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Fish 460 Report

Introduction

Marine environments of today's Anthropocene are far from simple systems, with changes in climate, ecology, and economics driving the many ways we as humans are able to interact with them. Our interactions through the Alaskan snow crab fishery are an example of robust and rapid changes to marine environments completely shifting human markets and driving further need for understanding more about the systems we extract so much from. The snow crab (*Chionoecetes opilio*) fishery in Alaska supported a nearly US$200 million industry with lucrative management processes and research conducted by the National Marine Fisheries Service. Despite these efforts, however, the Bering Sea snow crab population effectively disappeared from 2019-2021, and the reason why remains hidden beneath thousands of feet of water. What is known is that a marine heatwave swept through the North Pacific in the latter half of the 2010's (Hu et al. 2024), which could have strained the snow crabs' thermal tolerance, causing either a mass migration to cooler northern or deeper waters, or metabolic hikes resulting in starvation (Szuwalski et al. 2023). Another factor speculated to have played a role in this event is tied to the phenomenon of bitter crab disease, a fatal dinoflagellate-borne illness with correlation to temperature increase and population density (Balstad et al. 2024). Ectothermic stress response can be significantly impacted by temperature increases (Shields 2019), and when critical temperatures are reached, the risk of infection and illness may pose a crucial threat to the immune response.

Understanding the immune response under increased thermal regimes can be done in the lab using a proxy for snow crabs. To do this, hairy shore crabs (*Hemigrapsus Oregonensis*) offer an opportunity to use an ecologically significant specimen that is feasible in a lab setting while still providing insight into the physiological responses of ectothermic decapods at local relevance. Hairy shore crab is found along shorelines of the North American Pacific coast and is smaller than snow crabs, with roughly 30 mm wide carapaces and green-grey coloring (Dana, 1851). Hairy shore crabs have a larger thermal range of 3°C-27°C, than snow crabs, with a thermal range of -1°C-5°C. The deep-water environment in which snow crabs live has historically created a less variable thermal regime, such that their tolerance has adapted to a narrow scope, whereas shore crabs and taxa that inhabit the intertidal are often at the hand of much more variable environments and thus have a much larger thermal scope. This more variable environment at the intertidal level also results in higher taxa richness of bacteria and parasites (Wang et al. 2012). The presence of a diverse array of pathogens in intertidal environments, along with greater risk posed by increasing temperatures is described by Shields (2019) with crustaceans lowered threshold to infection when exposed to heightened temperatures, and Adamo (2012) outlines the lacking functions of the immune system compromised by infection as haemocyte count and stress hormones. To better understand how locally present parasites interact with *H. oregonensis*, heat stress and parasitism exposure will be monitored using physiological assays of haemocyte count, respiration, glucose levels, and righting time.

Methods

The experiment on hairy shore crab immune response as a response to heat stress was conducted over 3 weeks in the month of May, 2025, in the School of Aquatic and Fishery Sciences, Fisheries Teaching Building. Crab, *H. oregonensis,* specimens were collected from mixed substrate (shell, sand, pebble) off of Lion's Park Boat Launch, Bremerton, WA, between 11:30 and 13:34. Mud samples were collected from the streambed of a marginal freshwater outlet to Puget Sound near the shore within

Golden Gardens Park, Seattle, Wa at 13:10. Mud collection was done at a likely spot for parasitic biota presence as a nearshore freshwater system sediment mosaic including shell, sand, and biological debris.

4 groups were formed: a control group contained in cold water and no mud; one group contained in cold water with mud; one group contained in warm water and no mud; and one group contained in warm water with mud. With 6 crabs per treatment, excluding the control (18 total), 2 tanks (10 cm x 20 cm x 14.5 cm) containing mud were first hand-filled with 3.81 cm mud substrate and then filled with 1.5 L of water at an initial salinity of 33 ppt. 1 tank containing no mud was filled simply with 1.5 L of water at an initial salinity of 33 ppt. In all 3 tanks, 2 oyster shells serving as hides (15.96-33.6 cm^2) were placed in the tanks, one on either side of a mesh wall dividing the tank into two halves, with one side for unused specimens and one for used specimens. Non-manipulated control specimens were left in a large cool tank and not disturbed until measurements were made in week 2. Healthy, to-be-manipulated specimens, 6 per tank, were hand placed in each of the 3 tanks by hand. One crab in the cold water with no mud tank was missing a claw. Once tanks were set up, the two warm treatment tanks were set in a water table to be heated to 27°C, the one cold water treatment tank was set in another water table to be cooled to 13°C. Air stones supplied oxygen to each treatment tank for the duration of the study.

