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Impacts of temperature on immune response in *Hemigrapsus Oregonensis*

Introduction:

In recent years, marine heat waves have grown more frequent, and overall sea surface temperature has risen, with 2024 being the warmest year on record (EPA). Rising temperatures can have severe impacts on species survival. One notable example is the snow crab, *Chionoecetes opilio*, a valuable fishery species in Alaska. From 2018 to 2019, the Alaska snow crab population declined by over 90%, collapsing the 227-million-dollar fishery (NOAA). Changes in ocean temperature can cause major thermal stress in crabs, although this was not thought to be the leading cause of snow crab mortality. Instead, an infection called bitter crab disease is seen as a leading culprit. This disease is caused by infection from a microscopic parasite and is considered fatal to crustaceans (NOAA).

As global temperatures rise, microbial communities are expected to flourish with higher metabolic and growth rates (Shields 2019). These increasing pathogen abundances pose a threat to crab fishery stocks such as Dungeness crabs in the Pacific Northwest. Additionally, the impacts of the temperature stress associated with climate change weaken crustacean immune systems (Shields 2019). Researchers investigating the activity of hemocytes –crab's immune cells– in *Carcinus maenas* found that hemocytes decrease in antibacterial activity at higher temperatures (Chisholm 1994). In some invertebrate species, heat stress can result in immune response suppression (Adamo 2012). The combination of a decreased ability to mount a defense against pathogens and an increase in pathogen populations is a dangerous combination for commercial species.

In this study, we used the shore crab *Hemigrapsus oregonensis* as a proxy for Dungeness crabs to evaluate the impact of temperature on immune response. This species is native to the Pacific Northwest, and commonly burrows into the mud. Previous research shows that shore crabs that spend most of their time burrowed into mud have increased pathogen and parasite loads compared to those that inhabit rocky intertidal zones (Dittmer 2011). Based on these findings, we exposed groups of *H. oregonensis* to mud collected in the field to provide a realistic immune challenge at control and elevated temperatures. We hypothesized that crabs exposed to warmer conditions and immune challenge will exhibit a lower hemocyte count due to immune suppression, as well as decreased hemolymph glucose levels, longer righting time, and increased respiration due to prolonged stress. In using *H.* oregonensis as a model species, we intend to provide insight on the compounding impacts of immune challenge and heat stress on valuable fishery species.

Methods:

In this experiment, we used *H. oregonensis* collected from Lion's Park (47˚35'07"N, 122˚38'42"W). The crabs were placed in the control tank at 16:21 on 4/27/2025. Two days later 4/29/2025, we divided 18 crabs into three different treatment tanks: 13˚C with mud, 27˚C with mud, and 27˚C with no mud. We did not create a control tank, as communal control crabs were kept in a larger tank at 13˚C with no mud. Treatment tanks were 10cm x 20cm x 14.5cm. Mud was collected from a stream output at Golden Gardens (47˚41'23",122˚24'10") on 4/25/2025 at 13:20, and was refrigerated until use on 4/29/2025. Each treatment tank was provided with two oyster shells to control for shelter (shells varied in size from 16-32cm^2). The control tank contained larger hides. The tanks were filled with 1.5L of 33ppt water, and mud treatments received 1.5in of well-mixed mud in the bottom of the tank. After filling and transporting crabs by hand to each treatment, tanks were placed in a water bath to maintain the desired temperature (13˚C or 27˚C).

The following week on 5/6/2025, we measured righting time using a stopwatch and weight of four crabs from each treatment (only 3 crabs were measured in the 13˚C with mud group due to one mortality). Righting time is used to indicate stress, with a longer righting time typically indicating

higher stress levels. Two crabs from each treatment were then used in a modified Resazurin assay to measure respiration rate, and in turn, stress. This assay was performed in beakers filled with 35mL of working solution with one crab per beaker (148 mL seawater + 333 µL resazurin stock solution + 150 µL DMSO + 1.5 mL antibiotic solution= 150mL of working solution). The other two crabs per treatments had hemolymph extracted for hemocyte concentration and hemolymph glucose assays using a 1mL syringe and needle. Hemolymph was extracted between the inner leg and the abdomen. This hemolymph was emptied into a microcentrifuge tube for storage. Hemocyte concentration was determined using a hemocytometer (cell counting slide) and a compound microscope. Cells were counted in the 0.25mm grid squares of a Neubauer Ruling hemocytometer. Hemocyte counts from each grid were averaged and converted to cells/µL (1 mm x 1 mm x 0.1 mm = $0.1mm^3$ = 0.1µL). Hemocyte concentrations were used to indicate the strength of immune response, with a greater hemocyte concentration indicating a greater immune response. Remaining hemolymph was frozen for the glucose assay, another measure of stress level. We installed a plastic mesh divider in each treatment tank, and crabs used in week 1 assays were placed into a separate side of the tank from un-handled crabs.

