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The Effects of Physical Crowding and Crowding-Induced Chemical Cues on the Metabolic Activity of *Hemigrapsus oregonensis*

Introduction

In Seattle, Washington, a shockingly steep 92% of all the shoreline within the city is armored (Boonzaier, 2018). This means that, rather than preserving vital intertidal habitats, shorelines are now reinforced with man-made walls or bulkheads, large rocks, sandbags, or other structures meant to control erosion and protect coastal communities and buildings from tides and wave action. In addition to shoreline armoring, other anthropogenic effects such as climate change threaten the vulnerable shoreline ecosystem. The melting of ice sheets and glaciers, as well as thermal expansion of the oceans due to rising global temperatures has led to a rise in sea level of 8-9 inches since the year 1880 (Lindsey, 2023). The combination of these two impacts results in removal of shoreline from both the terrestrial, and marine side of the beach habitat, a term coined as "coastal squeeze" (Pontee, 2013). Coastal squeeze rapidly accelerates the loss of intertidal habitat for native organisms (Pontee, 2013).

Hairy shore crab (*Hemigrapsus oregonensis)* is an example of one such native species in the intertidal of Puget Sound. Hairy shore crab can be found in a variety of intertidal and salt marsh habitats including mudflats, sandy beaches, seagrass beds, and estuaries from Resurrection Bay, Alaska, as far south as Baja California, Mexico (Visser et al., 2004). *H. oregonensis* burrows in mud or hides underneath habitat features such as rocks to avoid predators and maintain abiotic equilibrium (Visser et al., 2004). They are known to be herbivores or scavengers that live in high densities (Visser et al., 2004). In the face of coastal squeeze, hairy shore crabs will experience shrinkage of their preferred intertidal habitat. Despite living in high density already, a lack of available space will force them into crowded conditions that may exceed their natural density.

Past studies involving similar species of crabs have shown increasing amounts of agonistic behavior, including the use of physical force and defensive displays, when faced with crowded conditions (Hazlet, 1968). One past study used heart rate monitors to assess the energy required during fights between shore crabs in heart rates of crabs and found that their physiology changed significantly, peaking during fights and remained elevated for some time afterwards (Rovero et al., 2000). Based on these findings, we predict that as *H. oregonensis* habitats shrink due to coastal squeeze, individuals will face increasingly crowded conditions that elevate metabolic demand via fighting and increased competition for resources; an energetically taxing state likely to reduce their overall fitness. To test our theory, we analyzed metabolic activity using the oxygen consumption rate and righting time of *H. oregonensis* specimens held for one week in treatments representing uncrowded and crowded conditions, as well as uncrowded conditions with added chemical cues derived from crowding.

Methods

For this experiment, 210 *H. oregonensis* specimens were collected by hand on April 27th, 2025, from mixed substrate habitats at Lion's Park boat launch and trestle (47° 35' 07" N 122° 38'42" W; -1.68-foot tide) and placed within a control tank prepared with 13°C saltwater with a salinity of 33 ppt within seven hours of collection.

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Trial one:

To test the effect of crowding on the metabolic activity of the crabs, two treatments were set up (Figure 1). One treatment, used to represent uncrowded conditions, contained 1000mL of saltwater at 18.5°C and a salinity of 34 ppt in a plastic rectangular enclosure. Another treatment used to represent crowded conditions was set up in a small jar containing 200mL of saltwater with the same parameters. Air stones were placed within the containers to provide oxygen. Eighteen crabs from the control tank were randomly chosen and placed into one of the treatment tanks from the control tank, totaling nine crabs per treatment.

After a period of seven days, three crabs were removed from each container and flipped onto their backs. The time it took for them to right themselves was recorded using a stopwatch and they were then returned to their respective containers. Resazurin was then utilized to assess oxygen consumption. 35 mL of prepared resazurin solution was pipetted into small cups. Three crabs from each of the two treatments and two crabs from the control tank were placed individually in a cup which was then closed with a lid. 20 µL samples were removed from each cup every 30 minutes for 90 minutes and placed into separate wells within a 96 well plate. A fluorescence plate reader was then used to measure the Relative Fluorescence Units (RFU) of each sample to assess the change in color of the resazurin solution, which was used as a proxy for oxygen consumption.

