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FISH 460

The Effects of Crowding on Metabolic Activity in *Hemigrapsus oregonensis*

Introduction

Shoreline modification and urban development are expanding along the Puget Sound, where over a quarter of the coastline has been modified with structures such as bulkheads, seawalls, and riprap. These forms of armoring can change beach composition and restrict intertidal zonations, reducing the available habitat for native species. The combined forces of sea level rise and shoreline development prevent natural landward migration of intertidal organisms, and they get increasingly restricted to narrower spaces. This is a phenomenon known as "coastal squeeze." This spatial compression raises concerns about potential crowding stressors, which may increase physiological stress and disrupt ecological interactions. Understanding how crowding affects the physiology of common intertidal organisms such as the hairy shore crab (*Hemigrapsus oregonensis*) offers critical insights into the ecological consequences of shoreline armoring and inform future conservation efforts to vulnerable habitats.

One likely consequence of shrinking intertidal space is increased population density, leading to crowding and competition among species. In crustaceans, crowding has been shown to elevate stress responses and influence behavior. Previous studies show that hermit crabs (*Pagurus bernhardus*) exhibit more aggressive encounters at higher densities (Hazlett, 1968), and European green crab (*Carcinus maenas*) experience elevated heart rates in response to conspecific intrusions (Rovero et al., 2000). Increased aggression and longer interactions raises oxygen consumption and expends more energy, as seen in swimming crabs breathing more quickly and irregularly during escalated fights (Smith & Taylor, 1993).

The hairy shore crab (*Hemigrapsus oregonensis*) is a small native crab commonly found along the Puget Sound. *H. oregonensis* is an ideal model organism for intertidal ecological studies due to its abundance, and is relevant to this study as they exhibit density-dependent behavioral changes, including competitive aggression for shelter (Visser et al., 2004; Jensen et al., 2002). In crowded environments, *H. oregonensis* often remain close to their burrows and show increased defensive behaviors during encounters with interspecifics, particularly while foraging. While behavioral responses to crowding in *H. oregonensis* are previously studied, the physiological consequences of such interactions remain largely unexplored. Furthermore, there is a knowledge gap regarding whether environmental changes from crowding, such as shifts in water parameters like salinity and possible water chemistry changes could affect metabolic performance. Therefore, the objective of this study is to assess how physical crowding and exposure to crowd-conditioned water influence the metabolic activity of *H. oregonensis*. We hypothesize that both physical crowding and crowded conditioned water will increase metabolic activity, as measured through oxygen consumption using a resazurin-based assay. Our findings aim to provide insight into how coastal squeeze from shoreline armoring may impose physiological stress on native intertidal species.

Methods

We designed a two-phase experiment to test the effects of both physical crowding and altered water chemistry on crab metabolic activity. In Experiment 1, crabs were assigned to two density treatments to simulate physical crowding: nine crabs were placed in a small tank (150 cm^2, 1000 mL seawater) to create a crowded condition, and nine crabs were placed in a larger tank (212 cm^2, 1000 mL seawater) as the uncrowded condition. A separate group of crabs were

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maintained in a control tank with sufficient space to imitate uncrowded, habitable conditions. To determine the dimensions for our experimental tanks, we initially referenced the average reported density (427 crabs/m^2) and the maximum reported crab density (624 crabs/m^2) from Jensen et al. (2002) and scaled it based on a total of 20 crabs. After a week in their respective environments, three crabs from each treatment were randomly selected and timed for righting response. These individuals were then gently dried and weighed to standardize RFU data before being transferred into individual containers, each containing 35 mL of resazurin dye solution. Crabs were incubated for 90 minutes, and 20 µL subsamples were collected at 30, 60, and 90 minute increments for fluorometric analysis to measure the reduction of resazurin, which serves as a proxy for oxygen consumption and aerobic respiration.

