Too Close for Comfort: The Effects of Crowding on the Behavioral and Physiological Stress Responses of *Hemigrapsus Oregonensis*

Introduction

Across the Pacific Northwest, rising sea levels and aggressive shoreline modification are compressing intertidal zones and reducing habitat availability for native species (Jackson and Mcllveny, 2011). In Seattle, over 92% of the coastline has been altered with hard armoring such as riprap, seawalls, and bulkheads (Boonzaier, 2018). These structures block the landward migration of intertidal habitats in response to sea-level rise, leading to a phenomenon known as *coastal squeeze* (Pontee, 2013; Long et al., 2011). As habitat area declines, population densities may rise, intensifying competition and crowding (Jackson and Mcllveny, 2011; Pombo et al., 2023). This effect is particularly problematic for species that rely on burrowing, as shoreline development and erosion can degrade sediment quality and reduce structural complexity, both critical for burrow construction (Visser et al., 2004; Jackson and Mcllveny, 2011).

The hairy shore crab (*Hemigrapsus oregonensis*) is a native intertidal crab commonly found across estuaries and mudflats along the Pacific coast. Like many intertidal decapod crustaceans, *H. oregonensis* depends on access to soft sediments to construct and retreat into burrows, which serve as shelter from predators, dessication, and competition (Visser et al., 2004). These crabs typically remain close to their burrows and avoid encounters with conspecifics while foraging. However, when placed in confined conditions, males exhibit frequent intraspecific conflict, suggesting sensitivity to proximity and density. Given their burrowing behavior, site fidelity, and widespread distribution, *H. oregonensis* is a well-suited model for studying stress responses to crowding in coastal environments affected by habitat loss.

While behavioral responses to crowding in crustaceans are well documented, physiological consequences remain poorly understood. Some studies show that chemical cues from stressed conspecifics can alter aggression (Huntingford et al., 1995) or reduce survival and growth in crustaceans (Nga et al., 2004). Yet few studies have tested whether either physical crowding or exposure to crowd-conditioned water causes measurable physiological stress. One likely mechanism is that crowding increases physical aggression and conspecific intrusions into established territories, both of which have been shown to elevate oxygen consumption in crustaceans (Smith & Taylor, 1993; Rovero et al., 2000; Hazlett et al., 1968). Because respiration reflects internal metabolic strain, it serves as a useful proxy for stress. Righting time, the time it takes for a crab to flip itself over after being displaced, is another well-established indicator of vitality and physiological condition. Righting time is a reflex that requires muscle coordination and neurological control, so a decline in righting ability can signal a decline in overall health and well-being (Wilson et al., 2022; Maraschi et al., 2024).

This study investigates whether *H. oregonensis* exhibits behavioral and physiological stress responses to physical crowding and whether exposure to chemical cues from crowded environments contributes to those responses. To distinguish the effects of physical crowding from chemical cues, crabs were exposed to four conditions: crowded, uncrowded with crowd-conditioned water, uncrowded, and a control. Including the crowd-conditioned water treatment allowed us to assess whether *H. oregonensis* stress responses were driven by direct contact or by chemical signals alone. Oxygen consumption was measured using a resazurin-based assay, and righting time was measured as a behavioral indicator of stress and vitality. These findings will help clarify how increasing habitat compression along armored shorelines may influence the resilience of intertidal crabs facing limited space and altered social conditions.

Methods

A two-phase experiment was conducted to evaluate whether *H. oregonensis* exhibits behavioral and physiological stress responses to physical crowding and potential chemical cues from crowded environments. Oxygen consumption was measured as a physiological indicator of metabolic activity, and righting time was used as a behavioral proxy for vitality and neurological condition. A total of 210 *H. oregonensis* were collected from Lion's Park boat launch (47°35'07"N, 122°38'42"W) on April 27, 2025, during a -1.68 ft tide from mixed substrate (sand, shell, pebble) between 11:30 and 13:34. Ambient water temperature and salinity at the time of collection were 15°C and 30 ppt. Crabs were transferred to a control tank (13°C, 33 ppt) within 7 hours of collection.

Phase 1:

Eighteen crabs were randomly selected from the control tank and assigned to two density treatments. Nine crabs were placed in a crowded container (200 mL water volume, ~40 cm^2) and nine in an uncrowded container (1000 mL water volume, ~195 cm^2). Both containers were aerated and initially filled with seawater at 34 ppt at 13°C, then held in a shared water table from April 28 to May 5 to maintain a consistent temperature. Salinity was measured at the beginning of the treatment and again on May 5 to track any changes throughout the treatment period.

