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Introduction

Crustaceans are vulnerable to increasing ocean temperatures due to climate change driven thermal stress. Within the Bering Sea, there were mass mortalities of Red King Crab and Snow Crab during a marine heatwave in 2022. These two species have high commercial status, and their great decline in population led to a large disruption in aquaculture (Fisheries, 2023). The linkage between the heatwave and the fatality event was due to the higher metabolism from the increased temperature, which outpaced food availability as caloric requirements elevated leading to starvation (Ramirez, 2024). As ocean temperatures rise due to climate change, the resilience of overlooked non commercial species like *Hemigrapsus oregonensis*, comes into question. The yellow shore crab (*H. oregonensis*) without being a non commercial status holds importance within their ecological role as an intertidal crab within the North Pacific. How does thermal stress affect glucose levels in *Hemigrapsus oregonensis* under different feeding states?

Within decapod crustaceans, the stomatogastric ganglion controls movement of the foregut as an important nerve control over transit time, which vary by species and environmental factors (McGaw & Curtis, 2012). Within a study of Green Crabs, Carcinus *maenas*, found that Green Crabs in increased temperature conditions had the highest contraction rate of the pyloric sac per minute (McGaw & Curtis, 2012). The pyloric region is where movement of food is regulated into the hepatopancreas, meaning that the food is being passed through the digestive tract at a faster rate than normal decreasing the efficiency of nutrient absorptions. This illustrates the physiological mechanisms that go into the higher energy demands leading up to starvation within an increased temperature environment. While digesting after a meal in decapods, absorption of food leads to a mobilization of glucose within the hemolymph to various tissues, allowing glycogen storage in their muscles or their hepatopancreas (Hu, 1958). In this study we investigate how feeding status between different temperature situations impacts *H. Oregonensis* metabolism using glucose as a parameter. Additionally, we will be evaluating the use of a glucometer as a proxy for glucose levels, an immediate blood sugar level tester that has been known to work for hemolymph and crustaceans (Caldari‐Torres et al., 2018). Through examining the impacts of increased temperature on metabolism within Yellow Shore Crabs, we aim to broaden our understanding on climate driven impacts on crab population health, and assess other methods of monitoring metabolism within crustaceans through the use of the widely available glucometer tool.

Methods

H. oregonensis were collected from intertidal habitats along the Pacific Northwest. Fifteen crabs were randomly assigned to three treatment tanks, five crabs per tank with consistent 2L volume, salinity, oxygen levels, and no substrate. One tank was set up for a fed group made of five crabs within 13°C temperature, mimicking a natural temperature from their natural environment. Two tanks for a fasted and fed group were kept in a 27°C temperature condition to imitate extreme ocean warming. A separate larger control tank was set up with around 30 crabs in fasting conditions at 13°C.

Individuals were selected to ensure similar size across groups to minimize size related metabolic differences between the three treatment tanks. The temperature conditions were set up one week prior to starting the glucose testing, and during this week all crabs in all three tanks were fasted and had no access to food since they were removed from their natural environment.

For trial 1, the fed groups were given a single forcep scoop amount of bull kelp (*Nereocystis luetkeana*) 1.25 hours before the first hemolymph extraction. Hemolymph

extraction was done with a hypodermic large guage needle, inserted within the back leg joints where there is an access into their open circulatory system. For each crab, a drop (around 0.5 μL) of hemolymph was immediately applied to a glucometer strip for in situ glucose reading. The remaining hemolymph from that extraction was transferred to micro tubes and stored in the freezer for glucose assay analysis. Four samples were collected every 30 minutes for each treatment group after the first hemolymph extraction (five total). Every crab that was used for a reading was avoided for being picked for the remaining to minimize any injury or risk of death from the hemolymph extraction, every crab selected from the control tank was placed separately to avoid resampling.

The Trial 2, fed groups received a single forcep scoop amount of bull kelp and had the first hemolymph extraction at 3 hours and 55 minutes post feeding time. Sampling intervals and procedure for hemolymph extractions for glucometer use and saved for glucose assays was the same as those in trial 1.Glucometer readings were used for real-time assessment of hemolymph glucose concentration by the ReliOn premier COMPACT that has a range of detection 20-600 mg/dL and a required 0.5μ*L* sample. Stored hemolymph samples were later analyzed via glucose assay for comparison to glucometer readings.

Results

On the day of trial 1, prior to feeding the fed groups, we observed fed 13°C group had two mortalities. One of the mortalities was a whole crab while the other had no limbs, only the eyes and the cephalothorax intact, indicating that this male individual had been eaten by the other crabs in the group. We obtained a glucometer reading for the first trial every 30 minutes at 1.25 hours after feeding for every group. Readings were done one through five; 1.25, 1.75, 2.25, 46.83, and 47.33 hours after feeding respectively. Reading one through three for all groups glucometer displayed “lo”, indicating it to be below the range of the glucometer device (20-600 mg/dL), shown in Table 1 as being <20 mg/dL. Additionally, when there were no accurate readings, the glucometer would display an “Er” implying error in reading the strip and we would redo that reading. On the s econd day of trial 1 (readings four and five), the fifth reading for the fed 13°C group demonstrated a reading of 22 mg/dL on the glucometer.

