The Impacts of Higher Temperatures on the immune response in *Hemigrapsus oregonensis*

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Introduction

Rising ocean temperatures have big impacts on crustaceans, which has begun to impact fisheries as stock crashes occur. This research aims to examine how the rising water temperatures impact crab immune systems, in the hope to further understand how disease rates may increase, causing more issues such as bitter crab disease

As climate change continues to increase ocean temperatures, the impacts of these temperature increases have begun to appear in the immune system of animals. Higher temperatures have been found to increase pathogen survival and growth rate, while reducing the ability of animals to fight off said infections (Shields, 2019). The year 2024 had the warmest sea surface temperature recorded, and with the increase in marine heat wave frequency, this trend is expected to continue. Heat has been found to impact species' distributions, reproductive success, and metabolic rates.

The physiology of ectotherms, such as crabs, can be heavily impacted by temperature changes. One area stress has been seen is in the immune system of crustaceans, with thermal stress being linked to a decrease in the threshold barrier to infection. Both high and low-temperature extremes have been found to heavily impact crab immune systems (Truscott and White, 1990). Additional stressors have also been found to cause a decrease in immune response in invertebrates (Adamo, 2012).

In 2018-2019, the snow crab population in the Bering Sea declined by up to 90%, which caused a crash in the crab fishery depending on that population, losing millions of dollars. When examining potential causes, heat stress was mentioned as a possible cause for increased bitter crab disease rates in the population (Balstad et al. 2024). Bitter crab disease is a fatal parasitic infection to crabs caused by dinoflagellates. If thermal stresses continue to increase, diseases like bitter crab disease may increase in frequency, putting more crab fisheries at risk of collapse.

The susceptibility of hosts under thermal stress to pathogens has been studied; however, how locally present pathogen species interact with crabs has not. Local pathogen examination could help determine future host-pathogen interactions in local marine areas. In the Salish Sea the crab *Metacarcinus magister* (Dungeness crab) is an economically crucial species, as one of the most important seafood industries on the west coast of North America. Bitter crab disease has been an issue in the past for this species, making the continued examination of immune responses in these crabs an increasingly important area of research.

To examine host-pathogen interactions in commercially significant crustaceans such as the Dungeness crab (*M. magister)* in the Salish Sea, the crab *Hemigrapsus oregonensis* (hairy shore crab) was chosen as a proxy. The hairy shore crab is a small (between 1.25 and 1.4 inches long) crab found in intertidal zones, such as mudflats, and is tolerant of temperatures from 3-27°C making it a fitting proxy organism (Dehnel, 1960).

This research aims to examine how temperature affects immune responses in *H. oregonensis* by measuring through haemocyte concentration and stress assays. The project's alternative hypothesis is that higher temperatures will result in increased stress levels and decreased haemocyte concentrations in *Hemigrapsus oregonensis* when exposed to pathogens.

Methods

To set up the experiment, roughly mixed mud (enough to lay 1.5” at the bottom of two of the three tanks (10 x 20 x 14.5 cm)), was collected at 1:10 pm Friday, April 15th from Golden Gardens Park, Seattle, WA, 98105 and kept in the fridge at ~4°C until the set-up date. All three tanks were then filled with 1.5 L of water, initially at 33 ppt, and the 18 crabs were split equally into three tanks by hand indiscriminately. The treatments were set up as follows: mud and water temperature set at 27˚C, mud and water temperature set at 13˚C, and no mud and water temperature set at 27˚C. The crabs were then left for one week in their respective treatment tanks. At the one-week mark, four crabs were taken from each treatment for testing (totaling twelve crabs) and were given numbers between 1-4. The crabs were then weighed, and the righting times were performed. Two crabs from each test were used to run resazurin assays and two were taken for haemolymph extraction. At the two-week mark the remaining crabs, plus one repeated crab from the mud and heat treatment (due to mortality), were taken, numbered, and put through the same tests as the week before, being separated in half for the resazurin/haemolymph tests as before.