Trial two:

To test the effect of chemical cues and water chemistry changes because of crowded conditions two treatments were set up. First, 200 mL of used water from the crowded treatment in trial one was combined with 800 mL of seawater and mixed thoroughly. This water was used to create a 1000 mL treatment in a rectangular enclosure. Another treatment was set up with 200mL of freshly prepared saltwater in a small glass jar. Five crabs used in the previous trial were randomly selected for use in each of the two treatments. Air stones were then placed in each treatment, and they were placed in a shared water table. After seven days, resazurin assays were prepared using the same process as in trial one for three crabs from each treatment and three crabs from the control tank. Samples were once again taken from the resazurin solution in the same manner as in trial one, every 30 minutes for 90 minutes. Afterwards, righting trials were conducted however, due to a limitation in the number of specimens because of the loss of three crabs that escaped from the crowded treatment, in this trial one individual from each treatment that had not been used in the resazurin assay were righted three times each, and an average of the times recorded was used for analyses.

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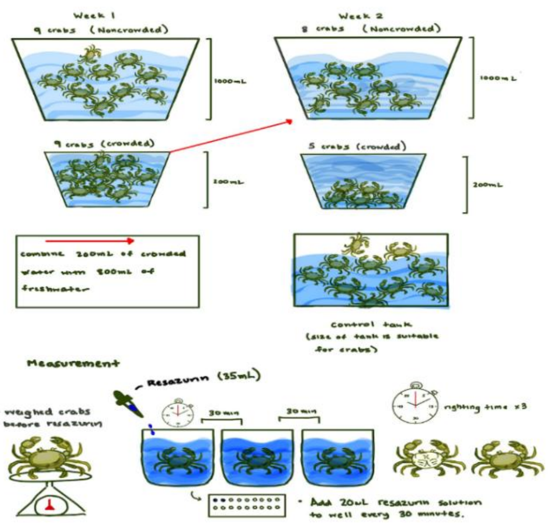

Figure 1. Graphical representation of the two trials conducted over a two-week period. Week one (left) included a noncrowded and crowded trial with nine specimens in each. Week two (right) included a noncrowded trial prepared with diluted water from the crowded treatment in trial one, as well as a crowded trial prepared with fresh seawater. Eight specimens were randomly placed in each treatment. During trial two, three specimens escaped from the crowded treatment, leaving only five specimens in the treatment by the end of the trial. In both trials, the righting time and the rate of oxygen consumption, measured via resazurin assay, were used to assess changes in metabolic activity and stress (bottom). Diagram created by Seila Lai.

Results

Trial one:

After the period of seven days, visually noticeable changes in the water of both treatments were observed. The water in both treatments was dirtied, with the crowded treatment's water being notably greener and cloudier. Differences in the condition of crabs were also observed between the two treatments. In the crowded treatment, there was a loss of one limb and death of two specimens, whereas neither incident was observed in the uncrowded treatment. To measure the rate of oxygen consumption over time, the RFU of each sample throughout the 90-minute period was normalized by weight for each crab and the median was then calculated. The change in oxygen use over time was estimated using a linear regression trendline equation (Figure 2, left). It was found that the rate of oxygen consumption over time (slope of the regression line) differed significantly from the control for both the uncrowded and crowded treatment (Figure 2, left). Among the three treatments, the uncrowded treatment experienced the greatest rate of oxygen consumption during the 90-minute test period with a slope of 6.4159 (Figure 2, left). The crowded treatment had the next highest slope value of 4.8797 compared to the control treatment, which had a slope value of 2.0979 (Figure 2, left). While statistical comparisons were not performed due to low sample size, differences in regression slopes suggest treatment effects. Average righting time did not vary significantly between treatments and control (Figure 3, left).

Trial two:

After the seven-day period in trial two, there were once again visually noticeable changes in water quality of both treatments, with the water in the crowded treatment being remarkably greener and dirtier. Notably, during this trial, three individuals escaped from the crowded treatment, which reduced the final sample size to five specimens. Additionally, in the crowded treatment the crabs experienced one limb loss and one death. The results of the resazurin assay in this trial showed that over the 90-minute-long test time, the individuals from the crowded treatment consumed the most oxygen with a regression slope value of 4.8603 (Figure 2, right). The uncrowded treatment with diluted crowded water had the second highest slope value of 2.8145 compared to the control treatment, which had a slope value of 0.917

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(Figure 2, right). Average righting times did not vary significantly between individuals from either treatments or control (Figure 3, right).

