Coming out of their shells: The effects of decreased pH on shell selection in the grainyhand hermit crab (*Pagurus granosimanus*)

by

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Abstract
Ongoing ocean acidification, due to increasing atmospheric carbon dioxide levels, threatens not only the physiological functioning of marine organisms, but alters their behaviour and resource-assessment by compromising chemo-sensitivity. Hermit crabs make a particularly good model organism for the investigation of such decision-making behaviours. The goal of our study was to explore how decreased sea water pH influences the shell selection behaviour of *Pagurus granosimanus*, the grainyhand hermit crab, in the presence and absence of predator effluent, in both acidified and untreated sea water. Our results, which revealed increased hiding in treatment groups exposed to predator effluent ($P = 0.03$), support previous findings that hermit crabs are sensitive to these olfactory cues. Furthermore, we observed that hermit crabs in damaged shells tested in reduced pH conditions took longer to make contact with a provided “optimal” shell ($P = 0.049$), suggesting a reduced ability to detect and assess potentially suitable shells, which forms such a critical component of their survival and fitness. However, we found no difference in the number of hermit crabs that made contact with the shell ($P = 0.375$), or the number of times they switched (0.0962), between treatments.

Key words [chemo-reception, chemo-sensitivity, effluent, ocean acidification, olfactory]

Introduction
As atmospheric carbon dioxide (CO$_2$) levels continue to rise, predicted to reach 800 parts per million by 2100, the resulting changes in ocean acidity are expected to have extensive consequences on marine biota (Doney *et al.*, 2009). Relative to pre-industrial values, surface ocean pH has already dropped by 0.1 units, and is projected to decrease a further 0.3 to 0.4 units by the end of the century (Orr, 2005). This acidification, due to the absorption of anthropogenic CO$_2$ emissions by the globe’s oceans, alters fundamental chemical balances (Kleypas *et al.*, 1999). The
resulting changes pose several challenges to marine organisms; for instance, lowered calcium carbonate, and aragonite saturation states impact shell-formation in calcifying marine organisms (Kleypas et al., 1999; Orr et al., 2005; Doney et al., 2009). While such physiological impacts are relatively well studied; our current understanding of how decreasing pH impacts organism behaviour is, by comparison, much more limited.

As a growing number of studies suggest, ongoing ocean acidification threatens not only the growth, metabolism and calcification of marine organisms (Pörtner et al., 2004), but disrupts their chemo-responsive behaviour. Decreased sea water pH has been shown to interfere with the ability of marine fish to correctly differentiate distinct odours or to detect them at all. For example, when reared and tested in an acidified environment, settlement-stage larvae of tropical fish were, in fact, attracted to the smell of predators (Dixson et al., 2009). Disruption of chemosensory sensitivity in fishes due to decreased pH extends to adult behaviours, impacting their homing ability (Devine et al., 2012). In the common hermit crab Pagurus bernhardus, acidification of sea water influences their response to a food odour, leading to longer times to locate the odour source, and decreased time spent in contact with it (de la Haye et al., 2011). The effects of ocean acidification suggested by these findings could
have far-reaching consequences in an ecosystem where organism behaviour is so
heavily reliant on chemical cues for foraging, maximizing reproductive success and
avoiding predation (Atema, 1995).

Hermit crabs make an excellent model for investigating resource assessment and
decision-making behaviours (Reese, 1963; Mesce, 1982; Côté et al., 1997). As an
inhabitant of the intertidal zone, they are already subjected to fluctuations in sea water
pH during tidal cycles (Truchot, 1968). However, such natural variation is likely to be
exacerbated by ocean acidification, with the potential to impact chemo-sensory
behaviour over increasingly extended periods of time (Caldeira and Wickett, 2003).

Hermit crabs rely heavily on chemoreception not only to detect food, potential mates
(Goshima et al., 1998), and predators (Rosen et al., 2009), but the calcium released by
shells (Mesce, 1982). Proper shell selection impacts every aspect of their lives; the
“optimal” home is snug enough to minimize the weight carried, while sufficiently
spacious to allow for complete withdrawal, reducing the likelihood of predation, and
allowing for growth and reproduction (Childress, 1972; Bertness, 1981; Mima et al.,
2003; Rotjan et al., 2003). Predation has been identified as a key factor impacting
shell choice, influencing not only the shape and weight of preferred shells, but even
the species selected (Rotjan et al., 2003; Rosen et al., 2009). While recent research
has suggested that shell assessment is compromised in decreased pH sea water (de la Hay et al., 2011), questions remain as to how ocean acidification, and the potential consequential reduction in chemo-sensitivity, may impact the interaction between predation pressure and shell choice.