Righting Time:
A piece of paper towel was placed on the counter and an iPhone stopwatch was set up. One crab would be taken and placed on its back. One finger was pressed gently on the crab’s abdomen to prevent the crab from moving. At the same moment, the finger was removed from the crab, and the stopwatch was started and was stopped once the crab got from its back onto its front again.

Resazurin Assay:
For week one (two crabs per treatment) and week two (one crab per treatment), crabs were taken from their treatment tank and placed in a 2 oz condiment cup chamber with 35 mL of working stock. The stock resazurin solution (10 mL): 0.5 g resazurin salt, 10 mL DI water, 10 µL DMSO was kept in a dark fridge. The solution was then used to create the working stock (150 mL): 148 mL seawater (DI water with Instant Ocean adjusted to 23-25 ppt), 333 µL resazurin stock solution, 150 µL DMSO, 1.5 mL antibiotic solution (100x Penn/Strep & 100x Fungizone frozen in dark freezer). The working stock was stored at 4°C in a dark fridge. The resazurin was then run using the FISH460 Resazurin Assay Preparation & Protocol (2025).

Haemolymph Extraction:
For week one (two crabs per treatment) and week two (one crab per treatment), crabs were handheld upside down and their legs were gently pressed backward, exposing the coxa joint. A syringing needle was pressed into the joint, breaking through the membrane, and haemolymph was drawn. Multiple coxa joints were used until enough haemolymph was collected for analysis.

Haemolymph Concentration Count:
A haemocytometer was used to count the haemocyte cells in the four corners, the average was then multiplied by 10 to find the total count per uL.

Glucose:
Haemolymph collected was stored in a 96-well culture plate and frozen at ~4°C. The glucose was measured using the Standard Operating Procedure (SOP) for Agilent BioTek Synergy HTX Multi-Mode Microplate Reader.

Results

Some notes from the research done. One crab was missing its claw initially in the no Mud and 13°C treatment, there was one mortality in the Mud and 13°C treatment (5/6/2025), and one more mortality in the Mud and 27°C treatment (5/13/2025).

The Haemocyte concentration count results were higher for the mud and heat treatment than the other treatments tested. The haemocyte counts were high in the first week for the mud and heat treatment at 1400 uL or higher, falling closer to the level of the mud and cold treatment in week two at near 600 uL. The haemocyte results for the heat, mud and cold, and control treatments were all around 400 uL or under for both weeks.

Table 1: Haemocyte cells per uL for crabs from the three treatments and one control.

Treatment	*Crab #*	*Haemocyte cells per uL*
Mud + Heat	1	1800
	2	1540
	3	597.5
Mud + Cold	1	425
	3	290
Heat only	1	130
	2	50
	3	357.5
Control	1	187.5
	2	55