Tank dimensions were informed by reported field densities of *H. oregonensis* in Puget Sound. According to Jensen et al. (2002), average natural densities reach approximately 427 crabs/m^2. This value was scaled to accommodate nine crabs per treatment. The uncrowded condition was housed in a container with a 3-inch by 6.5-inch base (~195 cm^2), closely matching the scaled natural density of these crabs. The crowded condition was housed in a smaller container with a 2.5-inch by 2.5-inch base (~40 cm^2), simulating a high-density environment.

Three crabs per treatment were randomly selected for righting time trials, with additional control data (5 individuals) used to supplement comparisons. Each crab, plus three crabs from the control tank, was gently dried, weighed (dry mass), and placed in 35 mL of resazurin solution for 90 minutes. Samples of 20 µL were collected at 30, 60, and 90 minute intervals and pipetted into a 96-well plate for fluorescence analysis. The plate was read using a fluorescence plate reader, and average RFU values (relative fluorescence units) were calculated for each well. Crabs were rinsed and returned to their original treatment containers following testing.

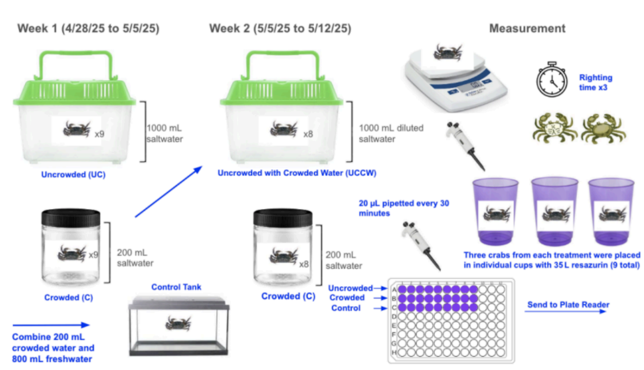

Figure 1: Methodological design.

<u>Phase 2:</u>

On May 5th, to assess the effect of waterborne chemical cues, the same containers from phase 1 were reused. Eight surviving crabs from the uncrowded treatment were transferred to their cleaned original container, now filled with 1000 mL of a mixture of 800 mL clean seawater (33 ppt) and 200 mL of reused water from the previous crowded treatment. Eight surviving crabs from the original crowded treatment were returned to their original cleaned container, with 200 mL of fresh seawater (13°C, 33 ppt). Both treatments were aerated. They were both held in the shared water table for an additional week. Salinity was rechecked on May 12 at the end of the treatment period.

Due to two mortalities and three escapes from the crowded condition, a single crab from each treatment was reused for three separate righting trials before being set aside from further resazurin testing to minimize repeated handling stress. To complete the final sampling, three new crabs per treatment, plus three crabs from the control tank, were randomly selected, weighed (dry mass), and tested in resazurin solution to assess oxygen consumption. The same resazurin assay was completed for these crabs.

Raw RFU data at 30, 60, and 90 minutes were divided by individual mass and averaged across individuals per treatment and time point. These values are shown as points in Figure 1 (created in Excel), where linear regressions were fit to treatment-level means. Slopes represent estimated oxygen consumption rates. Using the same raw data, Figure 2 was generated in R (Version 2024.09.0+375) with ggplot2; individual respiration slopes were calculated and plotted with treatment means ±SD. Figure 3 was created in Excel using raw righting time data per crab. Tables 1 and 2 were generated from raw data, using the readr, knitr, and kableExtra packages in RStudio.

Results

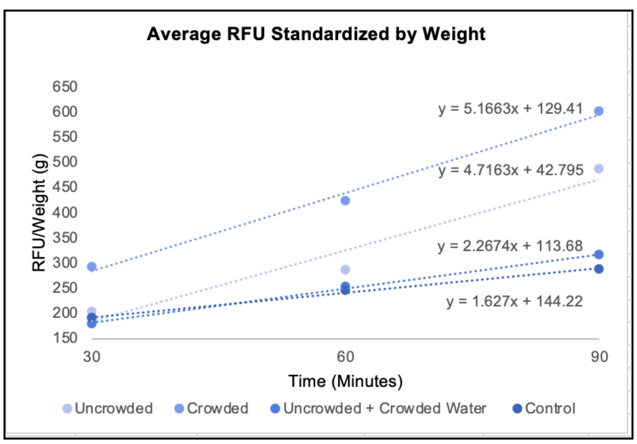

Figure 1: Average Relative Fluorescence Units (RFU), standardized by individual crab mass (g), measured at 30, 60, and 90 minutes across four treatment groups: Uncrowded (UC), Crowded (C), Uncrowded + Crowded Water (UC + CW), and Control. For each time point, RFU values from three individual crabs from each treatment were divided by their masses (g), and then those three values were averaged to generate a plotted point. Each point represents the mean mass-standardized RFU value from three individuals per treatment per time point. Linear regressions were fit to treatment-level mean values to estimate oxygen consumption rates over time. Slope equations are shown. Steeper slopes indicate higher oxygen consumption rates and greater metabolic activity.

