Does physiological tolerance to acute hypoxia and salinity change explain ecological niche in two intertidal crab species?

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Intertidal biota is subjected to significant fluctuations in environmental parameters such as salinity and dissolved oxygen (DO). In the current study, the effects of salinity and DO on metabolic rate, critical oxygen partial pressure (Pcrit), heart rate and osmoregulation in two intertidal crab species commonly found on New Zealand coastlines, Hemigrapsus crenulatus and Hemigrapsus sexdentatus, were measured. Based on its habitation of burrows in the lower intertidal zone, H. crenulatus was predicted to be more resilient to these environmental stressors than H. sexdentatus, which is distributed in the mid to high tidal zone. However, relative to the full-strength seawater control, there were no consistent salinity-dependent changes in respiratory or cardiovascular endpoints in either species following acute 6-h exposures mimicking a tidal cycle. Analysis of haemolymph osmolality and ions determined that both crab species were strong osmotic and ionic regulators over the 6-h exposure period. However, the threshold salinities at which significant changes in osmotic and ionic regulation occurred did differ and generally indicated that H. crenulatus was the better regulator. Respiratory and cardiovascular responses to DO were prominent, with a strong bradycardia observed in both species. Changes in osmolality and sodium ion regulation were also seen as DO declined. The effect on sodium ion levels had its onset at a higher oxygen partial pressure in H. sexdentatus than in H. crenulatus, indicative of a relatively poorer hypoxia tolerance in the former species. The relative resilience of respiratory, cardiovascular and osmoregulatory processes to salinity and DO variations likely contribute to distinct habitat distributions of the two crab species on New Zealand shorelines, although behaviour and inter-specific interactions may also play important roles. Environmental change, in the form of coastal erosion and anthropogenic contamination of estuaries, has the potential to disturb the delicate niche separation that exists between these species.

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Introduction

Intertidal environments are in constant flux. Over the course of a tidal cycle, an aquatic organism living in an intertidal setting may experience changes in water availability, variable water salinity, temperature oscillations and fluctuations in dissolved oxygen (DO). For example, tidal pool DO has been shown to range from 10 to 955 μmol l⁻¹ (~0.8–87 kPa) over the course of 6 h (Legrand et al., 2018), whilst salinity in estuaries can range from near zero to hypersaline depending on the magnitude of freshwater influx (Montagna et al., 2018). This instability of environmental physicochemical factors challenges organism homeostasis. Animals that live in these settings must employ physiological strategies that allow them to maintain function despite rapid environmental fluctuation and/or enact behaviours that minimize their exposure to these factors.

Crabs are common inhabitants of intertidal zones and employ a number of physiological mechanisms that enable them to withstand fluctuations in environmental variables such as salinity and DO. Osmoregulating crab species maintain haemolymph osmolality and ion concentrations through the co-ordinated actions of epithelial transporters (Péqueux, 1995). However, epithelial transport may incur a metabolic cost that can be manifested as an increase in oxygen consumption (Shinji et al., 2009). Altered energy demands may also be reflected in changes in cardiovascular parameters. For example, tachycardia is commonly reported in crabs exposed to dilute salinities (Hume and Berlind, 1976; Taylor, 1977; McGaw and McMahon, 1996). This increase in heart rate may facilitate oxygen loading at the gills and thereby fuel the increased metabolic demands of osmoregulation (Taylor, 1977). In conditions of declining DO, bradycardia is frequently reported in crabs (Airriess and McMahon, 1994; McMahon, 2001; McGaw and McMahon, 2003), a response that slows haemolymph flow through the gills, facilitating oxygen transport under conditions where the oxygen uptake gradients are compromised relative to normoxic waters (McMahon, 2001). However, at a certain critical oxygen partial pressure (PO₂), known as the Pcrit, regulation fails and the animal transitions from oxyregulation to oxyconformation (Yeager and Ultsch, 1989). This is associated with a greater reliance on anaerobic metabolism. Critically, because of the metabolic costs associated with osmoregulation and the reduced energy provided by anaerobic metabolism, a decrease in DO might ultimately compromise the ability of a crab to maintain extracellular fluid osmolality and ion concentrations (Lucu and Ziegler, 2017).