The hemolymph saved for glucose assays had some losses due to the lack of sample acquired. The applicable glucose levels from the assays are graphed on Figure 2 (a). Fasted 13°C started off the lowest at around 0.01 mg/dL then increased steadily to peak at the fourth reading 0.1 mg/dL then had a sharp decrease to 0.07 mg/dL. Fasted 27°C started 0.1 mg/dL then dropped very quickly to 0.006 mg/dL, increased sharply to 0.16 mg/dL and ended at 0.09 mg/dL. Fed 13°C first reading 0.1 mg/dL led to an increase to 0.18 mg/dL and ending up as the highest at 0.31 mg/dL. Lastly, the fed 27°C started at 0.2 mg/dL then dropped on readings three and four, to end at a slight increase at 0.12 mg/dL.

At the start of our second trial prior to feeding, there was one intact death in the fasted 27°C group. All of the five glucose readings were done on 5/12/25 starting every 30 minutes; 3.92, 4.42, 4.92, 5.42, 5.92 hours after feeding respectively. All of the glucometer readings demonstrated a low reading, indicating that the glucose levels are <20 mg/dL for every group, as shown in Figure 1 (b). For the second trial there were more losses of saved hemolymph through too small an amount of some samples for glucose assays, graphed available data points for the glucose assays are graphed on Figure 2 (b). Fasted 13°C started at 0.1 mg/dL and had a straight decrease to 0.03 mg/dL. Fasted 27°C started at 0.14 mg/dL and had an increase to peak at 0.24 mg/dL and decreased sharply to end at 0.03 mg/dL. Fed 13°C started at 0.27 mg/dL to peak at

the highest point 0.31 mg/dL and decrease to 0.2 mg/dL. Fed 27°C started at a low 0.04 mg/dL and increased to 0.26 mg/dL at the fifth reading.

Figure 1: Glucometer Results

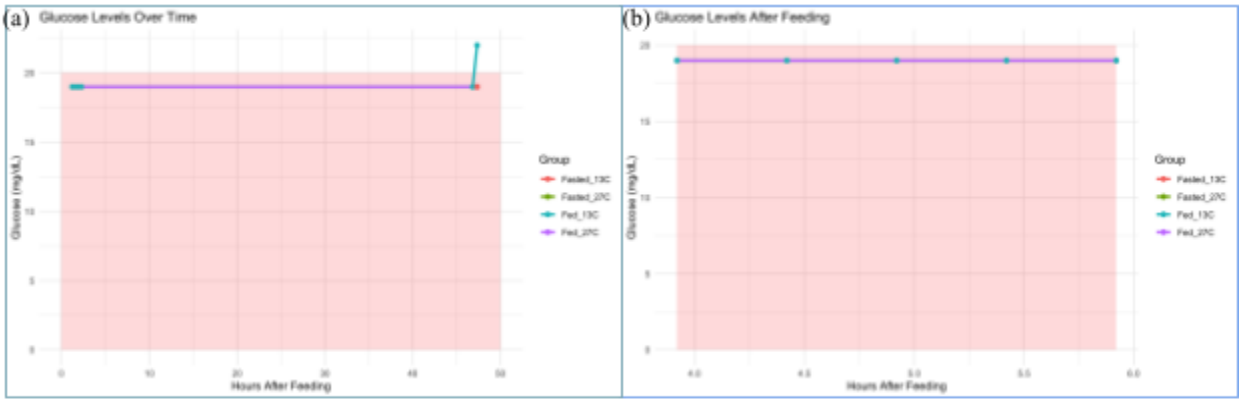

Fig. 1: Trial 1 (a) glucometer results starting after 1.25 hours after feeding, values under red highlighted area indicates having a reading of <20 ml/dL. Trial 1 Fed 13℃ had their fifth reading at 22 ml/dL. Trial 2 (b) glucometer results starting after 3.92 hours after feeding with all readings in each group being in the <20 ml/dL range within the red highlighted area.

