine respiration rates were all excellent fits with R^2 averaging 0.981 and ranging from 0.878 to 0.999. Respiration rates did not differ between blue and red king crabs under all conditions (Table 2, Figure 2). There was a significant interactive effect between pH treatment and exposure time for both species; the respiration rate at the initial exposure in pH 7.5 water was higher than at either pH 7.8 or ambient water, but after 3 weeks there was no difference among the treatments (Table 2, Figure 2). In the initial measurements, respiration in pH 7.5 water was 73% higher than in ambient water in red king crab, and 178% higher in blue king crab. Feeding ration did not differ between species, among pH treatments, or with exposure time (Table 2, Figure 3).

Although growth was not of primary interest in this experiment, sufficient red king crab moulted that we present the data for comparisons to other studies. There was a significant interactive effect between pH treatment and moulting on growth of red king crab (Table 3). Under all pH treatments, red king crab that did not moult increased in mass by ∼10% over the 3-week experiment (Figure 4). However, there was a large difference in growth among treatments for red king crab that did moult, with crab in ambient water growing more than those in pH 7.5 (Figure 4). Indeed, at pH 7.5, red king crab that did moult had a smaller

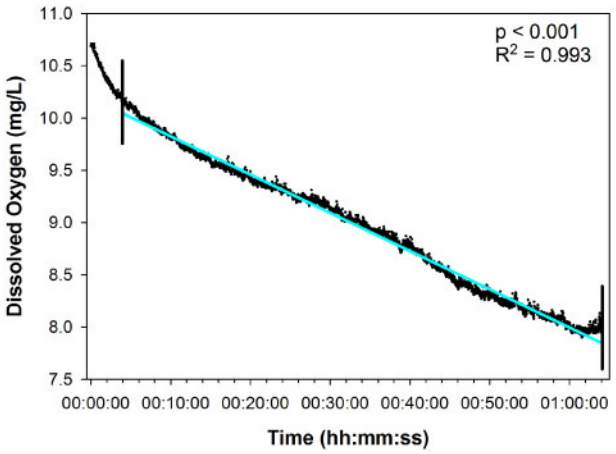

Figure 1. Example of data collected during respirometry trial on a blue king crab. Dots are individual measurements of dissolved oxygen within the cell, vertical lines indicate the range of data chosen to determine respiration rate. The line is the best fit linear regression for the data and the p and R^2 are given. Note data indicating higher rate of respiration during the initial part of the trial, likely caused by handling and air exposure, which was not used to determine the respiration rate.

(albeit non-significant) increase in mass than those that did not moult (Figure 3) and the average increase in mass was statistically indistinguishable from 0 (one sample t-test, $p = 0.281$). Ambient crab also grew 83% more than those in pH 7.8 (Figure 4), but the difference, though large, was not quite significant ($p = 0.064$). Blue king crab that did not moult increased in mass by 1.7 ± 1.1% and those that did by 21.8 ± 10.5% (s.e.).

Discussion

This study helps to shine light on the energetic and physiological underpinnings of the macro-responses of red and blue king crabs to ocean acidification. The initial higher respiration rate in both species at the lowest pH as compared to ambient pH suggests an energetic cost from the crabs buffering their haemolymph in response to an immediate change in water pH; although given the crab's sizes we were not able to directly measure this buffering response. This higher metabolism at the onset of the exposure did not lead to increased food intake, however. After 3 weeks in treatment water, respiration rates were the same as those in ambient pH water and feeding rates were unchanged; however, decreased growth [which occurred here for red king crab, but has been demonstrated more robustly for both species in longer-term studies (Long, Swiney, Harris, *et al.*, 2013; Long *et al.*, 2017)] suggests that the crab continued to expend a greater amount of energy on

osmo-/iono-regulation in acidified water, compared to crabs held in ambient water without an increase in feeding, thus reducing the energy available for growth. As the two species used in this study were at different ages and stages it must be acknowledged that any direct comparisons between the two in this study could have been affected by this; however, this does not change the interpretation of the results in regards to the physiological effects of ocean acidification. Furthermore, for all measurements other than the initial respiration rate, the effect of tub could not be estimated because of a lack of replication at the tub level; however, given the flow rates and the isolation of the crabs within the tubs (see Methods section) the effects of tub are assumed to be negligible. More experiments will be necessary to completely understand the physiological response of these crabs to reduced pH water, and to estimate the degree to which acclimation and adaptation may ameliorate the negative effects of ocean acidification at a population level.