On New Zealand coastlines, two species of crabs belonging to the genus Hemigrapsus are regularly encountered. Hemigrapsus sexdentatus (Hilgendorf) (formerly H. edwardsii; McLay and Schubart, 2004), the purple rock crab, has a relatively wide ecological niche. It is mainly distributed in the purple rock crab, a relatively wide ecological niche. It is mainly distributed in estuaries (Yeager and Ultsch, 1989). This is often associated with freshwater inputs (Williams, 1969) and as a semi-terrestrial species, largely avoids fluctuations in seawater (SW) salinity associated with tidal rhythms. Conversely, Hemigrapsus crenulatus (Milne-Edwards), known as the hairy-handed crab, mostly occupies estuaries and lower intertidal zones. It is commonly associated with mud-flats and in these and other soft substrates, it will burrow (McLay, 1988). Sheltering in the burrow during a tidal cycle will expose the crab to a declining DO, whilst riverine inputs at low tide reduce ambient salinity. Both of these crabs are tolerant to variable salinities (Hicks, 1973) and are known to be good osmoregulators, maintaining haemolymph osmolality above SW osmolality in dilute waters (Taylor and Seneviratna, 2005; Lee et al., 2010; Urzúa and Urbina, 2017). To date, however, little is known regarding the tolerance of either of these species to low DO. Given the propensity of H. crenulatus to burrow into potentially anoxic, organic-rich, muddy sediments (McLay, 1988), this species would be predicted to be more hypoxia tolerant than H. sexdentatus.

The aim of the current study was to investigate the responses of H. crenulatus and H. sexdentatus to acute changes in DO and salinity, characteristic of those occurring during a tidal cycle. Our hypothesis was that differences in physiological capacity for homeostasis in response to environmental stressors such as low DO and reduced salinity, may contribute towards the distinct ecological niches of these two Hemigrapsus species on New Zealand coastlines and may ultimately determine their capacity to withstand anthropogenic pressures on their habitats.

Materials and methods

Animal collection and maintenance

Male H. crenulatus (mean ± standard error of mean (SEM) mass = 11 ± 2 g) were collected from the Avon-Heathcote Estuary/Ihutai (43°33’S, 172°43’E), whilst male H. sexdentatus (33 ± 3 g) were collected from Waipara Beach (43°09’S, 172°48’E), both locations in the Canterbury province of New Zealand. Crabs were immediately transported to the University of Canterbury, where they were held in recirculating natural SW (salinity ∼ 35) maintained at 15°C and subjected to a 12-h light:12-h dark photoperiod, for at least a week before experimentation. During acclimation to holding conditions, crabs were fed every other day on fresh mussels, although feeding was withheld 24-h prior to experimentation. All animal procedures were approved by the University of Canterbury Animal Ethics Committee.

Oxygen consumption and determination of critical PO₂

Oxygen consumption was measured using closed-boxed respirometry, via methods described previously for freshwater crayfish (Broughton et al., 2017). Briefly, a 450-ml Perspex
The normoxic MO$_2$ of *H. crenulatus* varied significantly as a function of exposure salinity (one-way ANOVA, $P = 0.004$; Fig. 1A). A fall in exposure salinity from full-strength SW (35) to 50% SW (18) resulted in a significant 1.5-fold increase in MO$_2$. At lower salinities and in the hypersaline test condition, the MO$_2$ of *H. crenulatus* was unchanged relative to the control. This pattern was distinct from that observed for *H. sexdentatus* (Fig. 1B). In this species, there were no significant effects of salinity on MO$_2$, although a trend towards increasing MO$_2$ with decreasing salinity could be observed (one-way ANOVA, $P = 0.132$).
the heart rate of *H. crenulatus* (one-way ANOVA, *P* < 0.001; Fig. 3B). The mean heart rate of 79 ± 9 beats min⁻¹ at a salinity of 18 (50% SW) was significantly lower than that at the two salinity extremes [0.7 (2% SW) 123 ± 8 beats min⁻¹; 53 (150% SW), 131 ± 7 beats min⁻¹]. There were, however, no significant differences in *H. sexdentatus* heart rate in any salinity relative to the full-strength SW control.