After one week of containment in respective treatments, measurements were collected for haemocyte count, respiration, glucose, and righting time. Haemocyte count was obtained by collecting haemolymph from crabs by way of needle syringeal extraction at the arthrodial membrane in an optimally non-lethal procedure. Haemolymph was read using a hemocytometer to count the concentration of haemocytes. O2 respiration was obtained indirectly through metabolic rate by way of resazurin assays conducted following standardized protocol. As a proxy for respiration, fluorescence of stock solution in which the crab specimens are inoculated for 90 minutes is measured through the reduction of blue resazurin to red resorufin, read by a fluorescent spectrophotometer at 30-minute intervals. Glucose was obtained through the haemolymph extractions and oxidized following Cayman Chemical protocol to produce hydrogen peroxide and gluconic acid. Hydrogen peroxide was then measured using peroxidase and chromogen. Prior to each haemolymph extraction, crabs were submitted to a righting time test in which they were placed dorsal side down on a flat surface, and pressed down until release, at which point a stopwatch measured the amount of time it took for the crabs to reorient themselves. Haemocyte count, respiration, glucose, and righting time were collected two times per treatment, one week apart. Crabs were to be used only once and subsequently placed on the used side of their respective tanks for the remainder of the study. Once data was collected, simple statistical analysis was performed using Google Sheets and Microsoft Excel to produce readings for the four metrics obtained.

Results

Haemocyte Count

5 specimens had haemolymph extracted as part of the first week's measurement, while 5 specimens had haemolymph extracted as part of the second week's measurements, including 2 from the control group. Haemocyte concentration was highest in the mud and heat treatment specimens with an average of 180 and 154 cells per microlitre in the first week, and a standard deviation of 90.24 and 43.13 respectively. In the second week, average cells per microlitre were 59.75 with a standard deviation of 6.56. In week 1, the mud and cold treatment had the second highest haemocyte concentration with an average of 42.50 and standard deviation of 15.93. The control treatment of no mud and cold had the third highest haemocyte count with 18.75 and 5.5 cells per microlitre, and a standard deviation of 5.85 and 2.08 respectively. The no mud and heat treatment had the lowest haemocyte count in week 1 with an average of 13 and 5, with a standard deviation of 2.16 and 3.37 respectively. In week 2, the no mud and

heat treatment had the second highest haemocyte count with 35.75 cells per microlitre and standard deviation of 18.14. The mud and cold treatment had the third highest haemocyte count with an average of 29 cells per microlitre and a standard deviation of 12.25. The control group had the lowest average haemocyte count in the second week.

Respiration

The mud and cold treatment group had the highest average resazurin calculations (Figure 2.) Mud and cold individuals had an average RFU/g of 486.44 at 90 minutes with a standard deviation of 270.95 in week 1 and an average RFU/g of 495.81 at 90 minutes in week 2 without a standard deviation due to there only being one measurement per treatment. No mud and heat individuals had the second highest average resazurin calculations with an average RFU/g in week 1 of 308.70 at 90 minutes and standard deviation of 42.34. At 90 minutes in week 2 the no mud and heat average RFU/g was 176.84 with no standard deviation again due to there only being one specimen per treatment. Third, the mud and heat treatment had an average RFU/g in week 1 of 243.66 at 90 minutes with a standard deviation of 7.26 while week 2 had an average RFU/g of 157.39 at 90 minutes, once again, without standard deviation.