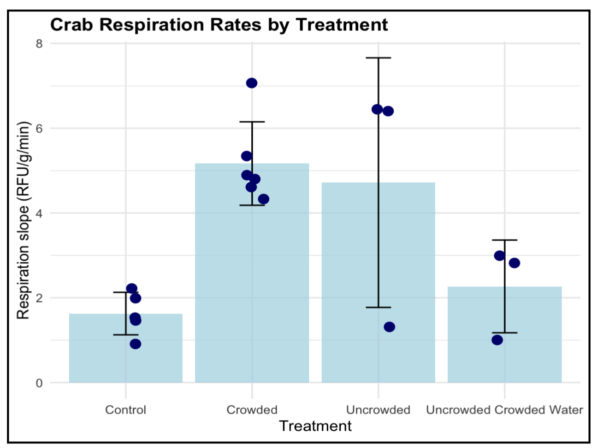

Figure 2: Averaged respiration slopes (RFU/g/min) for *H. oregonensis* across four treatments, based on resazurin fluorescence units measured every 30 minutes over 90 minutes. Error bars indicate standard deviations and individual points represent the respiration slopes of individual crabs.

Each treatment displayed different RFU trajectories over time, reflected in the differing slopes of the lines of best fit in the 90-minute resazurin assay (Fig. 1). Steeper slopes indicate greater oxygen consumption and, therefore, greater overall metabolic activity. Because slopes are calculated as RFU per gram per minute, the slopes for each line of best fit indicate average respiration rates for each treatment. The crowded group showed the highest average respiration rate (5.17 RFU/g/min), followed by uncrowded (4.72), uncrowded + crowded water (2.27), and control (1.63). At 30 minutes, average RFU values for the control, uncrowded, and UC + CW groups were similar and much lower than those in the crowded group. By 60 minutes, the RFU in the uncrowded treatment rose above both UC + CW and control, though UC + CW and control followed nearly identical trajectories throughout the assay. Figure 2 displays average respiration rates for each treatment, with overlaid points representing individual crab respiration rates. The crowded group had the highest average respiration rate, followed by uncrowded, UC + CW, and control. However, overlapping error bars between crowded, uncrowded, and UC + CW treatments suggest that differences may not be statistically significant. The uncrowded group also exhibited the greatest variability between respiration rates of individual crabs, indicated by the wide error bar. Despite this, the overall trend of increased respiration under physical crowding is consistent across both figures (Fig. 1 & Fig. 2). Righting time was also measured as a behavioral indicator of physiological stress across all treatments (Fig. 3). Median righting times were similar among the crowded (0.76 s), uncrowded (1.00 s), and uncrowded + crowded water (1.21 s) groups. However, the control treatment had a much quicker median righting time of 0.10 seconds. Mean righting times followed a similar trend: control (0.38 s) was markedly faster than the others, while uncrowded (1.03 s) and UC + CW (1.18 s) were nearly identical. The crowded treatment showed a slightly longer mean righting time (1.45 s), and also exhibited the largest interquartile range, indicating higher variability in individual righting responses. This increased variation was primarily driven by one crab that failed to right itself within 5 seconds, the set cutoff time used to standardize response measurement. Despite similar mean and median values across crowded, uncrowded, and UC + CW treatment groups, the slower, on average, and more variable righting behavior in the crowded group suggests elevated stress under physical crowding conditions.

