INTRODUCTION

Hemigrapsus oregonensis, commonly known as the yellow shore crab, is found in the west coast of North America, and typically resides in Puget Sound, Washington. Researching how population density affects marine organisms like *H. oregonensis* is important for understanding species-specific consequences of anthropogenic modifications to coastal environments. One consequence that has recently gained attention is *coastal squeeze*, which is a chronic process where rising sea levels, combined with other factors, push coastal habitats toward land resulting in a retreat of the shoreline and habitat loss (1) (2). Factors contributing to coastal squeeze include land subsidence, sediment deficit, increased storminess and the occupation of space by infrastructure (1) (2). These pressures are often further exacerbated by the combined effects of tourism, climate change and urbanization (2). A reduction in habitat availability in the intertidal combined with a constant abundance of organisms will inevitably lead to higher organism densities which can alter biotic interactions, such as predation and competition (3). Although *H. oregonensis* thrives in high-density environments with its own kind (472-624 crabs m^2) (4), alterations to organisms' abundance impacts their viability, persistence and risk of extinction (3). Therefore, it is valuable to study the potential consequences of increased population density on metabolic rate, injury and mortality in intertidal marine invertebrates affected by coastal squeeze.

In aquatic systems, organisms may respond to chemical cues, which are compounds that convey information about stress, predation risk or the presence of conspecific organisms and are released through excretion, injury or digestion (5) (6). In crustaceans, the presence of chemical cues has been found to lead to physical and behavioral changes, such as reduced movement, aggregations, courtship and even aggressive behaviour (5) (6). Besides this, seawater conditions and characteristics influence organisms. Therefore, it is important to be able to distinguish between the effects of physical crowding and chemical signals when evaluating stress response of *H. oregonensis*.

Here, stress response in *H. oregonensis* was assessed using mortality, injury (limb loss), righting time and metabolic rate during 90 minutes (used as a proxy for recovery from prolonged stress). Metabolic rate is a well established indicator of physiological stress and energy consumption in organisms, therefore it is valuable in assessing long-term stress response. Righting behavior is a reflex that requires muscle coordination and neurological control, so a decline in righting ability can signal a decline in well-being and health (1). Three experimental conditions were tested: crowded (high density), uncrowded (low density), and uncrowded with water from the crowded condition. This two-stage experimental design replicates that of a previous study (7) on shrimp, allowing differentiation between effects of mechanical/physical crowding and chemical cues from the water.

METHODOLOGY

H. oregonensis were collected from Lion's Park boat launch between 11:30 and 13:34 on April 27th, 2025. At the time of collection, the water temperature was 15℃, salinity was 30 ppt, and the tide was -1.68 m. Eighteen crabs were randomly selected for experimental trials, not including the control crabs. During the three week experiment, four experimental conditions were tested: control, crowded, uncrowded, and uncrowded with crowded water.

Week 1: All trials used seawater with a temperature of 18.5 ℃, and using a refractometer, salinity was measured to be 34 ppt. For the crowded condition, 200mL of this water was added into a small container measuring 2.5x2.5 in. Nine randomly selected *H. oregonensis* crabs from the control tank were placed inside this container and left for seven days. The conditions were spatially constrained, and the crabs often piled on top of each other. A bubbler was used to maintain oxygen levels. The uncrowded tank had dimensions of 3x6.5 in and used 1000mL of the same seawater. Nine randomly selected individuals from the control tank were placed inside the container and left for seven days. In this container, the crabs were not spatially constrained and could readily separate from each other. However, neither the crowded nor uncrowded conditions included natural substrate or hiding places. Both containers were submerged in a temperature controlled freshwater tank, to maintain thermal conditions across all treatments. The control crabs were kept in a separate, much larger tank with substrate and hiding spots, mimicking elements of their natural environment. This tank was held at a temperature of 13 ℃ and a salinity of 33 ppt.

Week 2: Following week 1, salinity in each container was measured. Then, three crabs from each condition were randomly selected to test their righting times, which can aid in measuring their stress response. The time it took each crab to reorient themselves was recorded, and the six individuals were returned to their respective containers. Next, another three crabs from each condition were weighed and individually placed into small beakers to measure their metabolic rate as a proxy for recovery from the prolonged one week stress event. This was done by adding 35mL of resazurin to each beaker. At 30, 60 and 90 minutes 20 μL resazurin was pipetted from the beaker with the crab into a 96-well plate, taking note of where each sample was pipetted. The well plate was run through a fluorescence reader. After 90 minutes, the crabs were washed and returned to their containers.