Table 2. RM-ANOVA results examining the effects of pH and exposure time on respiration and feeding ration in red and blue king crabs.

	df	Mean squares	*F*-ratio	*p*-Value
Respiration				
Time	1	0.002	0.073	0.788
pH	2	0.044	1.790	0.184
Species	1	0.010	0.422	0.521
Time*pH	2	0.142	5.732	**0.008**
Time*species	1	0.000	0.019	0.892
pH*species	2	0.026	1.032	0.368
Time*species*pH	2	0.016	0.637	0.536
Mass	1	0.036	1.469	0.235
Crab (pH*species)	34	0.021	0.830	0.702
Error	31	0.025	–	–
Feeding ration				
Time	1	0.845	0.005	0.941
pH	2	24.318	0.158	0.855
Species	1	119.780	0.778	0.385
Time*pH	2	175.866	1.142	0.332
Time*species	1	148.843	0.966	0.333
pH*species	2	274.258	1.781	0.185
Time*species*pH	2	37.859	0.246	0.784
Mass	1	74.651	0.485	0.492
Crab (pH*species)	34	370.709	2.407	**0.008**
Error	31	154.023	–	–

Time is either initial or final measurements, pH the pH treatment, species the crab species, mass the mass of the crab, and crab the individual crab on which multiple measurements were done. Significant *p*-values (<0.05) are highlighted in bold.

To maintain homeostasis, crustaceans exposed to decreased pH/increased pCO_2 must respond by either buffering their haemolymph or reducing CO_2 production. Failure to do so results in decreased protein function and the accompanying disruption of biological processes. One mechanism crustaceans use is to reduce metabolism and thus internal production of CO_2, as is the case for both the Dungeness crab, *Metacarcinus magister*, and the krill, *Euphausia pacifica* (Hans *et al.*, 2014; Cooper *et al.*, 2016). An alternative, non-exclusive mechanism is active transport of bicarbonate into the haemolymph at the gills, primarily through Cl^-/HCO_3^- exchange, which buffers the haemolymph and reduces, reverses, or prevents acidosis (Whiteley, 2011). This mechanism is very common in marine and estuarine crustaceans (Pane and Barry, 2007; Dissanayake *et al.*, 2010; Knapp *et al.*, 2015). Given the near-ubiquity of this response and the fact that active ion transport is energetically costly, it seems likely that the initial higher respiration rate that occurred in both red and blue king crabs in low pH water compared to those in ambient water was at least partially due to the energetic cost of buffering the haemolymph with bicarbonate. This conclusion is further substantiated by the fact that all red king crab life history stages examined, including larval, juvenile, and adult, have either increased or unchanged calcium content in acidified water (Long, Swiney, and Foy, 2013; Long, Swiney, Harris, *et al.*, 2013). Increasing the bicarbonate ion concentration in the haemolymph may make the internal calcification process employed by crustaceans more effective, thus explaining the increase in calcification that is a frequent result of exposure to CO_2 acidified water in this taxa (Ries *et al.*,