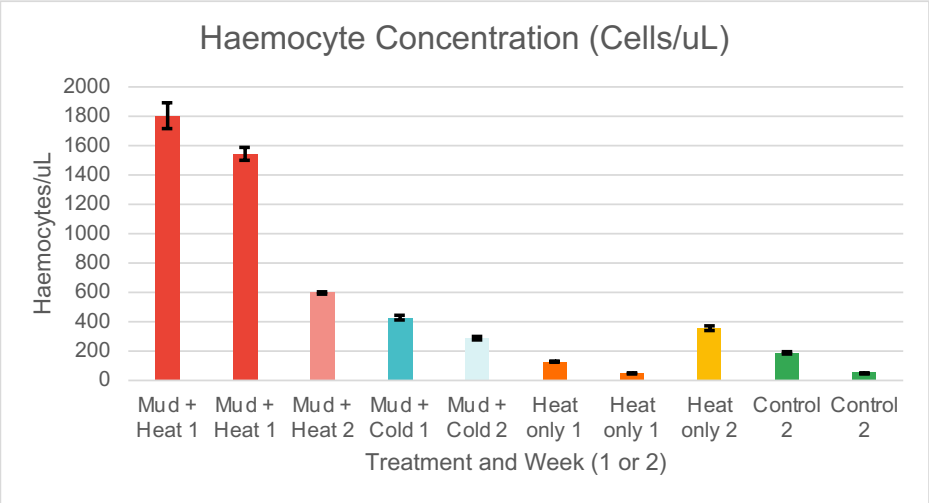

Figure 1: Haemocyte Concentrations per Treatment by Week

The resazurin results, when averaged and normalized by weight, showed the highest resazurin results for the mud and cold results for both week one and two. Both treatments with raised temperatures had lower resazurin levels, with mud and heat week two having the lowest results overall.

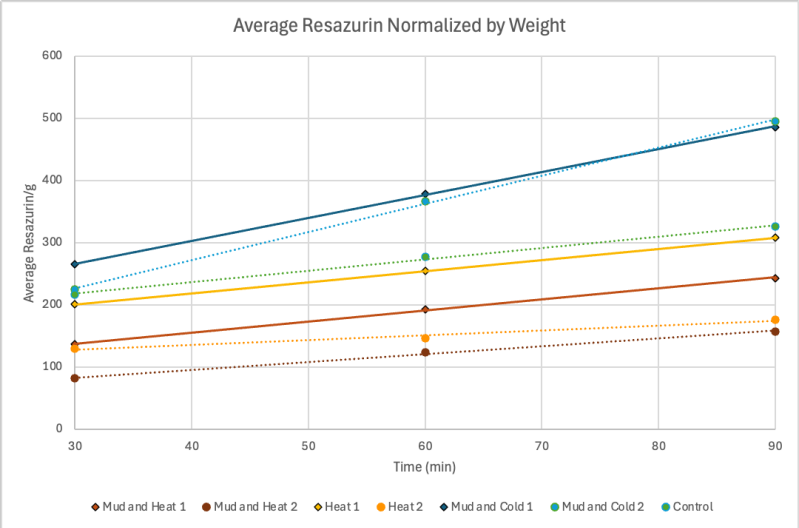

Figure 2: Average Resazurin levels normalized by weight for both weeks for 30, 60, and 90 minutes.

The glucose level results (Figure 3) for all treatments have an increase in the second week (seen in pink) when compared to the control samples. There is large variety in the mud and cold treatment week two sample.

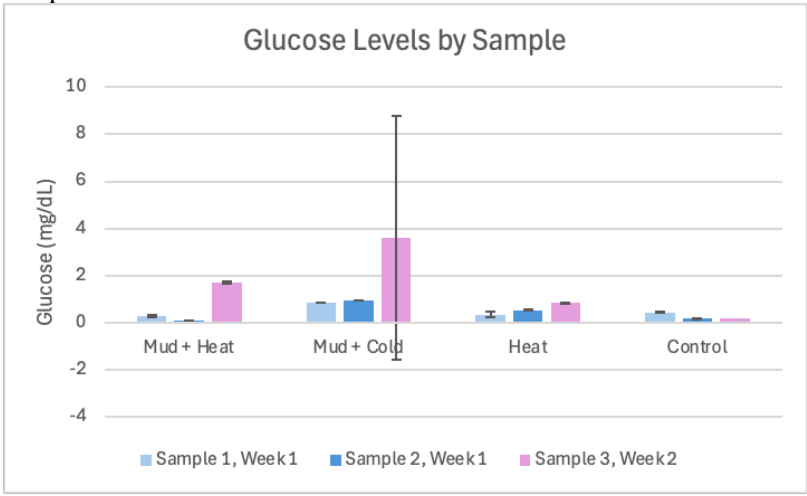

Figure 3: Glucose levels by sample for week 1 and 2

The righting times had large variance with overlapping error bars, though mud and heat treatments have a much larger range than the control and heat only tanks.