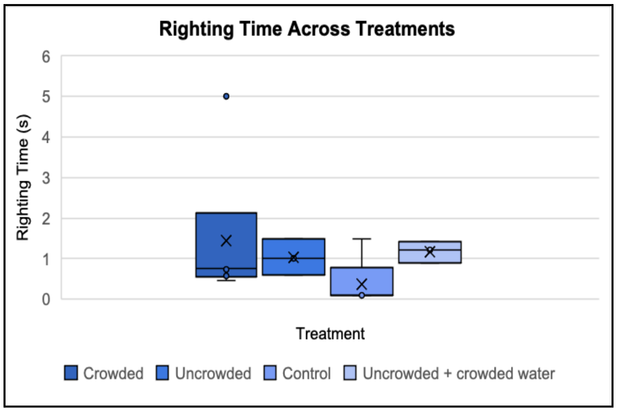

Figure 3: Righting time (seconds) of *H. oregonensis* across four treatments: Crowded, Uncrowded, Control, and Uncrowded + Crowded Water. Each box represents the interquartile range (IQR), with the horizontal line in the middle representing the median and the "X" representing the mean. Whiskers extend to the minimum values within 1.5 times the IQR. Any dots beyond this range represent outliers. In week 1, three unique individuals were tested per treatment. In week 2, the same individual was tested three times per treatment.

Condition	Mortality	Limb Loss	Escape
Crowded Phase 1	2	1	0
Crowded Phase 2	1	1	3
Unrowded	0	0	0
Control	0	0	0
Uncorwded + Crowded Water	0	0	0

Table 1: Counts of mortality, limb loss, and escape events observed in *H. oregonensis* across treatment conditions: Crowded, Uncrowded, Control, and Uncrowded + Crowded Water. All events occurred only in the crowded treatment. In Crowded phase 1 (includes the duration of the initial crowding

Treatment	Salinity (Start)	Salinity (End)
Crowded Phase 1	34	40
Crowded Phase 2	34	41
Uncrowded	34	34
Uncrowded + Crowded Water	36	36
Control	34	34

Table 2: Salinity measurements (ppt) taken on day 1 (start) and day 7 (end) of each treatment: Crowded Phase 1, Crowded Phase 2, Uncrowded, and Uncrowded + Crowded Water. Salinity for the control condition remained stable throughout both phases of salinity measurement. Increases were observed only in the crowded treatments, while salinity remained stable in the other groups.

Mortality, limb loss, and escape events occurred exclusively in the crowded treatment (Table 1). In phase 1 (treatment period and May 5th righting trials), two crabs died, one during trials and one in the container, and one limb loss occurred during the treatment period. In phase 2 (treatment period and final trials on May 12th), one crab died during trials, three escaped during the treatment period, and one additional limb loss occurred.

Salinity increased in both crowded treatments, rising from 34 to 40 ppt in Phase 1 and to 41 ppt in Phase 2 (Table 2). No change occurred in the Uncrowded + Crowded Water treatments, though this treatment had an elevated starting salinity (36 ppt) due to the mixing of crowded water with clean seawater (34 ppt) before the Phase 2 treatment period began. The uncrowded and control treatments remained stable at 34 ppt throughout their entire duration.

Discussion

This study investigated behavioral and physiological stress responses in *H. oregonensis* to physical crowding and crowding-induced chemical cues. Righting time, oxygen consumption (via average RFU at 30, 60, and 90 minutes), mass-specific respiration rates (RFU/g/min), mortality, limb loss, escape events, and salinity changes were measured to assess stress under these conditions.

Crowded crabs had the highest RFU values throughout the 90-minute resazurin assay and the quickest respiration rates, likely due to physical crowding and the intrusion of conspecifics into territory, which induces aggressive behavior in decapod crustaceans linked to elevated metabolic rates (Smith & Taylor, 1993; Rovero et al., 2000; Hazlett et al., 1968). Aggression was frequently observed in crowded conditions, supporting this interpretation. Additionally, crowding led to increased salinity in both treatment phases (rising from 34 to 40-41 ppt), likely due to the build-up of waste that carries salt ions (Lin & Chen, 2001). Although *H. oregonensis* is euryhaline, it typically inhabits salinities of 17-32 ppt (Hiebert, 2015), so elevated salinity likely led to osmotic stress that increased respiration rates and oxygen consumption (Lin et al., 2022). The similar RFU values in the control, UC + CW, and uncrowded treatments at 60 minutes suggest those groups were acclimating to the resazurin exposure, while the crowded group's elevated RFU indicates a heightened and sustained stress response that exceeded the baseline adjustment seen in other conditions.

In contrast, crabs that were exposed to crowded water (UC + CW) did not show a large increase in oxygen consumption compared to control conditions. Their RFU slopes remained similar to that of the control crabs, suggesting that chemical cues alone were insufficient at elevating metabolic activity. One explanation is that chemical cues may have led to reduced locomotion as a defense mechanism, reducing energy expenditure and the need to increase metabolic rates, a scenario that has been observed in other decapod crustacean species (Hazlett, 2010). While UC + CW crabs had slightly slower mean and median righting times than UC, their RFU values and oxygen consumption rates were lower, possibly due to this reduced locomotion effect, as a result of exposure to chemical cues from stressed conspecifics.