New experimental conditions were prepared. To test the influence of chemical cues, 200mL of water from the previous crowded conditions was mixed with 800mL of fresh seawater (34 ppt, 18.5 ℃) and placed in the uncrowded container that now had 8 crabs (two mortalities occurred in the crowded treatment). The crowded conditions were re-established with 8 individuals in the same manner as in Week 1.

Week 3: After another seven day period, salinity, righting time and recovery rate were measured as before. Due to high mortality and escape in the crowded conditions, righting time was only measured on one individual, but repeated three times. To avoid handling stress, crabs used for righting time were not used in metabolic rate tests. The same procedure from Week 2 was followed to assess recovery rate using resazurin fluorescence. Data analysis was performed in excel and graphs were made in RStudio. To quantify metabolic activity, RFU values at 30, 60 and 90 minutes were plotted against time. Metabolic rates have been found to be proportional to body weight (8). Therefore, a linear regression was used and the resulting slope was used to represent the rate of fluorescence increase per gram of crab for each condition.

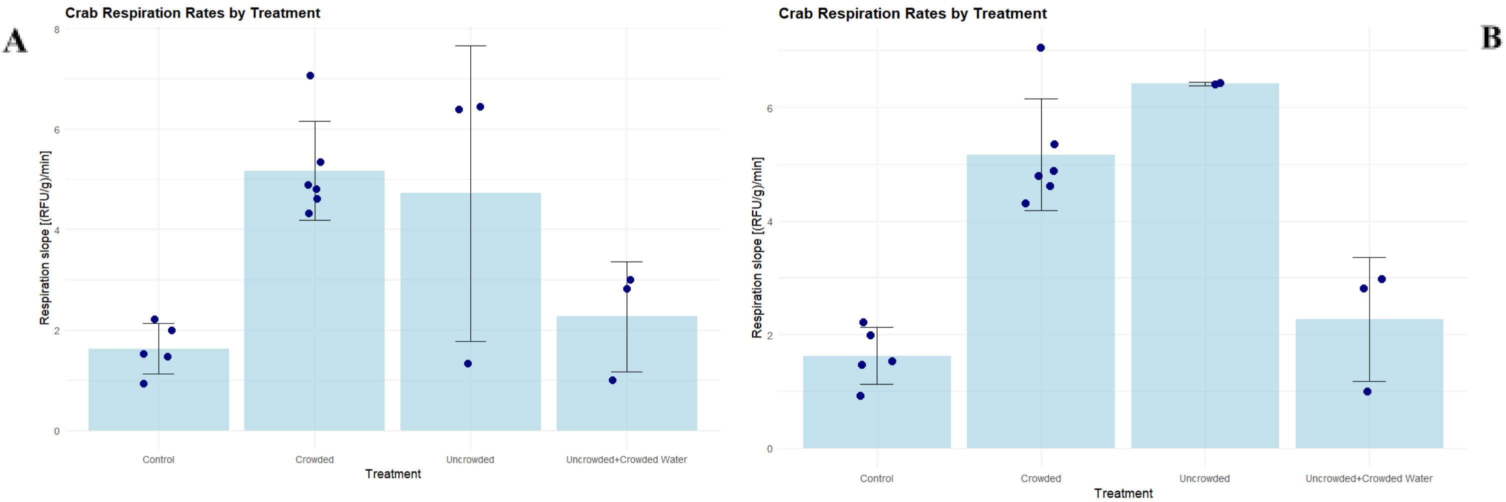
RESULTS

All four experimental conditions showed an increase in RFU over 90 minutes, indicating ongoing metabolic activity. Each condition had different average slopes, however, it is important to note that the number of individuals tested varied across treatments: crowded (n=6), control (n=5), and both uncrowded and uncrowded + crowded water conditions (n=3). A steeper positive slope represents higher metabolic activity and greater oxygen consumption. This may also suggest a longer recovery time following prolonged stress, as metabolic activity remains elevated post-stress.

As shown in Figure 1A, the control condition had the smallest slope (1.63), followed by uncrowded + crowded water (2.27). The uncrowded condition showed a steeper slope (4.72), and the crowded condition showed the overall steepest slope (5.17). These results suggest that the crowded condition had the longest recovery period following stress.