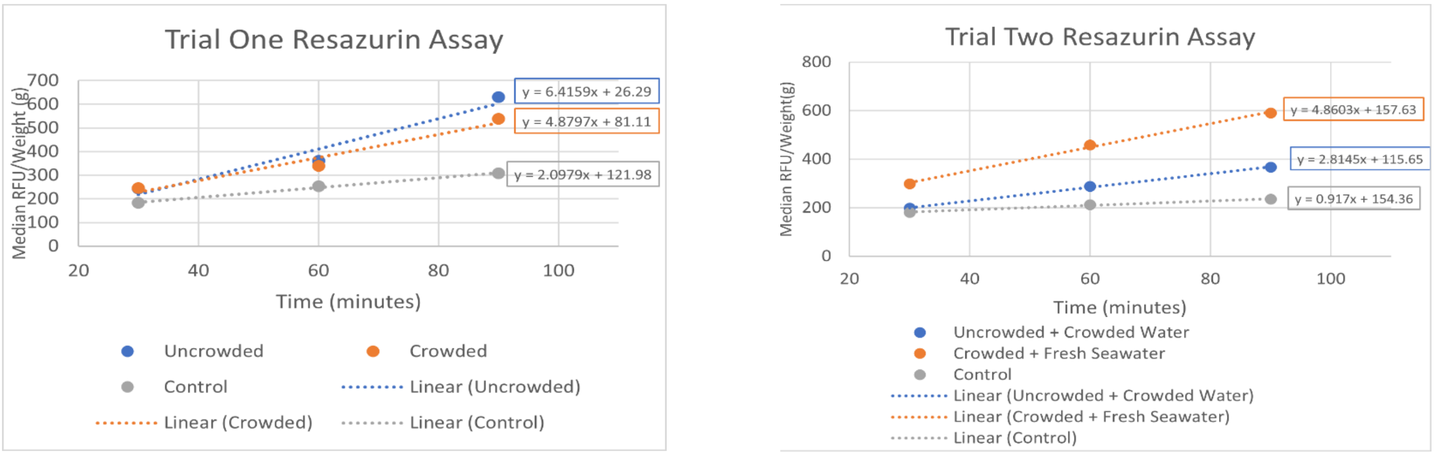

Figure 2. Resazurin assays were used to observe oxygen consumption every 30 minutes over a 90-minute period. Median RFU, normalized by individual weight, is shown for uncrowded (n=3, blue), crowded (n=3, orange), as well as control treatments (n=2 for trial one, n=3 for trial two, grey). Dashed lines represent linear fit for each treatment across sampled time points. Equations indicate slope value and y-intercept of each corresponding regression line.