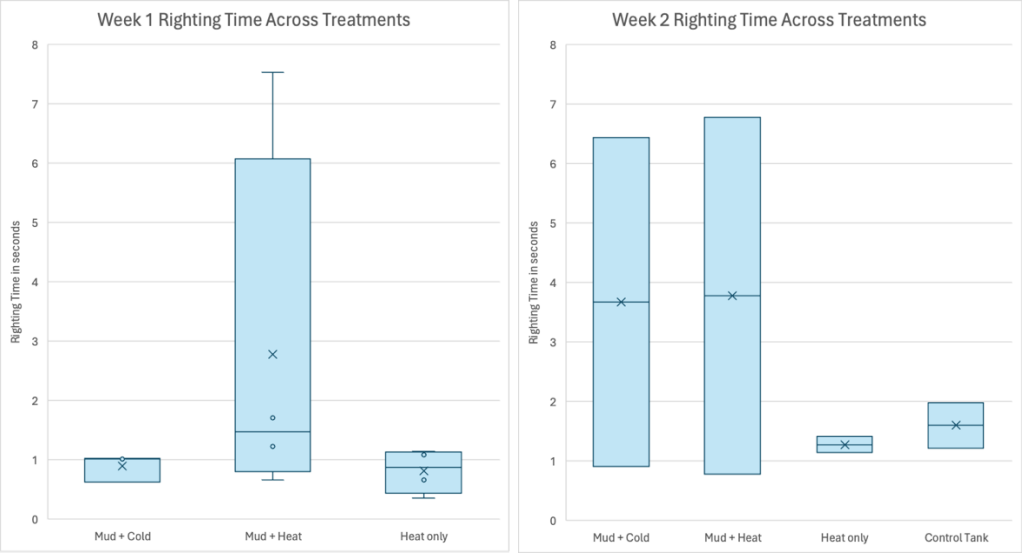

Figure 4 & 5: Righting Times results (in seconds) across treatments for Week 1 (left) and Week 2 (right)

Discussion

As shown in Figure 1, significantly higher haemocyte concentrations were seen for the heat and mud treatment in week one (between 1540 and 1800) compared to all other treatments that week (below 500). At three or more times the concentration of other treatments, this finding shows a significantly larger immune response in the mud and heat treatment. These higher concentrations were followed by a dramatic drop in the heat and mud haemocyte concentration in week two, at just below 600. This drop to around half the concentration suggests either a collapse in the immune system or some immune suppression due to chronic stress.

The resazurin assay results for treatments with raised temperatures were lower than those in colder water. An increased immune response is energetically taxing, which could cause an exhaustion response and lower the oxygen consumption rate. It could also be due to low health or low activity. The cold and mud treatment had higher resazurin results when compared to the control. This is possibly due to a slight immune response due to the presence of mud but without the immune suppression or exhaustion due to the increased heat the other treatments experienced.

Glucose and righting times both had a wide variety, making them less reliable measures of fitness or stress. This plus the small sample size makes it hard to determine outliers or patterns in these tests. Some areas of error in our research may be due to the small sample size, only two periods of data collection, one crab was eaten, and one crab had to be retested. Small amounts of haemolymph collection mean that few tests could be run, in future research lactate and BCA proteins should be tested to further examine the impact higher temperatures have on crab's immune systems. A change to using Dungeness crabs could also help in this area, as the crabs are much larger and will have larger haemolymph storage. This would allow for more testing opportunities and would allow for the examination of the impacts of heat on bitter crab disease rates specifically.

Future research should also examine if immune suppression is occurring, by examining the effects for a longer period than two weeks and taking samples more often to get a clearer image of timeframes and effects. Another area that could be examined is whether different mud treatments have different pathogen impacts, this could be done by sterilizing the substrate and adding known pathogen loads, or by collecting mud from different locations.

The haemocyte concentration results of this experiment strongly suggest that an immune reaction followed by a form of immune suppression likely occurred in crabs in the hot and mud treatment tank. The other results were not statistically significant enough to reject the null hypothesis, however, they did suggest an immune reaction or immune suppression occurring in the mud and heat treatment.

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