Glucose

Glucose levels rose in week 2 from week 1 across the three treatment groups (Figure 3.). The mud and cold treatment had the largest total increase in glucose levels from an average of 0.91 mg/dL with a standard deviation of 0.09 to 3.64 mg/dL with a standard deviation of 5.15. The mud and heat treatment saw a rise in average glucose mg/dL from 0.19 with a standard deviation of 0.12 in week 1 to 1.71 with a standard deviation of 0.04 in week 2. No mud and heat treated specimens had an average glucose mg/dL of 0.45 and standard deviation of 0.03 in week 1 and increased to 0.84 mg/dL with a standard deviation of 0.02. The control (No mud and cold) averages were lower than all treatment groups week 2 averages at 0.15 mg/dL and a standard deviation of 0.03

Righting Time

Righting time showed an overall increase from week 1 to week 2 (Figure 4.). Righting time for the control treatment was an average of 1.60 seconds. For the mud and cold treatment, righting time was an average of 0.89 seconds in week 1 and an average of 3.68 seconds in week 2. In the no mud and heat treatment, righting time was an average of 0.81 seconds in week 1 and an average of 1.28 seconds in week 2. Lastly, for the mud and heat treatment, righting time was an average of 2.78 seconds in week 1 and an average of 3.78 seconds in week 2.

Discussion

In the advent of rising sea temperatures, developing the scientific understanding of interactions between commercially and culturally significant species and parasites bolsters our abilities to steward healthy populations. The proper functioning of the immune response under thermal stress will better allow ectothermic crustaceans to deal with infection as both nearshore and deep-water environments warm and develop to be more suitable for bacteria and parasites to thrive. In this study, hairy shore crabs were exposed to temperatures within their thermal range, at 13°C and 27°C, likely not resulting in treated crabs eliciting extreme forms of stress response. The heightened levels of haemocyte count in the two heat treatments after 2 weeks support this, indicating stress response being induced but not overhauled by exhaustive energetic demands. Resazurin levels and glucose levels were both highest in the mud and cold groups. These measurements can act as a proxy for thermal and normal stress (Dehnel 1960). Finally, righting time results were highest in week 2 mud treatments. Excluding resazurin, the results from week 2 indicate that the specimens maintained in the mud treatments had a greater level of stress response than those kept in no mud treatments. The nature of this study makes it hard to

determine whether pathogenic presence was the cause for this outcome as this was left up to a high degree of chance in using mud as a medium, rather than direct admittance of parasites, which would be a worthwhile avenue to explore with proper resources. However, in assuming that mud treatments subjected crab specimens to parasite presence, the higher levels of stress response give way to the idea that crabs within their thermal range are able to fend off against infection. As a proxy for snow crab, hairy shore crabs in this study promote the idea that within thermal tolerance, the stress response can be effective, and while this indicates the optimal outcome being anthropogenic actions aimed towards the climate remaining tolerable to marine life, future work will excel in discovering the abilities of crabs and important ectothermic species to adapt to a changing seascape.

Figures and Graphs

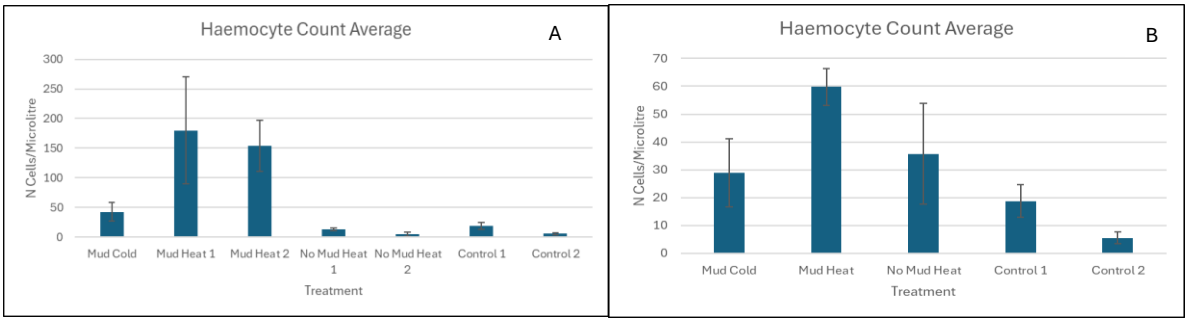

Figure 1. (A) Average haemocyte count per treatment after one week (Control count collected with week 2 samples on 05/13/2025). (B) Average haemocyte count per treatment after two weeks (Control count collected with week 2 samples on 05/13/2025). Bars represent SD.