During week 2 on 5/13/2025, we measured righting time and weight from two crabs from each treatment, and two crabs from the communal control tank. One crab from each treatment underwent the modified Resazurin assay, and one crab received hemolymph extraction. Hemocyte concentration counts were taken, and the remaining hemolymph was frozen for the glucose assay. The Cayman Chemical glucose concentration assay was followed for all samples.

All analysis was performed using Excel, and no statistics were run due to small sample sizes. Significant difference in data was determined by average values and standard deviation.

Results:

Righting time had very large variation, with no significant differences in righting time across groups from either week (Figure 1). Overall, the mud and 27˚C group had the most variation and highest mean righting time, with an average of 3.12 +/- 3.16 seconds across both weeks (Figure 1).

The Resazurin assay also had large amounts of variability. The average rate of respiration (represented by the rate of increase in RFU/g body mass) is highest in the mud and 13˚C group at a slope of 3.96 RFU/minute compared to the rates of other treatments ranging from 1.45-1.66 RFU/minute (Figure 2).

The hemolymph glucose concentration assay had large variability in the week 2 mud and 13˚C treatment (Figure 3). The week 1 mud and 27˚C treatment did not show significant difference from the control glucose concentrations (Figure 3). Measurements from week 1 13˚C with mud, week 2 mud and 27˚C, and the 27˚C only treatment from both weeks had significantly elevated hemolymph glucose concentrations compared to the control group (Figure 3).

Hemocyte concentration had less variability across groups, and the mud and 27˚C treatment was the only group with significantly elevated hemocyte concentrations, with an average of 1312.5 +/- 632 hemocytes/uL hemolymph (Figure 4). Overall, mortality across treatments was very low. In the mud and 13˚C group there was one mortality between 4/29/2025 and 5/6/2025, and in the mud and 27˚C group there was one mortality between 5/6/2025 and 5/13/2025.

Discussion:

Due to small sample sizes, there was significant variability in our data, which impacts its reliability for drawing conclusions. For instance, righting time had a very large standard deviation, meaning that it is likely not the best metric for measuring stress in our *H. oregonensis*.

The Resazurin assay also had some variability, especially in the Mud and 13˚C treatment, although respiration rates tended to be consistent within the other treatment groups (Figure 2). The finding that the respiration rate of the mud and 13˚C treatment was over double that of other

treatments and control group was unexpected, as warmer temperatures tend to increase metabolic and respiration rates. This result may be due to small sample size. Previous studies suggest that the lower respiration rate in our heat treatments groups is possibly due to heat depression (Dehnel 1960). Another possibility for this variation was handling stress, although we attempted to handle all crabs equally to reduce this bias.

The hemolymph glucose levels observed in our treatments are also unexpected based on previous research. We hypothesized that the mud and 27˚C group would have significantly lower hemolymph glucose due to energy depletion over time, however, it was not significantly different from the control crabs. Between weeks 1 and 2, one of the crabs in the mud and 27˚C treatment was cannibalized by the other crabs, which is likely responsible for the increase in hemolymph glucose between weeks. The mud and 13˚C group had higher glucose than any of the other treatment groups. This is potentially an artifact of our small sample size, although it is possible that the crabs were able to consume organisms living in the mud they were treated with. The mud and 27˚C treatment may have had the same confounding aspect, leading them to not have depleted glucose concentrations, but more experimentation is needed to confirm this. To prevent this in the future, we could use aquarium sands with known pathogens added instead of mud collected from the field. We fail to reject the null hypothesis that an immune challenge under increased temperature does not significantly impact hemolymph glucose. Due to high variability, more replicates are needed to confirm this finding.

The hemocyte concentrations we measured also contradicted our initial hypothesis. The mud and 27˚C treatment crabs had far higher hemocyte concentrations than all other treatments. This means that the elevated hemocyte concentration is most likely due to the mud treatment since the 27˚C only treatment did not have elevated hemocyte concentrations (Figure 4). We initially hypothesized that crabs exposed to mud and 27˚C waters would have low hemocyte concentrations due to immune suppression by high temperatures (Adamo 2012; Shields 2019). In the absence of immune suppression, it is possible that crabs exposed to higher temperatures would have the high hemocyte concentrations we measured, as bacterial and microbial growth rates typically increase with increasing temperature (Shields 2019). This means that the crabs in the mud and 27˚C treatment likely experienced a greater immune challenge than those in the mud and 13˚C treatment, causing a greater immune response.