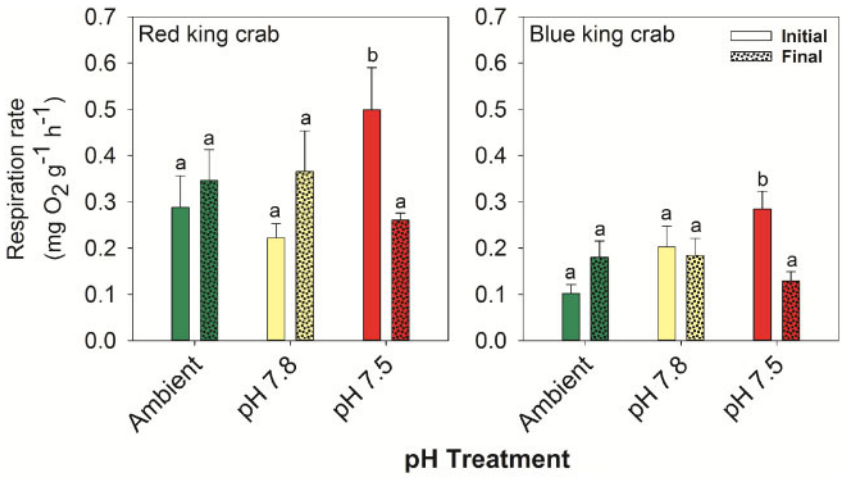

Figure 2. Effect of pH and exposure time (Initial: immediately after exposure, solid bars; Final: after 3 weeks' exposure, stippled bars) on the respiration rates in juvenile red and blue king crabs. Error bars are one standard error. Bars with different letters above them differ within each species.

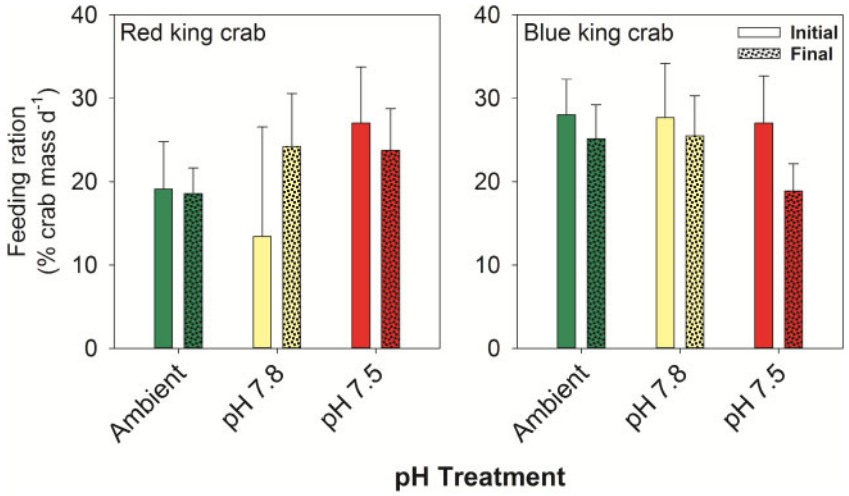

Figure 3. Effect of pH and exposure time (Initial: immediately after exposure, solid bars; Final: after 3 weeks' exposure, stippled bars) on the feeding ration in juvenile red and blue king crabs. Error bars are one standard error. No significant differences were found.

2009); however, calcium content is not necessarily correlated with other measures of exoskeleton structure or function (Coffey *et al.*, 2017; Glandon *et al.*, 2018).

In this study, neither red nor blue king crab increased their feeding rate under acidified conditions in either the short or long term despite a very high initial increase in respiration. This could be because both species are at their maximum possible feeding rate at this temperature. In endotherms, feeding rates increase with temperature (Brett, 1971). In this study, both species fed more (based on interpolation between measurements made at 2 and 6°C for red king crab and 4 and 6°C for blue king crab) at 5°C than in previous studies (Stoner *et al.*, 2010; Long and Daly, 2017) suggesting that these crab may be incapable of increasing feeding, at least at this temperature. This may be why high food availability did not reduce or eliminate negative effects of ocean acidification as can occur in both barnacles, *Amphibalanus improvisus*, and the Olympia oyster, *Ostrea lurida* (Hettinger, Sanford, Hill, Hosfelt, *et al.*, 2013; Pansch *et al.*, 2014). Long-term trials suggest that slightly (+2°C) increased temperatures may reduce negative effects on mortality in red king crab juveniles (Swiney *et al.*, 2017), and this could be because higher temperatures allow for a higher feeding rate; however, a larger increase in temperature in the same study (+4°C) resulted in a synergistic increase in the mortality rate suggesting that at that point the increase in metabolic demand due to the combined stressors of pH and temperature could not be compensated for by increased feeding. Further work on the interaction between pH and temperature on the physiology of these species, particularity blue king crab, is warranted.