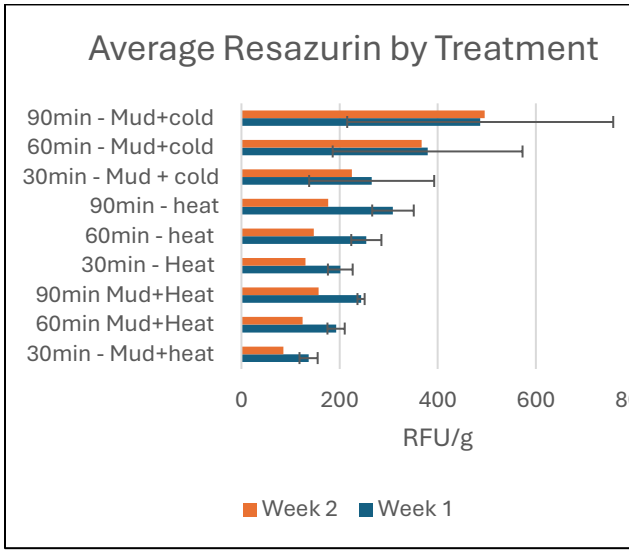

Figure 2. Average resazurin by treatment measured by RFU/g. Bars represent SD.

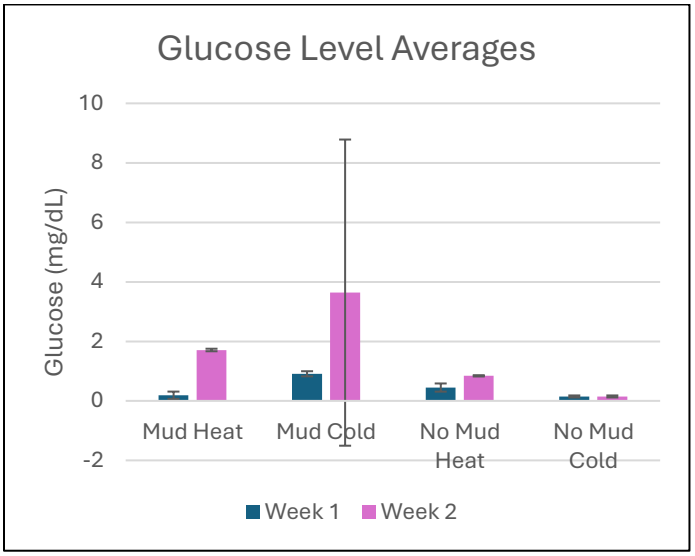

Figure 3. Average glucose levels (mg/dL) across treatments for weeks 1 and 2. Bars represent SD

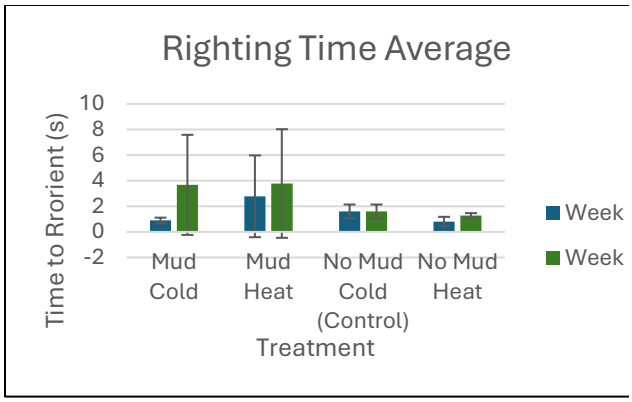

Figure 4. Righting time average across treatments for weeks 1 and 2 collected prior to haemolymph extraction/resazurin inoculation. Bars represent SD.

Table 1. Average haemocyte counts for weeks 1 and 2 with standard deviation.

	Week 1		Week 2	
	Average Haemocyte Count	SD	Average Haemocyte Count	SD
Mud, 13 C	42.5	15.92692	29	12.24745
Mud, 13 C	-	-	-	-
No Mud, 13 C (Control)	-	-	18.75	5.85235
No Mud, 13 C (Control)	-	-	5.5	2.081666
Mud, 27 C	180	90.24042	59.75	6.551081
Mud, 27 C	154	43.13545	-	-
No Mud, 27 C	13	2.160247	35.75	18.13606
No Mud, 27 C	5	3.366502	-	-

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