Uncrowded crabs had intermediate respiration rates but showed a sharp increase after 60 minutes. This may have been caused by repeated handling, as the same crabs were used for both righting time and resazurin assays for this treatment group. Previous studies have shown that habitat complexity supports vitality in crabs (Wilson et al., 2022), which may also explain the elevated respiration rates in uncrowded and crowded treatment groups and the greater mean and median righting times amongst crowded, uncrowded, and UC + CW treatment groups in comparison to control crabs who were placed in a structurally complex habitat. It is important to note that the error bars overlapping for the respiration rates of crowded, uncrowded, and UC + CW conditions hint that the differences observed between these treatments are not statistically significant. Despite this, weak conclusions can still be made, especially when analyzing the results from figures 1 and 2 together.

Righting time, a behavioral stress and vitality metric (Wilson et al., 2022), did not differ drastically across uncrowded, UC + CW, and crowded treatments in terms of median values. However, the crowded group showed greater variation and the longest mean righting time, driven by one crab that failed to right within the 5-second cutoff period. While not conclusive on its own, this variation may reflect compounded stress from both physical crowding and environmental factors like increased salinity and ammonia levels. Additionally, since higher salinity has been shown to counteract ammonia toxicity in crustaceans (Lin & Chen, 2001), and both UC + CW and crowded crabs likely experienced higher ammonia exposure, this may explain why their average and median righting times were aligned closely with those of uncrowded crabs. Elevated ammonia levels can cause neurotoxicity, potentially impairing the motor coordination required for righting responses (Lin et al., 2022), but higher salinities in the UC + CW and crowded conditions may have mitigated these effects and quickened their righting responses. Age, sex, and size were not controlled during righting trials, which may have introduced variability, particularly in the crowded treatment group that had the largest combined sample size across phase 1 and phase 2 of experimentation (Schembri, 1981; Penn & Brockman, 1995).

All mortality, limb loss, and escape events occurred in the crowded treatment. Two crabs died, and one lost a limb in phase 1. Another crab died, three escaped, and one limb loss was recorded in phase two. These outcomes occurred despite similar handling across uncrowded and UC + CW and crowded conditions, suggesting that physical crowding, not handling, was the key stressor. Although Wilson et al. (2022) attribute such outcomes to handling, our findings indicate that mortality and limb loss are valid indicators of crowding-induced stress in *H. oregonensis*. The observed escape events further bolster the argument that crowding was a stressful condition.

These results suggest that both physical crowding and environmental changes (e.g., salinity, ammonia buildup) may interact and influence crab stress. While chemical cues alone did not elicit a strong increase in metabolic responses, subtle effects on behavior were observed, with the more variable righting times and a higher mean righting time. Future studies should measure factors like ammonia and pH to better understand how these variables may have impacted the results observed. Additionally, standardizing crab size, sex, and age would potentially reduce variation in behavioral metrics like righting time and possibly physiological metrics like respiration rate.

These findings have broader implications for shoreline management. As shoreline development increases in the face of rising sea levels, intertidal zones shrink, and intertidal organisms like H. oregonensis may be forced into crowded, degraded habitats. This can elevate energy expenditure through increased metabolic activity, reduce energy available for growth and reproduction, and potentially have impacts on population health. The crowded treatment in this experiment mimics conditions found under coastal squeeze, where shoreline armoring reduces burrowable substrate and shelter (Jackson & Mcllvenny, 2011). Living shorelines are natural solutions to hard shoreline armoring that may include native vegetation, wood, compost, and substrates. Replacing hard shoreline armory with these living shorelines can increase habitat space and reduce changes to intertidal substrates. Therefore, an increase in the development of living shorelines could offer complex habitats that mitigate stress in intertidal organisms (Jackson & Mcllvenny, 2011; Smith et al., 2020).

In conclusion, physical crowding affected metabolic stress in *H. oregonensis*, likely due to aggression, restricted space, rising salinity, and potentially increased ammonia levels. While chemical cues did not elicit the same metabolic effect, they may still influence behavior. Together, these findings highlight the physiological consequences of coastal squeeze and reinforce the need for habitat restoration strategies to promote resilience in intertidal communities.

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