The initial higher oxygen consumption did not persist after 3 weeks exposure to low pH water in red or blue king crab which, combined with the lack of an increase in feeding, suggests that the crabs had reached a physiological equilibrium by that point. *Palaemon elegans* and *P. serratus* both suffer initial acidosis over at least the first 14 days, but after 30 days of exposure they are able to completely compensate for the reduced ambient pH (Dissanayake *et al.*, 2010) and Dungeness crabs do the same but within 24 h after exposure to acidified water (Pane and Barry, 2007). Thus, our timeframe for apparent compensation within 3 weeks is comparable to other crustaceans. However, the reduced growth in red king crab that occurred in this study, and the increased mortality and decreased growth that occurs in both species in acidified conditions in longer-term, 1 year for blue and 6 months for red king crab, experiments (Long, Swiney, Harris, *et al.*, 2013; Long *et al.*, 2017), suggest an ongoing energetic cost to maintaining acid/base balance against an ever-present pH gradient. This higher cost of maintaining homeostasis, if uncompensated by increased feeding, would necessarily divert energy away from other biological functions such as growth. A similar effect occurs in the brittlestar, *Amphiura filiformis*, which increases both metabolism and calcification under acidified conditions but suffers reduced muscle mass (Wood *et al.*, 2008). Future research should look at other functions that could be affected by decreased energy availability such as immune function (Meseck *et al.*, 2016) or, in mature animals, reproductive output (Dupont *et al.*, 2013).

More research will be necessary to better quantify and model the physiological response in these species. It is worth noting that in this experiment as well as in virtually all ocean acidification experiments, the pH change experienced by the experimental animals is more akin to an upwelling or other acute event rather than the gradual change in pH predicted from rising atmospheric CO_2 levels. Thus while the acute exposure experiments conducted to date suggest substantial population and fishery level effects within a few decades for at least red king crab (Punt *et al.*, 2014), there is potential for evolutionary adaptation in both species which should be investigated. In addition, carryover effects from previous life-history stages (Hettinger *et al.*, 2012; Hettinger, Sanford, Hill, Lenz, *et al.*, 2013) as well as transgenerational

Table 3. ANOVA results for effect of pH on growth as measured as a percent change in wet mass in red king crab.

	df	Mean squares	*F*-ratio	*p*-Value
Moulted	1	1 492.253	6.845	0.020
pH	2	1 018.971	4.674	0.028
Moulted*pH	2	938.653	4.305	0.035
Error	14	218.015		

Moulting represents whether the crab moulted during the experiment and pH the pH treatment.

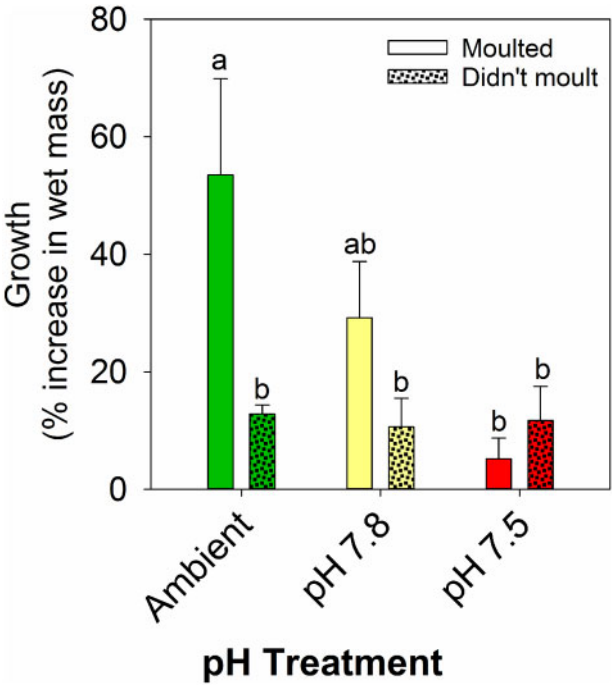

Figure 4. Effect of pH and moulting on growth in juvenile red king crabs. Error bars are one standard error. Bars with different letters above them differ statistically.

effects from parental exposure to low pH (Parker *et al.*, 2012; Long *et al.*, 2016) can both either ameliorate or exacerbate the effect on an organism and also need to be explored further for these species. Finally, co-occurring stressors, especially temperature (Swiney *et al.*, 2017), could alter the effects of ocean acidification, and should also be considered (Breitburg *et al.*, 2015).

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