The goal of our study was to explore how decreased sea water pH influences the shell selection behaviour of Pagurus granosimanus, the grainyhand hermit crab, in the presence and absence of predator effluent, in both acidified and untreated sea water. The structure and function of the olfactory organs of crustaceans are highly congruent across species (Hallberg et al., 1992). Therefore, any effects we observe could also apply to other marine crustaceans, which form a fundamental component of functioning marine ecosystems. Improving our understanding of how ocean acidification affects animal behaviour will be critical in the coming years, as this environmental pressure becomes increasingly substantial.
Materials and Methods

We collected grainyhand hermit crabs by hand at low tide, between the hours of 9:30 am and 1:00 pm, on May 29th 2013, from Dixon Island, British Columbia (48°51’9”N, 125°7’10”W). For each hermit crab, we measured the propodus length as an index for their size (Côté et al., 1997), and the height of their shell to the nearest 0.1 mm.

We measured a total of 119 crabs, 45 of which were kept to take back to the lab. Once in the lab, we housed the 45 collected hermit crabs in a sea table with a constant and consistent flow of natural sea water. For shelter, we provided them with rocks and seaweed. We kept the hermit crabs for no more than four days prior to the start of the experiments, and fed them every other day with fresh mussels, *Mytilus trossulus*. The temperature of the sea water in the table was maintained within 12.4 to 13.4°C, with a pH of 7.75. Using needle nose pliers and wire cutters, we broke back the outer lip of each hermit crab’s shell until their propodus was flush with the shell opening. We placed the now sub-optimal hermit crabs into individual plastic recovery containers for 12 hours. After their recovery period, we moved the hermit crabs into individual Plexiglas™ test tanks measuring 42 cm long, 9 cm wide and 25.5 cm high. We covered the bottom of the test tanks with a layer of sand 1 cm deep, and filled them with sea water 4 cm deep (1.41 L). Around each of the test tanks we placed an open-top box made of white plastic corrugated sheets (48 cm long x 28 cm wide x 26
cm high) to eliminate unwanted visual stimulation. We randomly assigned each hermit crab to one of four conditions: Natural sea water (pH = 7.75), with no predator effluent present; natural sea water, with predator effluent present; reduced pH sea water (pH = 7.00 ±0.03), with no predator effluent present; reduced pH sea water with predator effluent present. To create the predator effluent we placed two red rock crabs (Cancer productus) in a plastic container filled with 3.09 L of standing water. We removed the predators after four and a half hours, immediately prior to starting the experiment.

Using a gas-water reactor and Flynn Accumet® portable laboratory digital pH metering system, we created a bucket of reduced pH sea water from which we filled the decreased pH test tanks.

Prior to beginning the experiment, we gave each hermit crab 20 minutes to acclimatize to their test tank and sea water condition, without the addition of any predator effluent. After this acclimatization period, we placed an un-occupied black tegula shell at the opposite end of the tank. We selected each shell based on the propodus length of the individual hermit crab, and the corresponding “optimal” shell height determined from our field data (refer to Figures 1 and 2). For the predator
effluent treatments, we removed 200 mL of the test tank sea water and replaced it with 200 mL of the predator effluent, at the appropriate pH, immediately prior to the shell drop. Following the shell drop, we observed each crab for 15 minutes. For the hermit crabs that never made contact with, we recorded the latency period as 15 minutes. We ran a total of 9 trials for each treatment, totaling 36 hermit crabs.

We recorded all data in Microsoft Excel 2010 ®, and transferred it into Minitab 16 ® for statistical analysis. We used an ANOVA to test for statistical differences in latency between treatments, and a Chi-square test to compare the number of hermit crabs that made contact with the provided shell, as well as the number of them that were observed hiding, between each treatment. We used VasserStats® to perform a Kruskal-Wallis test comparing the number of times the hermit crabs in each treatment group switched shells. We then generated graphical representations of our results in Minitab 16 ®.

**Results**

We found that the ratio of shell height to propodus length for the measured grainyhand hermit crabs on Dixon Island yielded a normal distribution (refer to Figure 1). When we plotted shell height against propodus length, it showed a clear relationship (refer to Figure 2). Between the four treatments, we found a significant
difference in the number of hermit crabs who hid (X^2 = 9.11, P = 0.03; refer to Table 1). Comparing the number of hermit crabs that made contact with the provided “optimal” shell within the 15 minute observational period, we found no statistically significant difference between treatments (X^2 = 2.9, P = 0.375, refer to Table 2).