Analysis of haemolymph osmolality showed that both crabs were hyperosmotic regulators in dilute salinities (Fig. 4A and B). In both *H. crenulatus* and *H. sexdentatus* haemolymph osmolality dropped significantly in crabs exposed for 6-h to a salinity of 18 (50% SW) relative to crabs maintained at a salinity of 35 (100% SW; overall one way ANOVA, *P* < 0.001 for both species). Thereafter, further dilution of the exposure medium had no further significant effect on the osmolality of *H. sexdentatus* haemolymph, whereas at a salinity of 0.7 (2% SW), *H. crenulatus* osmolality was significantly lower than that at all other salinities. In hypersaline conditions (salinity = 53; 150% SW), both species displayed a significant increase in haemolymph osmolality.

Patterns for haemolymph ions were generally similar to those for osmolality (Fig. 4C–H). Relative to the control (salinity 35, 100% SW), crabs of both species exposed to dilute salinities displayed lower haemolymph ion concentrations, whereas crabs exposed to higher salinities displayed elevated haemolymph ion concentrations (one way ANOVAs, all *P* < 0.001). The threshold salinities, at which ion concentrations became significantly different, varied between ions and species. For sodium, potassium and chloride in *H. crenulatus*, statistically significant differences relative to the control were observed in crabs exposed to salinities of 0.7, 9 and 9, respectively (Fig. 3C, E and G). For sodium, potassium and chloride in *H. sexdentatus*, statistically significant differences relative to the control were observed in the haemolymph of crabs exposed to salinities of 9, 18 and 9, respectively (Fig. 4D, F and H). The single exception where an increase in exposure salinity to 53 (150% SW) did not lead to a statistically increased haemolymph ion concentration was for chloride in *H. sexdentatus*.

A 6-h exposure to lowered water DO had a significant overall effect on heart rate for both *H. crenulatus* (one-way ANOVA, *P* < 0.001; Fig. 5A) and *H. sexdentatus* (one-way ANOVA, *P* < 0.001; Fig. 5B). In the former species, heart rate was statistically unchanged relatively to the normoxic control (18.7 kPa), until exposure to 1.1 kPa. In crabs from this treatment, a mean heart rate of 43 ± 3 beats min⁻¹ was observed, a value just 35% of that measured in normoxic crabs of this species (122 ± 15 beats min⁻¹). For *H. sexdentatus*, a significant fall in heart rate with declining DO was observed at 4.7 kPa, a treatment of higher PO2 than that observed to have the same effect in *H. crenulatus*. In the lowest PO2 (1.1 kPa), the heart rate of *H. sexdentatus* was 33 ± 2 beats min⁻¹, a value 32% of that recorded in normoxic crabs of this species (103 ± 10 beats min⁻¹).

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**Figure 1:** Effect of exposure salinity on normoxic MO₂ in *H. crenulatus* (A) and *H. sexdentatus* (B). Plotted values represent means (± SEM) of six replicates. Within panels, bars sharing lowercase letters are not significantly different (one-way ANOVA, *α* = 0.05).