However, the standard deviation of the uncrowded condition was extremely high, which raises concerns considering the small sample size (n=3). Therefore, a Dixon's Q test was run to determine if the lower data point (1.32) in the uncrowded condition could be excluded as an outlier. Using the formula: $Q = \frac{gap}{range}$, with n=3 at $\alpha = 0.05$, the critical value $Q_{critical} = 0.94$. A calculated Q value that is greater than the $Q_{critical}$ indicates that the point can be excluded as an outlier. The test results gave $Q = 0.99$, therefore this point can be excluded. The same test was run on the lowest value of the uncrowded + crowded water condition, but it did not pass the test for exclusion.

Figure 1B shows the results excluding the outlier (1.32) from the uncrowded conditions. A dramatic change can be seen, with the uncrowded conditions now having the steepest slope. Without more replicates for the uncrowded treatment, it is difficult to determine definitive conclusions, and results from other stress metrics should be taken into consideration along with this data.



Treatment	Mean Slope	Median Slope	Standard Deviation
Control	1.626967	1.544167	0.5013525
Crowded	5.166333	4.838333	0.9840502
Uncrowded	4.716278	6.393833	2.9439437
Uncrowded+Crowded Water	2.267444	2.814500	1.0938391

Figure 1. A) Crab respiration rates [(RFU/g)/min] for each experimental condition (control, crowded, uncrowded and uncrowded + crowded water) are plotted individually (blue points). Bar graphs represent the mean values for each condition, and error bars show standard deviation. B) Crab respiration rates plotted as in A), but excluding one data point in the uncrowded condition based on the Dixon's Q test results. C) Statistical summary (mean, median and standard deviation) for each treatment condition.

Righting time was used as a proxy for stress response in crabs. Figure 2A shows that the crowded treatment had the highest average righting time, however, it also had the greatest variability. This is because one individual failed to right itself during the 5 second observation period, resulting in a maximum value. In contrast, all the other individuals in the crowded, uncrowded and uncrowded + crowded water had similar righting times. This suggests that most of the crabs in the experimental conditions exhibited a similar stress response, while the control group showed lower righting times, indicating lower stress.

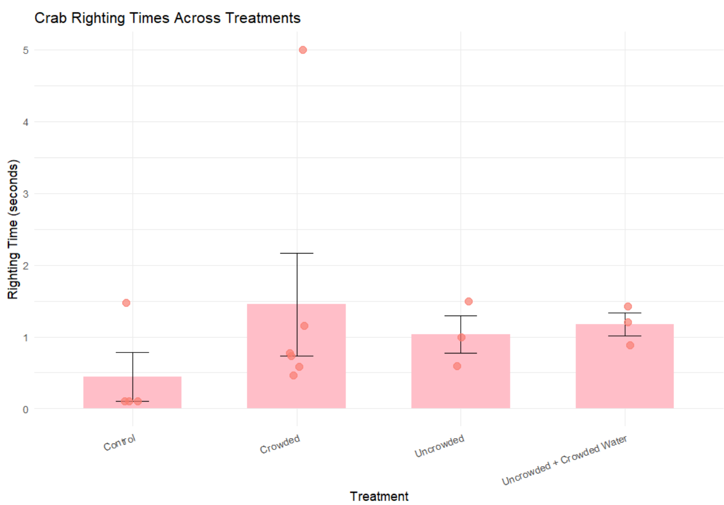


Figure 2B shows that mortalities and limb loss only occurred in the crowded condition with one instance of each reported per week throughout the experiment.

Treatment	Mortality	Limb Loss	Escape
Crowded	4	2	3
Uncrowded	0	0	0
Uncrowded + Crowded Water	0	0	0

Figure 2. A) Crab righting time across treatment conditions. Bars represent the average righting time and error bars indicate the standard deviation. Points represent individual crab righting times. B) Number of mortalities and limb loss events recorded per week for each condition.

Notably, there was a change in salinity in the crowded treatment. As shown in Figure 3, the salinity in the crowded treatments increased, reaching 41 ppt in week 1 and 40 ppt in week 2. The uncrowded + crowded water condition showed a stable salinity of 36 ppt and the uncrowded treatment remained stable at 34 ppt. In addition to salinity changes, observations of the conditions inside the containers were recorded. The seawater from the crowded treatment changed in color, odor and contained visible debris and fecal matter in it. Meanwhile, the seawater in the uncrowded treatment had a slightly cloudy and green color, but much less than the crowded treatment.