Figure 2: Glucose Assay Results

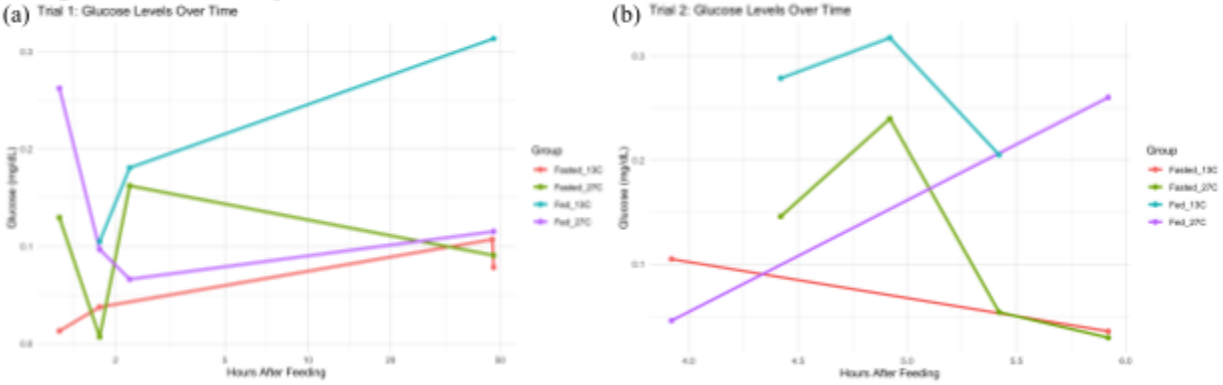

Fig. 2: Trial 1 (a) glucose assays for the four groups; fasted 13°C, fasted 27°C, fed 13°C, fed 27°C, starting after 1.25 hours after feeding. Trial 2 (b) glucose assays for the four groups; fasted 13°C, fasted 27°C, fed 13°C, fed 27°C, starting after 3.92 hours after feeding.

Discussion

This study explored how feeding status under thermal stress may influence glucose levels in *H. oregonensis* and connects to the larger concern of how climate driven ocean warming may impact crustaceans. The two trials of the glucometer results were all <20 mg/dL for each reading and group, except for the fifth reading of fed 13°C as 22 mg/dL in trial 1. Their corresponding hemolymph glucose assays identified the two fed groups (13°C and 27°C) with the higher glucose levels (Figure 2), and displayed the fed 13°C fifth reading as the highest. The two fasted groups finished with the lowest glucose levels, 13°C was the lowest in trial 1 and 27°C was the lowest in trial 2. This was expected as the fed groups did have access to a high carbohydrate diet of bull kelp before taking glucose readings. Within trial 1 we see a sharper decrease in fed 27°C

versus a direct increase in trial 2 (Figure 2), however, within trial 2, there are only two data points introducing unreliability in this trend. The limited amount of data reduces the certainty of observations, especially when combined with a low sample size and restriction of resampling for assessment of individual crab trends. The sharp decrease of the fed 27°C may be due to the heightened metabolism through an increase in transit time (McGaw & Curtis, 2012). Therefore, our findings align with concerns about increased metabolic demands outpacing nutrient absorption as seen in other decapods. As the fed group in the higher temperature conditions showed a different trend than crabs in the natural temperature suggesting thermal stress may alter glucose even in the presence of sufficient nutrients.

The patterns observed in our data may be explained by Crustacean Hyperglycemic Hormone (CHH) as a response to thermal stress (Fingerman, Jackson, & Nagabhushanam, 1998). CHH is secreted from the sinus gland from stressor stimuli and can directly impact the levels of hemolymph glucose through mobilizing glycogen storage (Miller, 2023). A study on Blue Swimmer Crab *Portunus pelagicus* had the highest amount of CHH under a temperature stressor, the lowest extreme at 24°C and the extreme highest at 32°C (Vasudevan & Rajendran, 2021). This shows that crabs that experienced heat shock have an increase in CHH, increasing hemolymph glucose through depletion of the hepatopancreas glycogen. This could be a possible explanation as to why there are higher glucose levels in the fasted 27°C group compared to the fasted 13°C group in some points of the data (Figure 2) as there may be an increase in CHH.

Within this study we have proved that the glucose range determined by the glucometer (<20 mg/dL) does match up with the glucose assay results, all being below 0.5 mg/dL. However, within trial 1 the fed 13°C read 22 mg/dL on the glucometer, which corresponded to a 0.31 mg/dL in the glucose assay. Although these two parameters did not match the same value of mg/dL, it was clear that the fifth reading of the fed 13°C had the highest glucose level across both trials in both the glucometer and the glucose assay. Even though the glucometer did work in terms of reading the in situ hemolymph glucose level, it lacked precision from only showing levels to be below 20 mg/dL. A consideration in the future for increasing the accuracy of the glucometer readings could be a dilution of the hemolymph with a known concentration of glucose, to then calculate the difference from the glucometer reading. Additionally, there should be more research for using these quick available methods of glucometer use to assess metabolism through any thermal impacts throughout different species of decapods. Especially in commercially important species in aquaculture where a single drop of 0.5uL is minimally invasive and enough to give a proxy on their metabolism. However, this method is not suitable for accurately quantifying low hemolymph glucose values. The use of a glucometer proved to be a convenient but limited proxy for measuring hemolymph glucose, especially when values are below its detection range. As ocean warming persists, understanding the negative physiological impacts on non commercial species like *H. oregonensis* becomes increasingly important for predicting broader ecological changes. Furthermore, applying reliable indicators on metabolism could help evaluate potential impacts on commercially important species from thermal stress that may lead to another detrimental change in the aquaculture industry.

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