Although our results do not characterize immune suppression under elevated temperatures, they do provide evidence of an increased immune response (Figure 4). The goal of this study was to use *H. oregonensis* as a model organism for Dungeness crabs to better understand the impacts of climate change on fishery health. An increased immune response like we observed in our study is energetically taxing and has the potential to be exacerbated by heat stress (Adamo 2012; Shields 2019). For optimum fishery productivity, it is important that Dungeness crabs are able to put energy towards growth and reproduction. Based on our results, we may expect to see decreases in crab fishery production due to increased pathogen exposure, and decreased barrier to infection. Additionally, Dungeness may have to contend with pathogens they have not previously been exposed to as pathogen ranges shift due to global warming (Shields 2019). This puts fisheries further at risk.

Our results provide a limited scope of the potential impacts of rising temperatures on *H. oregonensis* immune response and its implications for valuable fishery species. Future work can be aimed in many areas. Firstly, expanding sample size will allow us to better quantify the impacts of heat stress through additional hemolymph assays such as lactate and BCA protein. Using our experimental design with mud sampled from different locations could help to diversify pathogen exposures. Replacing mud with purchased sand and adding known pathogens could help us to minimize confounding variables. Extending the experiment duration would allow us to test for signs of immune suppression. Expansion and modification of our current experimental design would allow us to better understand the impacts of climate change on crustaceans.

Figures:

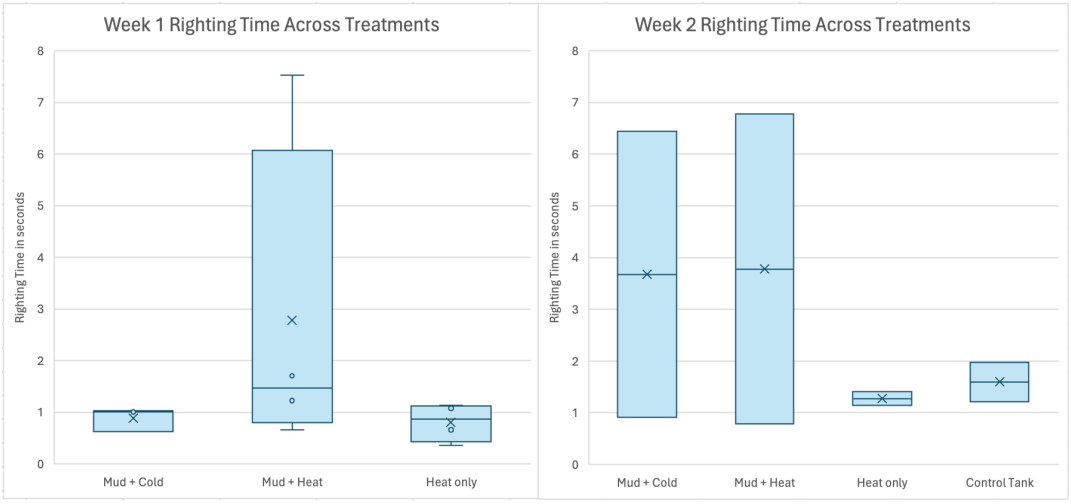

Figure 1: Righting times across treatments in week 1 and week 2. Control crab righting times were only taken during week 2. The average values are indicated with an X.