However, the latency values, defined as the time taken for the hermit crabs to make contact with the provided shell, revealed a significant difference (F = 2.92, df = 3, P = 0.049; refer to Figure 3). The hermit crabs that we tested in normal pH had shorter latency periods relative to their reduced pH counterparts, while latency periods increased for both sea water treatments with the addition of predator effluent.

Hermit crabs would occasionally switch back to their previous “sub-optimal” shell, before returning into the provided, fully intact shell. However, we found no significant statistical difference in the number of times hermit crabs switched shells between treatment groups (H = 6.34, df = 3, P = 0.0962; refer to Table 3).

Discussion

Ocean acidification, a consequence of rising atmospheric CO2 levels (Orr, 2005; Doney et al., 2009), threatens fundamental chemical balances affecting the growth, metabolism, and calcification of marine organisms (Kleypas et al., 1999; Orr et al., 2005; Doney et al., 2009). Of increasing concern, studies have also revealed dramatic
effects of decreasing sea water pH on the chemo-responsive behaviour of some
animals (Dixson et al., 2009; de la Haye et al., 2011; Dixson et al., 2012). In an
environment where organisms are so heavily reliant on chemical cues for foraging,
maximizing reproductive success and avoiding predation (Atema, 1995), decreased
chemo-sensitivity could have extensive consequences on ecosystem functioning.
As an intertidal species, exposed to fluctuations in pH likely to be exacerbated by
ocean acidification, the hermit crab makes an excellent subject for investigating
resource assessment and decision-making behaviours (Reese, 1963; Mesce, 1982;
Côré, 1997). These marine crustaceans use chemoreception not only to detect food,
potential mates (Goshim a et al., 1998), and predators (Rosen et al., 2009), but the
calcium released by shells (Mesce, 1982). Finding and selecting an optimal shell
requires the ability to accurately detect and assess the costs and benefits associated
with the decision (Childress, 1972; Bertness, 1981; Mima et al., 2003; Rotjan et al.,
2003). Previous studies have identified predation as a key factor influencing shell
choice (Rotjan et la., 2003; Rosen et al., 2009), and suggested that decreased pH sea
water compromises shell assessment. However, the potential consequences of reduced
chemo-sensitivity on the interaction between predation pressure and shell choice in an
acidified environment remained unclear.
The goal of our study was to explore how the shell selection behaviour of *Pagurus granosimanus*, in the presence or absence of predator effluent, is affected by decreased sea water pH. We assigned hermit crabs with damaged “sub-optimal” shells, to one of four conditions; the test aquaria containing either reduced pH or natural pH sea water, and either predator effluent or none. Field surveys confirmed a relationship between propodus length and shell height, which we used to select an “optimal” shell for each of the tested hermit crabs.

We observed a decrease in the frequency of hiding behaviour in hermit crabs exposed to predator effluent under reduced pH conditions, relative to natural sea water (P = 0.03). Such observations are supported by studies in the literature suggesting that hermit crabs are capable of detecting olfactory predator cues (Mima *et al.*, 2003; Rotjan *et al.*, 2004; Rosen *et al.*, 2009), and reinforced by studies investigating the effects of sea water pH using other organisms (Dixson *et al.*, 2009). This not only exposes the crabs to predation, but, given the key role of predators in shell selection (Rotjan *et al.*, 2003; Rosen *et al.*, 2009), may have a detrimental impact on any ensuing shell assessment. While pH had no impact on the number of hermit crabs that made contact with the provided shell (P = 0.375), reduced pH sea water significantly increased the amount of time they required to find them (P = 0.049). This increase in latency suggests a decrease in chemo-sensitivity to the calcium
released by shells; thereby potentially impacting shell availability and the likelihood of acquiring an optimal home. The number of times hermit crabs switched shells did not change between treatments (P = 0.0962).