Closed-box respirometry resulted in a decline in PO₂ as the crab consumed the oxygen in the respirometer (Fig. 2). Plotted values in this figure represent datapoints for all individual crabs within a given stressor level. In both crab species, in all water salinities, MO₂ remained relatively constant until a water PO₂ of ~5.5–7.5 kPa. Thereafter, the capacity of the crab to regulate oxygen consumption failed and a rapid decline in MO₂ with falling water PO₂ was observed. Using values calculated for each individual and then averaged, the *P*<sub>crit</sub> was determined to be 5.9 ± 0.9 kPa for *H. crenulatus* in full-strength SW (i.e. salinity = 35). Under the same exposure conditions, the mean calculated *P*<sub>crit</sub> for *H. sexdentatus* was 7.6 ± 0.2 kPa. Values of *P*<sub>crit</sub> did not differ as a function of exposure water salinity for either species (one way ANOVAs; *P* = 0.37 for *H. crenulatus*, *P* = 0.08 for *H. sexdentatus*; data not shown).

During a 6-h exposure to a range of water salinities, the heart rate of *H. crenulatus* remained unchanged (one-way ANOVA, *P* = 0.92; Fig. 3A), ranging from 106 ± 11 to 121 ± 14 beats min⁻¹ for salinities of 35 and 0.7, respectively. Conversely, there was a significant effect of salinity on the mean heart rate of *H. sexdentatus* (one-way ANOVA, *P* < 0.001; Fig. 3B). The mean heart rate of 79 ± 9 beats min⁻¹ at a salinity of 18 (50% SW) was significantly lower than that at the two salinity extremes [0.7 (2% SW) 123 ± 8 beats min⁻¹; 53 (150% SW), 131 ± 7 beats min⁻¹]. There were, however, no significant differences in *H. sexdentatus* heart rate in any salinity relative to the full-strength SW control.
Figure 2: Effect of salinity [0.7 (A and B), 9 (C and D), 18 (E and F), 35 (G and H), 53 (I and J)] on the relationship between water $\text{PO}_2$ and $\text{MO}_2$ in $H. \text{crenulatus}$ and $H. \text{sexdentatus}$. Plotted values represent all data for six crabs, with lines fitted and breakpoints (i.e. $P_{\text{crit}}$) derived via a piecewise regression performed using R statistical software.
and significantly as a function of exposure.

Haemolymph sodium concentrations also differed significantly as a function of exposure (one-way ANOVAs, both P < 0.001; Fig. 6C and D). For H. crenulatus, haemolymph sodium was maintained at normoxic control levels, until the lowest tested exposure PO2 (1.1 kPa), where the value of 463 ± 10 mM was significantly distinct from crabs in all other waters (Fig. 6C). Conversely, in H. sexdentatus dropping the PO2 from 18.7 to 9.3 kPa caused a significant 22% fall in haemolymph sodium (Fig. 6D). A further significant decline in H. sexdentatus haemolymph sodium was noted at 4.7 kPa. At 1.1 kPa, there was a small but significant increase, in haemolymph sodium relative to the 4.7 kPa treatment, but this value was still significantly reduced with respect to the control. Relative to normoxia, there were no significant effects of PO2 on haemolymph potassium (Fig. 6E and F) or haemolymph chloride (Fig. 6G and H), for either crab species. However, for H. sexdentatus, crabs from the 1.1 kPa exposure condition displayed haemolymph potassium values that were significantly reduced relative to the 4.7 and 9.3 kPa treatments.

**Discussion**

**Respiratory responses to acute salinity change**

The MO2 of H. sexdentatus acutely exposed to either hypo- or hyper-saline waters was unchanged across all tested salinities (Fig. 1B). In contrast, an increase in MO2 was observed in H. crenulatus as crabs moved from full-strength SW to 50% SW (salinity = 18; Fig. 1A). In other crab species, increases in MO2 with declining salinity are commonly reported (Taylor et al., 1977b; Shumway, 1983; Normant and Gibowicz, 2008), although some authors have reported MO2 increases in hypersaline waters (Ramaglia et al., 2018), or a lack of change in MO2 with salinity (Winch and Hodgson, 2007; Theuerkauf et al., 2018). Differences between studies are likely due to diverse experimental protocols (most notably whether the measurement occurs after acute exposure or after acclimation) and differences in the osmoregulatory capacity of the test species.