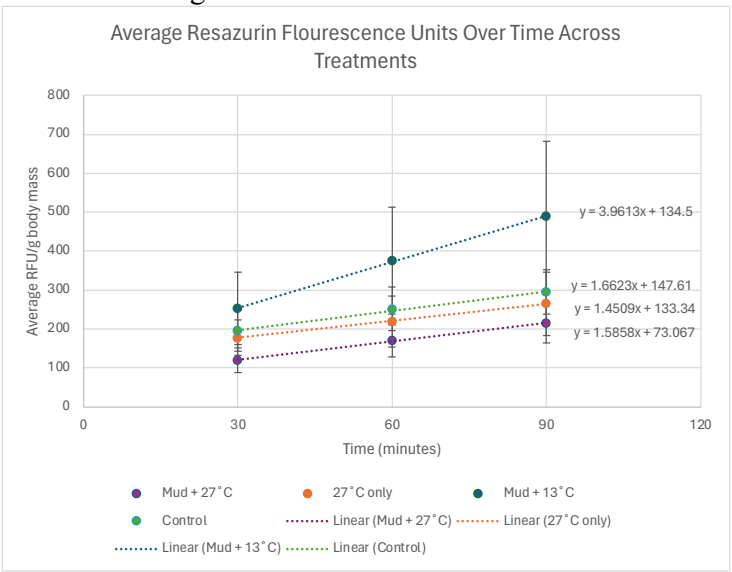

Figure 2: Mass standardized resazurin fluorescence units over time averaged across weeks 1 and 2. Error bars indicate standard deviations of each measurement and are overlapping in all groups except for the Mud and 27˚C treatment and the Mud and 13˚C treatment. A linear regression was applied to all points to determine a representation of respiration rate.

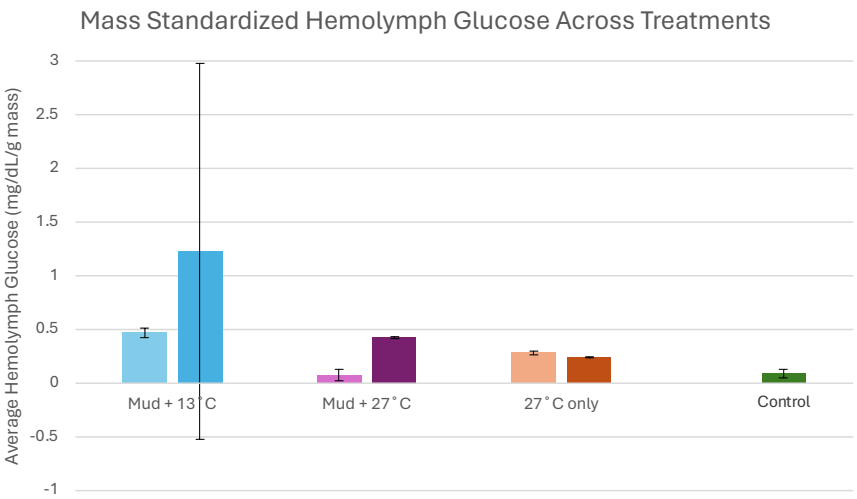

Figure 3: Mass standardized hemolymph glucose across treatments and weeks. The average week 1 values are displayed in light colors, average week 2 values are displayed in dark colors. Control measurements were only taken during week 2. Error bars represent the standard deviations of the average of replicate samples from each crab or multiple crabs. Averages for week 1 include two crabs for all treatments except Mud + 13˚C, and week 2 treatments each only contain data from one crab.

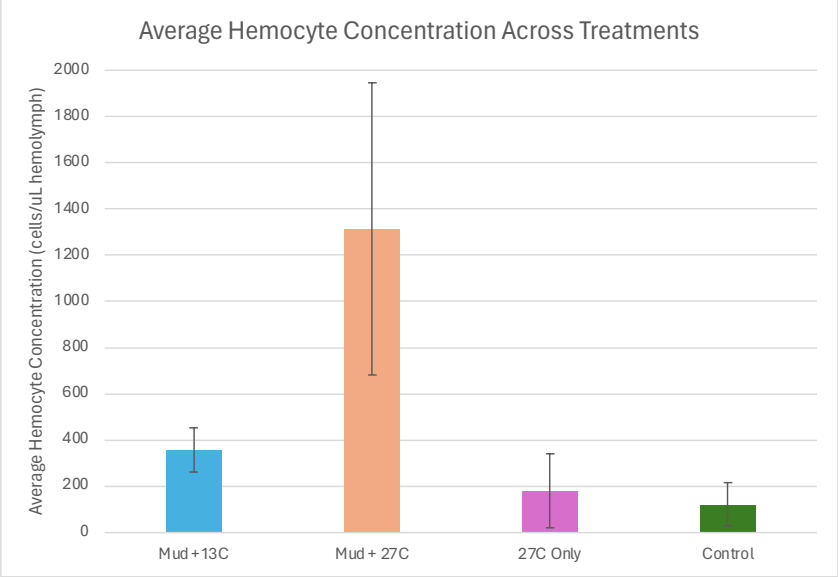

Figure 4: Average hemolymph hemocyte concentrations across weeks 1 and 2. Only the Mud + 27˚C treatment shows significant difference in hemocyte concentration from the other treatments. Error bars represent the standard deviation. Control samples were only taken during week 2.

References:

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