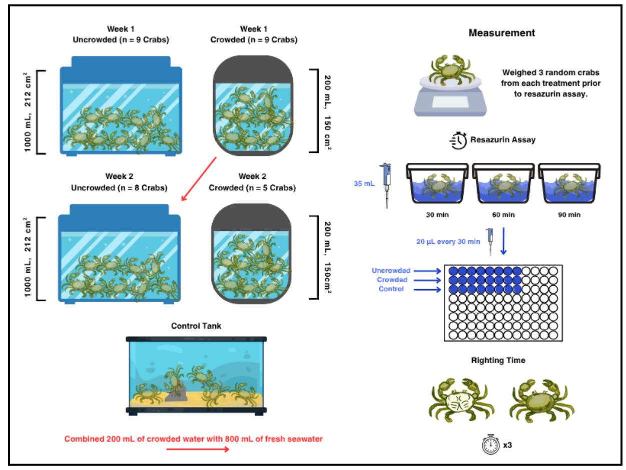

Figure 1. Overview of experimental design used to test the effects of physical crowding and chemically altered water on the metabolism of *Hemigrapsus oregonensis*.

In Experiment 2, we evaluated whether the water quality altered by crowding would affect crab metabolism. A new set of randomly selected crabs was assigned to two treatments. Five crabs were placed in the same small tank (150 cm², 1000 mL of seawater) to simulate crowding, while eight crabs were placed in a larger tank (212 cm²) containing 1000 mL of water composed of a 200:800 mL mixture of crowded water from Experiment 1 diluted with fresh seawater. After one week, three crabs from each treatment were randomly selected for resazurin assessment, and one individual per treatment was used for righting time with three replications. Due to observed mortality, the number of crabs and righting protocol were adjusted from the previous phase. All selected crabs were then dried, weighed, and placed into separate 35 mL resazurin containers. As in the previous phase, 20 µL samples were taken at 30, 60, and 90 minutes and analyzed for fluorescence. Throughout both experiments, salinity was measured at the start and end of each treatment period to ensure consistency and identify any changes within the water composition. All containers were thoroughly cleaned between trials to prevent cross-contamination. The following results summarize the observed trends in metabolic activity and behavioral responses across both experiments.

Results

To assess the physiological effects of crowding on *H. oregonensis*, we measured oxygen consumption over time using a resazurin-based assay. RFU (relative fluorescence units) increased across all treatments, but at different rates relative to different crowding conditions.

In Experiment 1, crabs in the crowded treatment exhibited consistently higher oxygen consumption compared to uncrowded and control groups across all three time points (30, 60, and 90 minutes) (Figure 2a). By the 90-minute mark, the mean RFU in the crowded group exceeded 610 RFU/g/min, compared to 486 RFU/g/min in the uncrowded group and 308 RFU/g/min in the control. These results suggest that physical crowding could elevate metabolic activity. However, the data does not support a statistically significant difference due to overlapping standard error bars. Formal statistical tests (i.e. t-test, ANOVA) were not conducted due to limited sample size, so these findings should be interpreted as preliminary trends rather than conclusive effects.

In Experiment 2, crabs in the uncrowded with crowded water treatment group showed oxygen consumption trends similar to the control group with only a slight increase observed at the 90 min-mark (Figure 2b). At that interval, the RFU values for the uncrowded group remained below 350 RFU/g/min, while the control group exhibited 275 RFU/g/min and the crowded group with 590 RFU/g/min. This suggests that water composition and chemistry altered by crowding may influence metabolism, though the effect appears weaker than direct physical crowding with no statistical significance shown.

When combining data from both experiments, the overall trend remained consistent: the crowded treatment resulted in the highest metabolic activity across time, followed by the uncrowded group, with the control and water treated group consistently lowest (Figure 3). While these patterns indicate that both physical crowding and crowd-induced environmental changes may elevate metabolic demand in *H. oregonensis*, the observed differences were not statistically significant and should not be considered as a definitive effect.