However, the current findings of a transient effect or lack of response of MO2 to salinity are distinct from previous studies in Hemigrapsus. For example, employing a protocol comparable to that of the current work, an acute (2–6h) salinity exposure in H. takanoi resulted in a higher MO2 in lower salinities (Shinji et al., 2009). We propose that differences in experimental outcomes reflect subtle differences in ecological niches, which are known to vary between Hemigrapsus species and between populations of the same species from different regions (e.g. McIay, 1988).

Over the course of a 6-h acute exposure neither crab species displayed changes in Pcrit. The Pcrit is widely used as an indicator of hypoxia tolerance in aquatic biota, representing the point at which an oxyregulator can no longer maintain metabolic rate and thereafter oxygen consumption declines as a function of declining PO2 (Yeager and Ultsch, 1989). Previous studies have shown that exposure of crabs (Carcinus maenas and Carcinus aestuarii) to dilute salinities leads to an increase in Pcrit (Taylor et al., 1977a; Rivera-Ingraham et al., 2016). This is likely a consequence of the greater oxygen demands of crabs in lower salinities in these studies, such that the onset of the transition between aerobic and anaerobic metabolism occurs at a higher PO2. Given that the crabs in the current study did not show a consistent pattern of increased metabolic costs in dilute salinities, then the finding of a salinity-independent Pcrit is not surprising.

**Cardiovascular responses to acute salinity change**

Exposure of crabs to salinity change is known to induce tachycardia (Hume and Berlind, 1976; Taylor, 1977; McGaw and McMahon, 1996), although in some osmoconforming
Figure 4: Effect of exposure salinity on haemolymph osmolality (A and B), sodium (C and D), potassium (E and F) and chloride (G and H) ion concentrations in H. crenulatus (A, C, E and G) and H. sexdentatus (B, D, F and H). Plotted values represent means (±SEM) of six replicates. Within panels, bars sharing lowercase letters are not significantly different (one-way ANOVA, α = 0.05).
and increased locomotor costs (Taylor, 1977). Consequently, metabolic costs associated with osmoregulation and/or that facilitates oxygen uptake, thereby fuelling enhanced response to salinity change has been proposed as a mechanism.

Figure 5: Effect of exposure PO2 on heart rate in H. crenulatus (A) and H. sexdentatus (B). Plotted values represent means (±SEM) of six replicates. Within panels, bars sharing lowercase letters are not significantly different (one-way ANOVA, α = 0.05).

species bradycardia may be observed (McGaw, 2006). However, in the current study, a change in exposure salinity had no impact on heart rate relative to controls where heart rates were monitored in full-strength SW. Tachycardia in response to salinity change has been proposed as a mechanism that facilitates oxygen uptake, thereby fuelling enhanced metabolic costs associated with osmoregulation and/or increased locomotor costs (Taylor, 1977). Consequently, our data showing an absence of tachycardia are consistent with our results showing a lack of consistent effect of salinity on MO2. Heart rate alone, however, does not always provide a complete picture of cardiovascular change. In crabs, cardiac output can change independently of heart rate due to altered stroke volume, such that heart rate may not accurately reflect changes in cardiovascular dynamics (McGaw and McMahon, 2003). It is therefore possible that although heart rates were relatively consistent across different salinities, cardiac output may not have been. However, even if changes in cardiovascular physiology occurred, it seems as though these acted to maintain MO2 rather than to meet increased costs associated with hypo- or hyper-saline exposure.