Several factors may have influenced our results. Given the time constraints of this study, hermit crabs were only acclimatized to their test sea water for 20 minutes. The significant increase in hiding frequency (P = 0.03) and latency time (P = 0.049) that we observed in the reduced pH treatment may therefore be partially attributable to the stress of this sudden environmental acidification. By selecting the hermit crabs at random, we included both males and females in our study. Any differences in shell selection between the sexes may have altered our results. Furthermore, we observed that, while switching, the abdomens of three hermit crabs were covered with eggs, these made them larger than predicted by the length of their propodus. With their additional mass, these females did not fit as well into the provided “optimal” shells, and switched more frequently between their damaged shell and the new one. Yet another confounding factor could be the disruption created by the addition of the predator effluent. We should similarly have added water to the other treatment groups to control for this disturbance. Despite the lack of hiding that we observed in response other disruptions or handling in the predator absent groups, we cannot definitively rule out the potential confounding effects due to the addition of effluent.
In conclusion, our results suggest that the grainyhand hermit crab’s chemo-sensitivity is affected by reduced pH; resulting in a compromised ability to detect predator effluent, and decreased sensitivity to the calcium released by shells. This compromise to chemo-responsive behaviour poses several threats in the face of ongoing ocean acidification; impacting their ability to make an appropriate assessment of the costs and benefits associated with their behaviour. While these findings are consistent with those in the literature, further research is required to clarify the effects of reduced pH on crustacean chemo-reception. Future studies could focus on how different pH treatments affect chemo-sensitivity, using more gradual changes and/or greater acclimatization periods. Investigating the effects of different predator effluents could provide interesting insight as to how food web interactions may be altered by sea water pH. Ocean acidification, however, is not the only environmental pressure that these hermit crabs will experience as a result of ongoing climate change; the effects of rising sea water temperature also warrant inquiry. Understanding the mechanism by which decreased pH interferes with chemo-reception could prove critical for taking mitigative and adaptive measures. Repeating these studies on other crustaceans and marine organisms could contribute significantly towards elucidating the impending effects of ocean acidification on marine ecosystems.
Acknowledgments

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References


Reese, E.S. The behavioral mechanisms underlying shell selection by hermit crabs. Behaviour, 21(1/2), 78-126.


Tables and Figures

Figure 1. Distribution of the ratio between shell height to propodus length of grainyhand hermit crabs collected and measured on Dixon Island, BC. Mean = 1.979, StDev = 0.3653, N = 119.

Figure 2. Propodus length and shell height of grainyhand hermit crabs sampled at Dixon Island, BC, with line of best fit (y = 0.9718x + 6.5019)
**Table 1.** Chi-square test for the number of hermit crabs that hid within their shells for 1 minute or more upon the start of experimental observation in normal and reduced sea water treatments, in the presence or absence of predator effluent (N = 9 per treatment).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal sea water pH (pH = 7.75)</th>
<th>Reduced sea water pH (pH = 7.00 ±0.03)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predator absent</td>
<td>Predator present</td>
</tr>
<tr>
<td></td>
<td>Predator absent</td>
<td>Predator present</td>
</tr>
<tr>
<td># of hermit crabs that hid</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 2. Chi-square test for the number of hermit crabs that made contact with the provided “optimal” shell within 15 minutes of observation in normal and reduced sea water treatments, in the presence or absence of predator effluent (N = 9 per treatment).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal sea water pH (pH = 7.75)</th>
<th>Reduced sea water pH (pH = 7.00 ±0.03)</th>
<th># of hermit crabs that made contact</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predator</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>5</td>
<td>2.9</td>
</tr>
<tr>
<td>Predator</td>
<td>Absent</td>
<td>Present</td>
<td>4</td>
<td>2</td>
<td>P = 0.375</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td></td>
<td>2</td>
<td>2.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Kruskal-Wallis analysis of the number of times hermit crabs switched shells during the 15 minutes of experimental observation in normal and reduced sea water treatments, in the presence or absence of predator effluent (N = 9 per treatment).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal sea water pH (pH = 7.75)</th>
<th>Reduced sea water pH (pH = 7.00 ±0.03)</th>
<th>Mean number of times hermit crab switched shells</th>
<th>H</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predator</td>
<td>Absent</td>
<td>Present</td>
<td>1.44</td>
<td>6.34</td>
<td>3</td>
<td>P = 0.0962</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>Absent</td>
<td>0.55</td>
<td>0.66</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>Present</td>
<td>0.55</td>
<td>0.66</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
<td>6.34</td>
<td>P</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3. Time taken for hermit crabs to make first contact with the provided “optimal” shell in normal and decreased pH sea water, with absence or presence of predator effluent. F = 2.92, df = 3, P = 0.049, bars are one standard error from the mean (N = 9 for each treatment).