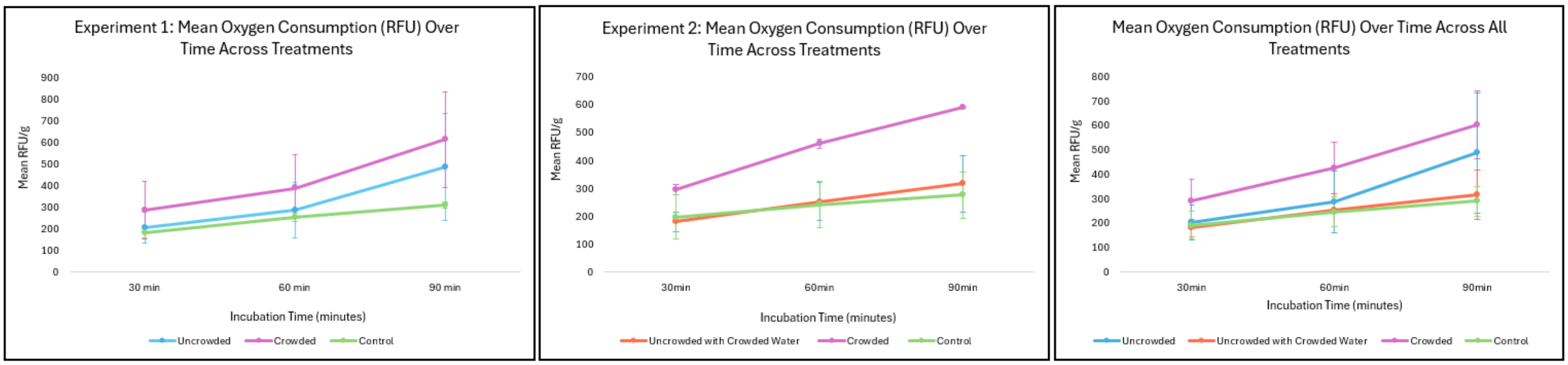

Figure 2. Mean oxygen consumption of *Hemigrapsus oregonensis* over time across treatments, measured by resazurin fluorescence (RFU). a) Experiment 1: Comparison of crowded, uncrowded, and control treatments. b) Experiment 2: Comparison of crowded, control, and uncrowded crabs exposed to water previously used in the crowded treatment (uncrowded with crowded water). c) Combined data from both experiments showing overall trends. Each point represents the mean RFU at 30, 60, and 90 minutes. Error bars indicate ± standard error.

In addition to oxygen consumption data, we recorded instances of mortality, limb loss, and escape across treatments (Table 1). Salinity was also recorded before and after each experimental period. All of the observed events occurred in the crowded treatment, with three mortalities, two cases of limb loss, and three escape attempts. No incidents were observed in the uncrowded, control, or uncrowded with crowded water groups. These outcomes suggest that the extreme spatial restriction for a prolonged period in the crowded tanks may have induced elevated physiological and behavioral stress which exceeded their tolerance threshold. Environmental conditions may also play part in amplifying the stress. Specifically, salinity levels in the crowded tanks increased more substantially than in the uncrowded treatments, with shifts from 34 to 40–41 ppt compared to minimal or no changes in other groups. The combined forces of elevated salinity with physical crowding could have exacerbated stress responses, leading to increased mortality and escape behavior.

Table 1. Observed mortality, limb loss, escape attempts, and salinity changes across *H. oregonensis* treatments. Recorded a week after the experimental period of each phase. Salinity changes reflect start and end values measured in week (1) and week (2).

Treatment	*Mortality*	*Limb Loss*	*Escape*	*Salinity Change*
Uncrowded	4	2	3	34 → 34 ppt
Uncrowded + Crowded Water	0	0	0	36 →36 ppt
Crowded	0	0	0	34 → 40 ppt (1) 34 → 41 ppt (2)
Control	0	0	0	NA

We also evaluated righting time as a behavioral indicator of stress across the three treatment conditions. Median righting times were similar across groups, ranging from approximately 0.7 to 1.2 seconds (Figure 4). The crowded treatment exhibited more variability as it included an outlier in which one individual was immobile for over 5 seconds. Given this relatively long response, we established a 5-second cutoff righting time to maintain consistency in interpreting the data. Each box plot displays the interquartile range (IQR), with the "X" representing the mean, whiskers extending 1.5 times the IQR, and dots representing outliers and inner points. Even though these results show no clear difference, the increased variability and presence of an extreme outlier in the crowded group showcase that some individuals experienced heightened stress under dense conditions.

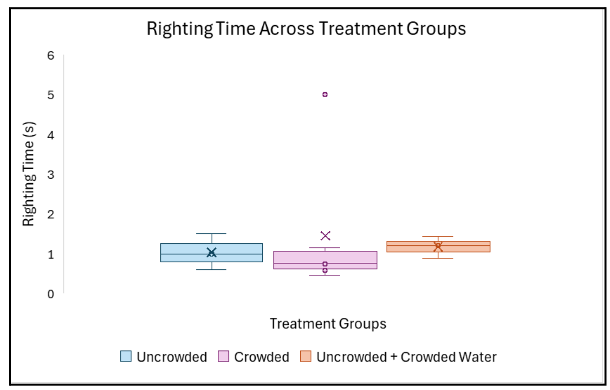

Figure 3. Righting time (in seconds) of *Hemigrapsus oregonensis* across three treatment groups: Uncrowded, Crowded, and Uncrowded with Crowded Water. Each box displays the interquartile range with the middle horizontal line inside the box representing the median. Whiskers extend to the minimum and maximum values within 1.5 times the IQR. The "X" symbol indicates the mean for each group, while individual circles represent outliers and inner points.