Osmotic and ionic responses to acute salinity change

Both H. crenulatus and H. sexdentatus maintained haemolymph osmolality and ion concentrations below and above, those of more dilute or concentrated salinities, respectively. Although the current study only determined osmolality after 6 h (i.e. a tidal cycle) and it can take up to 48 h for osmoregulatory status to develop completely (Lovett et al., 2001), our findings were consistent with previous work on these species (Taylor and Seneviratna, 2005; Lee et al., 2010; Urzúa and Urbina, 2017).

In both crabs, haemolymph osmolality dropped significantly as animals acclimated to full-strength SW were placed in 50% SW (salinity = 18; Fig. 4A and B). The effects of salinity on haemolymph ions were more distinct and highlight H. crenulatus as the stronger ion regulator. For example, the threshold salinity at which haemolymph sodium ion concentration differed significantly from the control was 0.7 and 9, for H. crenulatus and H. sexdentatus, respectively. For potassium ion, the salinity threshold was 9 for H. crenulatus and 18 for H. sexdentatus. Distinct patterns for haemolymph osmolality and haemolymph ions within the same treatment group are likely due to changes in unmeasured osmolytes (e.g. amino acids), which are known to fluctuate with external salinity (Findley and Stickle, 1978).

The finding that H. crenulatus is the better regulator over the course of a 6-h exposure is generally consistent with previous data regarding the tolerance of these two species to salinity change. For example, Hicks (1973) noted that H. crenulatus was more tolerant to dilute salinities than H. sexdentatus at an exposure temperature of 15°C. Conversely, Taylor and Seneviratna (2005), found that H. crenulatus was less tolerant to low salinities, although this study was conducted on early life-stages. This finding does, however, have some support from the data in the current work. At the lowest tested salinity (0.7), H. sexdentatus was able to maintain osmolality at a level statistically indistinct from that in 50% SW (18), whereas in 0.7 salinity water H. crenulatus osmolality was statistically lower than that of crabs at 50% SW. This ability to regulate in very dilute salinities has been attributed to the association of this species with freshwater inputs (Williams, 1969).

In the current work, the mass of the adult crabs differed markedly, with H. sexdentatus being three times larger than H. crenulatus. A recent study examining the capacity of H. crenulatus to regulate haemolymph sodium in response to decreasing salinity noted that larger crabs were better regulators (Urzúa and Urbina, 2017). It is therefore noteworthy that on the basis of mass differences alone, H. sexdentatus would be predicted as the better regulator. This indicates that the effects seen in the current study were not simply a consequence of body mass and that a more distinct separation of the osmoregulatory and ionoregulatory capacities of the two species may have been identified if experimental body sizes were equivalent.
Figure 6: Effect of exposure PO$_2$ on haemolymph osmolality (A and B), sodium (C and D), potassium (E and F) and chloride (G and H) ion concentrations in *H. crenulatus* (A, C, E and G) and *H. sexdentatus* (B, D, F and H). Plotted values represent means (±SEM) of six replicates. Within panels, bars sharing lowercase letters are not significantly different (all one-way ANOVA, except *H. crenulatus* potassium and chloride which were assessed via Kruskal-Wallis ANOVA; $\alpha = 0.05$).
Respiratory responses to hypoxia

Under conditions where crabs were exposed to declining PO2, resulting from their consumption of oxygen within a sealed chamber, both species initially oxyregulated. This pattern was then superseded by an oxyconforming response once \( P_{\text{crit}} \) was reached (Fig. 2). The measured \( P_{\text{crit}} \) values ranged from 5.5 to 7.6 kPa, in line with previous studies that have characterized \( P_{\text{crit}} \) in intertidal crab species. For example, in Carcinus a \( P_{\text{crit}} \) of 5.3 kPa was determined (Taylor, 1981), albeit at a slightly lower experimental temperature than that used in the current study (10 vs. 15°C). In general, intertidal crabs display \( P_{\text{crit}} \) values that are intermediate to the higher values measured in crustaceans that function in well-oxygenated subtidal environments (e.g. 4–11 kPa) and to the lower values determined for species that inhabit poorly oxygenated burrows (1.3–6.7 kPa; Whiteley and Taylor, 2013). Contrary to prediction, the more fossorial species, H. crenulatus, did not display a lower \( P_{\text{crit}} \), indicative of higher hypoxia tolerance. Given that differences in cardiovascular responses to hypoxia were discerned in the current work, this suggests that the \( P_{\text{crit}} \) may not be a useful indicator of relative hypoxia tolerance in these species.