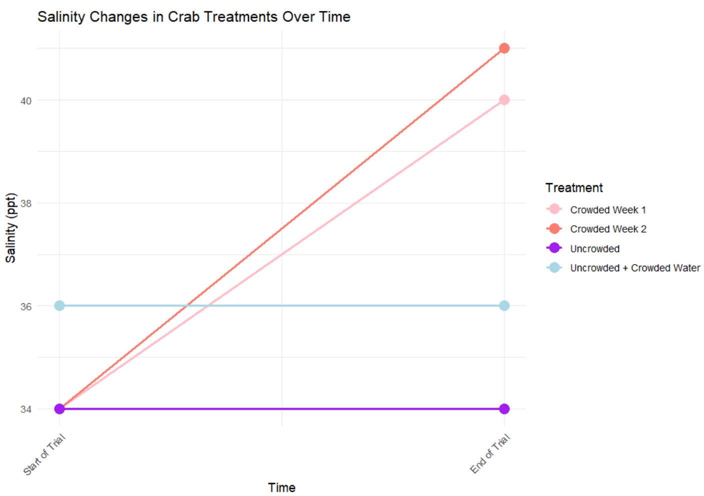


Figure 3. Salinity changes over time in each crab treatment condition. Lines represent the four different treatments.

DISCUSSION

This study aimed to investigate how crowding of *H. oregonensis* influences stress and recovery, using metabolic rate, injury occurrence, mortality and righting time as indicators. A two stage experimental design allowed to separate the effects of physical crowding from chemical cues in the water.

Metabolic rate during a 90 minute period following prolonged stress in crowded conditions was used as a proxy for recovery. Without the exclusion of the outlier in the uncrowded group, Fig. 1A shows that the crowded group had the highest slope in respiration rate, which could indicate that it was the most affected. However, in Fig 1B, with the outlier excluded, the uncrowded group appears to have the highest slope. Due to the small sample size, definitive conclusions cannot be drawn, however, in both figures it is clear that both conditions had higher respiration slopes compared to the control group. Studies on stocking density, habitat enrichment, survival and vitality in crabs have shown that excessive handling can lead to a decline in condition due to cumulative stress and deterioration in health, which can often have a greater impact than inappropriate stocking density (1). The elevated metabolic rate of both the crowded and uncrowded conditions compared to the control show trends similar to those findings. However, the uncrowded + crowded water condition showed a much lower respiration rate, more similar to the control than to the uncrowded and crowded groups. This may suggest that chemical cues from the crowded seawater could have a suppressive effect on metabolic rate. Previous studies have also shown that chemical cues from stress or injury can induce behavioral changes, such as reduced movement (5). This could explain the lower metabolic rate in the uncrowded + crowded water treatment and could be the objective of further studies.

Righting time across the experimental conditions was similar, except the control, which had a lower average righting time. Righting time has been found to be a good indicator of vitality (1), and in this study it is also being used to assess stress response. Therefore, the control group seems to have the least stress compared to the other conditions. Because the standard deviation is high in the other conditions, it is hard to draw definitive conclusions. Other studies have shown that righting time can vary vastly between individuals of the same species (5) (6), suggesting that standardization across sex, size, age and substrate may be necessary to analyze the results further. Therefore, future studies could aim to standardize these variables and increase the sample size to better distinguish the effects on righting time. Additionally, the other metrics of stress (limb loss and mortality) indicated that the crowded group was the most negatively affected by the physical conditions of crowding, since no other treatment experienced injury or death.

Salinity showed a notable difference between treatments in both weeks, with the crowded treatment experiencing an increase, reaching 41 ppt in week 1 and 40 ppt in week 2. After consideration, it can be hypothesized that this increase may be due to the smaller volume of seawater used in the crowded conditions. During both weeks, the crowded treatments only had 200mL of water, whereas the uncrowded and uncrowded + crowded water treatments each had 1000mL. This smaller volume would make the crowded treatment more susceptible to water loss through evaporation, which leaves salts behind and increases the salinity of the remaining water.

Ammonia was also considered to be a potential contributor to the increased stress in all the treatments, but especially the crowded conditions, since it is excreted into the environment (6) and at high levels it can be toxic to organisms (9). However, it has been found that higher salinities mitigate the toxic effects of ammonia (10). Therefore, the increased salinity in the crowded treatments may have reduced some of the toxic effects of ammonia, potentially helping to explain why righting times and metabolic rates in the crowded and uncrowded + crowded water conditions were similar to the uncrowded conditions.