Discussion

This study examined how physical crowding and physicochemically altered water conditions affect the metabolic and behavioral responses of *Hemigrapsus oregonensis*. Our results show that physical crowding significantly increases oxygen consumption over time as shown in Figure 2b, suggesting elevated metabolic demand under high-density levels. This may be explained by physiological responses to stress. In crowded conditions, crabs tend to engage in more frequent and aggressive interactions and exhibit heightened vigilance in a confined space. Additionally, ventilation efficiency could be poor due to immobility. All of these factors have potential to elevate energy cost and thereby increase respiration rate.

While exposure to water previously habited by crowded crabs also have slightly more respiration, the difference was much smaller and not significant, indicating that physical constrictions have a greater impact on metabolism than physicochemical cues (e.g. stress signals, metabolites, reproductive hormones) alone. These findings were not able to support our second alternative hypothesis and conclusively determine that water affected by crowding would induce stress.

We also observed an increase in salinity in the crowded condition. This change may be attributed to the increased excretory output and respiration rate from a high biomass of crabs within the same water volume. The accumulation of waste products can change the concentration of ions in the sealed water, potentially leading to the observed increase in salinity. Despite *H. oregonensi*s being euryhaline and having a wide tolerance range of salinities, it is most commonly found in environments where salinity typically ranges between 17 and 32 ppt (Hiebert 2015). Although it can tolerate salinity of seawater (around 34 ppt), conditions above this threshold are likely to induce osmotic stress and may negatively affect metabolic cost and survival over time. Therefore, this may act as another physiological stressor driven by crowding. Together, significant findings from the second experimental phase support our alternative hypothesis regarding stress induced by crowding, and combined with the salinity results reinforce concerns about the physiological impacts of coastal squeeze and shoreline armoring on the habitat and water.

A strength in our design was the use of both physical and chemical treatments, which allowed us to isolate the effects of crowding from those of waterborne signaling compounds. To improve upon our chemical treatments, recording pH, ammonia, and CO_2 could better assess the environmental changes associated with crowding. There were also several methodological limitations. First, due to crab mortality and escape in the uncrowded treatment, our sample sizes were slightly reduced, potentially reducing the crowding effect with more vacant space in the tank. Additionally, the handling of crabs during weighing and righting response assessments may have introduced additional stress, which could have contributed to elevated RFU values.

Our findings build upon prior research demonstrating that crowding can drive metabolic costs and behavioral stress. For example, Smith & Taylor (1993) showed that agonistic interactions in swimming crabs significantly increased oxygen consumption due to elevated muscular activity and irregular breathing. Rovero et al. (2000) also found that *Carcinus maenas* experienced elevated heart rates in response to conspecific instructions, indicating increased physiological stress. These studies support the observed heightened oxygen consumption in our crowded *H. oregonensis* treatments, where physical crowding and restriction likely induced stress-related responses.

In conclusion, this study demonstrates that crowding can elevate metabolic activity in *Hemigrapsus oregonensis*, likely due to physiological stress with elevated salinity contributing to the effects. While water composition changes alone had little effect, the combined stressors highlight the ecological risks of coastal squeeze. These findings have broader implications for understanding how shoreline development and sea level rise affect intertidal populations. As seawalls and other armoring structures continue to reduce available habitat, shore crabs may be forced into higher densities, potentially resulting in increased metabolic rates, greater energy expenditure, reduced growth, and shifting the competition dynamics within the ecosystem. Over time, these effects could influence population health, biodiversity, and species interactions in the intertidal zone. To further prevent the loss of critical intertidal habitats, allocating conservation resources to vulnerable shorelines by replacing hard armoring structures with living shorelines that incorporate natural materials like shellfish beds and native vegetation can not only provide effective erosion control but also promote ecological resilience (Smith et al., 2020). Continued research on long-term physiological impacts will be essential for guiding effective conservation like living shorelines in urban coastal environments.

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