Cardiovascular responses to acute hypoxia

In response to declining PO2, both Hemigrapsus species displayed bradycardia. This is a commonly observed response to hypoxia in crabs (Bradford and Taylor, 1982; Airriess and McMahon, 1994) and is one that is usually accompanied by an increase in stroke volume (McGaw and McMahon, 2003). Together, these changes are thought to aid oxygen loading at the gills by increasing the volume of haemolymph oxygenated and its branchial residence time, whilst also facilitating oxygen unloading at the tissues (McMahon, 2001).

Although both species in the current study displayed bradycardia, the onset of this response differed. For H. crenulatus, a significant bradycardia only occurred once PO2 reached 1.1 kPa, whereas the equivalent value for H. sexdentatus was 4.7 kPa. This suggests that H. sexdentatus is less hypoxia tolerant. In general, there is a correlation between bradycardia and hypoxia tolerance in crustaceans. In species such as Cancer pagurus, a relatively hypoxia-sensitive crab, heart rate decreases as PO2 declines (Bradford and Taylor, 1982). However, burrowing crustaceans are highly hypoxia-tolerant and often do not exhibit a bradycardia in waters of low PO2, instead relying on adaptations such as respiratory pigtails with high oxygen affinity to maintain metabolic rate (Whiteley and Taylor, 2015).

Osmoregulatory responses to acute hypoxia

In both H. crenulatus and H. sexdentatus, exposure to reduced PO2 resulted in decreases in haemolymph osmolality and sodium ion concentrations (Fig. 6A–D). In the freshwater prawn, a similar effect of hypoxia has been noted (Cheng et al., 2003). This was attributed to haemolymph dilution, which resulted from enhanced water influx associated with elevated ventilation rates. An alternate explanation is that the observed effects are the consequences of osmoregulatory compromise at the gill. A standard response to hypoxia in crabs is to increase ventilation rate, compensating for the reduced PO2 by bringing larger volumes of water in contact with the gill epithelia (McGaw and McMahon, 1996). This would exacerbate diffusive exchange of ions should any small differences in extracellular and environmental ion concentrations exist. Similarly, the reduction in MO2 as PO2 drops means that there is reduced energy available to restore osmotic balance (Lucu and Ziegler, 2017), which could also result in changes in haemolymph osmolality and ion concentrations.

The threshold of effects of PO2 on haemolymph osmolality was identical in H. crenulatus and H. sexdentatus. The drop in PO2 from 18.7 to 9.3 kPa, induced significant osmolality declines in both species. However, with respect to effects on sodium ion, H. crenulatus was better able to maintain concentrations (until a PO2 of 1.1 kPa), relative to H. sexdentatus (statistically significant drop in haemolymph sodium relative to normoxic control at 9.3 kPa). The mechanism for this is unknown, but if H. crenulatus had a superior anaerobic capacity to H. sexdentatus, then this could provide the energy to better sustain ion regulation over the duration of the short-term 6-h exposure. These data nevertheless suggest that H. crenulatus is the more tolerant of the two species to hypoxia, consistent with cardiovascular data and its habitation of environments with greater risk of exposure to low PO2.