Despite this possible mitigation, the physical consequences of crowding are still evident, as limb loss and mortality occurred only in the crowded conditions. This suggests that while chemical stressors of crowding may have been somewhat reduced, the direct effects of physical crowding still had an effect on the stress response of crabs. However, the low number of replicates and high mortality rates raise important questions about the cost-benefit balance of the study, especially given that decapod crustaceans are now recognized as sentient beings (11), which introduces ethical considerations.

Overall, these findings suggest that chemical cues and seawater conditions, such as ammonia and salinity, may influence aspects of *H. oregonensis* stress response and recovery, including righting times and metabolic rate. However, the physical effects of crowding are a source of harm, causing limb loss. These results should be taken into consideration when studying coastal squeeze and the reduction of habitat availability for intertidal species.

REFERENCES

1. Pontee, N. (2013). Defining coastal squeeze: A discussion. *Ocean & Coastal Management*, *84*, 204–207. https://doi.org/10.1016/j.ocecoaman.2013.07.010
2. Lithgow, D., Martínez, M. L., Gallego-Fernández, J. B., Silva, R., & Ramírez-Vargas, D. L. (2019). Exploring the co-occurrence between coastal squeeze and coastal tourism in a changing climate and its consequences. *Tourism Management*, *74*, 43–54. https://doi.org/10.1016/j.tourman.2019.02.005
3. Jackson, A. C., & McIlvenny, J. (2011). Coastal squeeze on rocky shores in northern Scotland and some possible ecological impacts. *Journal of Experimental Marine Biology and Ecology*, *400*(1–2), 314–321. https://doi.org/10.1016/j.jembe.2011.02.012
4. Jensen, G., McDonald, P., & Armstrong, D. (2002a). East meets west: Competitive interactions between green crab carcinus maenas, and native and introduced shore crab hemigrapsus spp.. *Marine Ecology Progress Series*, *225*, 251–262. https://doi.org/10.3354/meps225251
5. Hazlett, B. A. (2010). Chemical cues and reducing the risk of predation. *Chemical Communication in Crustaceans*, 355–370. https://doi.org/10.1007/978-0-387-77101-4_18
6. Kamio, M., Yambe, H., & Fusetani, N. (2021). Chemical cues for intraspecific chemical communication and interspecific interactions in Aquatic Environments: Applications for Fisheries and Aquaculture. *Fisheries Science*, *88*(2), 203–239. https://doi.org/10.1007/s12562-021-01563-0
7. Nga, B. T., Lürling, M., Peeters, E. T. H. M., Roijackers, R., Scheffer, M., & Nghia, T. T. (2005a). Chemical and physical effects of crowding on growth and survival of penaeus Monodon Fabricius post-larvae. *Aquaculture*, *246*(1–4), 455–465. https://doi.org/10.1016/j.aquaculture.2005.02.026
8. Leffler, C. W. (1973). Metabolic rate in relation to body size and environmental oxygen concentration in two species of xanthid crabs. *Comparative Biochemistry and Physiology Part A: Physiology*, *44*(4), 1047–1052. https://doi.org/10.1016/0300-9629(73)90241-7
9. Lorenzon, S., Giulianini, P. G., Libralato, S., Martinis, M., & Ferrero, E. A. (2008). Stress effect of two different transport systems on the physiological profiles of the crab cancer pagurus. *Aquaculture*, *278*(1–4), 156–163. https://doi.org/10.1016/j.aquaculture.2008.03.011
10. Motamedi-Tehrani, J., Peyghan, R., Shahriari, A., Razijalali, M., & Ebrahimi, E. (2025). The influence of ammonia-N and salinity levels on oxidative stress markers, hepatic enzymes, and acid phosphatase activity in Nile tilapia (Oreochromis niloticus). *Scientific Reports*, *15*(1). https://doi.org/10.1038/s41598-024-84136-2
11. Wilson, C. H., Wyeth, R. C., Spicer, J. I., & McGaw, I. J. (2022a). Effect of animal stocking density and habitat enrichment on survival and vitality of wild green shore crabs, Carcinus maenas, maintained in the Laboratory. *Animals*, *12*(21), 2970. https://doi.org/10.3390/ani12212970