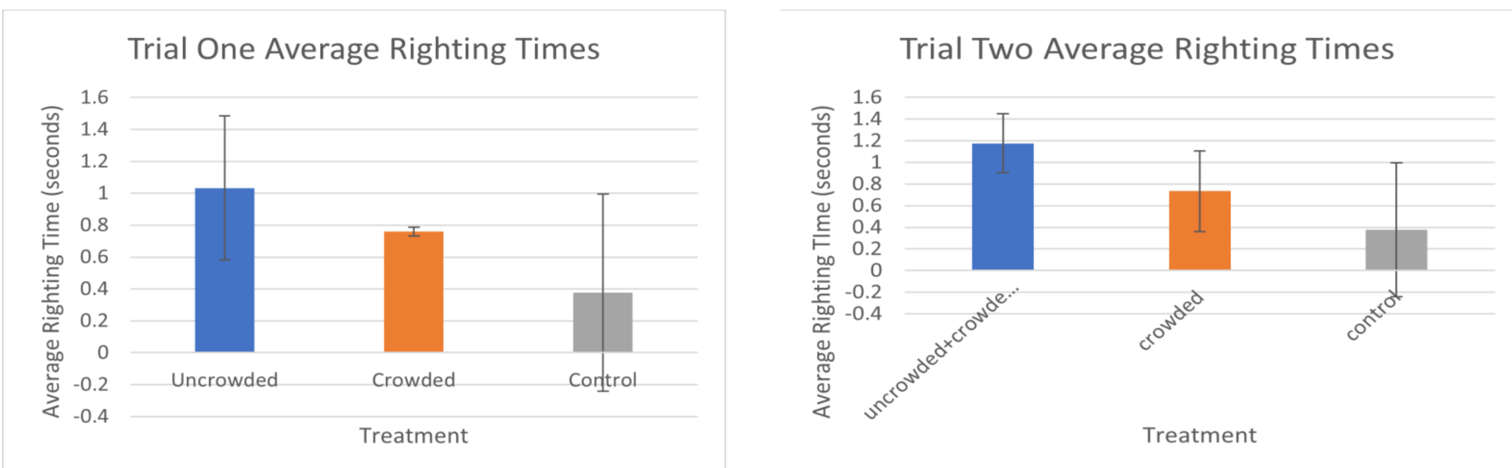

Figure 3. Righting time (in seconds) was measured for individuals across treatments. **Left:** Mean righting time is shown for three treatments in trial one: uncrowded (n=3, blue), crowded (n=2, orange), and control (n=5, grey). One individual was established as an outlier and removed from the crowded treatment data. **Right:** Mean righting time of three replicate measurements for a single individual (n=1) is shown for both uncrowded with crowded water (blue) and crowded (orange) treatments. The mean of five individuals tested once is shown for control treatment (grey). In both graphs, bars indicate standard deviation.

Discussion

In this study, we attempted to answer how physical crowding and chemical cues from crowding affect the metabolic activity of H. oregonensis. Our initial prediction was that crowded conditions and chemical cues from crowding will elevate metabolic demand, by increasing oxygen consumption and righting time. In trial one, we instead observed higher oxygen consumption in crabs from the uncrowded treatment than the crowded treatment. We also saw no significant difference in righting time between the treatments. These unexpected results, along with noticeable water degradation, limb losses, and mortality, may reflect deteriorated body condition in crowded crabs. Therefore, when measuring oxygen consumption, we may have seen lower rates of respiration due to energy consumption or metabolic suppression.

In the second trial, we saw that crabs exposed to crowding conditioned water consumed less oxygen than physically crowded crabs. Indicating that chemical cues did not have the same effect as physical crowding. However, both treatments experienced greater oxygen consumption rates than the control, indicating some amount of stress response. We also observed no significant difference in righting time in this trial. This lack of results may indicate that righting time is not a good indicator for stress in *H. oregonensis.* Additionally, escapees in this trial created limitations with how many crabs we had to choose from for righting, which resulted in the same crab being used multiple times and thus experiencing a greater amount of handling. In future studies, additional biomarkers such as lactate or cortisol may provide stronger results as well as greater sample sizes and longer holding times.

Although we did not get strong results regarding the metabolic rates of the crabs in our experiment, we did observe behavioral changes that aligned with the effect of crowding in past studies. For example, we observed the crabs in both treatments fighting, but at a greater frequency in the crowded treatment, supported by the fact that both crowded treatments across the trials experienced limb loss (Rovero et al., 2000). These findings suggest that as shoreline habitat continues to shrink because of coastal squeeze, intertidal species such as H. oregonensis will experience increases in density that may result in crowded conditions. As a result, it is likely that they will display increased fighting behavior amongst conspecifics, or even other intertidal species which may result in both physical injury and elevated metabolic costs. Consequently, lowering the fitness of the crabs.

References

Boonzaier, K. (2018). *Beaches rebound in the sound*. Washington Association of Land Trusts. https://walandtrusts.org/news/beaches-rebound-sound/

Hazlett, B. A. (1968). Effects of crowding on the agonistic behavior of the hermit crab *Pagurus bernhardus*. *Ecology*, *49*(3), 573–575. https://doi.org/10.2307/1934131

Lindsey, R. (2023, August). *Climate change: Global sea level*. NOAA Climate.gov. https://www.climate.gov/news-features/understanding-climate/climate-change-global-sea-level

Pontee, N. (2013). Defining coastal squeeze: A discussion. *Ocean & Coastal Management*, *84,* 204–207. https://doi.org/10.1016/j.ocecoaman.2013.07.010

Rovero, F., Hughes, R. N., Whiteley, N. M., & Chelazzi, G. (2000). Estimating the energetic cost of fighting in shore crabs by noninvasive monitoring of heartbeat rate. *Animal Behaviour*, *59*(4), 705–713. https://doi.org/10.1006/anbe.1999.1353

Visser, E. P., McDonald, P. S., & Armstrong, D. A. (2004). The impact of yellow shore crabs, *Hemigrapsus oregonensis*, on early benthic phase Dungeness crabs, *Cancer magister*, in intertidal oyster shell mitigation habitat. *Estuaries*, *27*(4), 699–715. https://doi.org/10.1007/bf02907654