Methodological and environmental considerations

In the current study, crabs were added into exposure chambers and immediately subjected to physiological investigation. This differs from approaches where the animals are acclimated to the chambers for several hours prior to medium manipulation (e.g. Broughton et al., 2017). Consequently, the responses measured may reflect stress associated with handling. However, analysis of continuous recordings in respirometry experiments showed that any initial elevations in oxygen consumption lasted less than 20 min (data not shown). Some authors have also utilized pauses in heart rate as an indicator of reduced stress in crabs (McGaw and Nancollas, 2017). In our study, the mean time of acardia onset in control crabs was 86 min (data not shown), suggesting that crabs settled relatively quickly into chambers. Nevertheless, we cannot rule out that a component of time-integrated measurements (i.e. haemolymph osmolality and ions) could reflect handling stress.

The ability of our study to draw conclusions regarding the importance of physiology in shaping niche habitation is also limited by a lack of knowledge of the physical and chemical variability in the habitats, and more specifically the microhabitats, of the study species. For example, it is possible...
that the tested levels of salinity and DO are beyond the scope of those experienced by the crabs in natural settings. Consequently, interpreting physiological tolerance at physicochemical extremes in the laboratory may be misleading if environmental values for salinity and DO fluctuate over narrower ranges. Furthermore, laboratory studies involve the maintenance of animals under controlled conditions. Given that crab species exhibit physiological responses that are entrained by environmental cues (e.g. McGaw and McMahon, 1998), then this represents an additional challenge when attempting to ascribe ecological niche habitation to physiological tolerance.

**Conclusion**

*H. crenulatus* appeared to be more tolerant to low environmental PO$_2$ than *H. sexdentatus*, befitting its habitation of environments where hypoxia may occur (i.e. burrows in muddy substrates; McLay, 1988). Similarly, lower onsets of salinity effects on haemolymph sodium and potassium ion concentrations in *H. crenulatus* relative to *H. sexdentatus* hint at a greater short-term tolerance of the former species to dilute salinities, at least in the 9–18 (25–50% SW) range. This would be consistent with a crab that inhabits the lower intertidal zone, where exposure to salinity fluctuations is more commonly encountered.

Overall, however, the effects of DO and salinity on *Hemigrapsus* physiology were relatively minor, suggesting that factors other than physiology will contribute to the distinct niches of these two species along New Zealand coastlines. For example, the current study exposed crabs under conditions where normal behavioural responses could not be enacted. The importance of responses such as emersion and avoidance in crabs exposed to environmental stressors is well-established (McGaw *et al.*, 1999; Bell *et al.*, 2009) and will be of relevance in determining habitat selection in natural settings. Inter-specific interactions will also play a role. Indeed, it is notable that in Chile *H. crenulatus* is the sole *Hemigrapsus* species and occupies a wider and more exposed environmental niche than it does in New Zealand (McLay *et al.*, 2011). It is also important to note that the current study only examined salinity and DO as stressors. Similar studies of sympatric intertidal crab species have identified temperature and tolerance to this stressor, as the major factor explaining differences in species distributions (Jensen and Armstrong, 1991; Stillman and Somero, 1996).

Finally, the current study has implications for conservation of these species. The coastal habitats of *Hemigrapsus* in the Canterbury region of New Zealand are subjected to anthropogenic change. For example, the Avon-Heathcote Estuary/Ihutai receives nutrient inputs that result in water quality metrics that exceed regulatory trigger values and which lead to extensive anoxia (Bolton-Ritchie and Main, 2005). Beach habitats are exposed to coastal erosion, which will worsen with predicted sea level rise (MfE, 2017). This can result in changes in tidal profiles and narrowing of ecological niches. This is exacerbated by modifications of coastal infrastructure such as sea walls, which may further condense optimal habitat and expose animals to more severe fluctuations in environment (Gittman *et al.*, 2016). The current work indicates that the *Hemigrapsus* crab species found on New Zealand coasts have sufficient physiological plasticity to enable them to adjust niche in response to environmental change. However, shifts in ecological niches may increase inter-specific competition and ultimately could lead to exclusion of the less robust species.

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