Assessing *Hemigrapsus oregonensis* Glucose Metabolism under Temperature and Nutrient Stress

Introduction:

Due to the growing effects of climate change, many crustacean species have been affected by increasing temperatures. Snow crab in the Bering Sea have been affected by a recent marine heatwave, leading to a decline in both juveniles and adults (Fisheries, 2023). This has been linked to starvation due to warming oceans, causing a great disturbance within Alaskan aquaculture (Fisheries, 2023). Crustacean metabolisms have been shown to be sensitive to thermal stress. When water temperatures increase, a crustacean's metabolism does as well. This can lead to an increase in caloric requirements for snow crab, which, when coupled with a lower supply of prey, would lead to starvation (Fisheries, 2023). A study with green crabs found that when temperatures increased, the pyloric contractions in the crab also increased. The pyloric region of a decapod crustacean is where the movement of food is controlled into the hepatopancreas. When contractions increase, digestion speeds up, leading to a higher metabolic rate (McGaw & Curtis, 2012). Glucose metabolism during feeding and the intake of carbohydrates increases blood sugar. This leads to the mobilization of glucose to various tissues, allowing glycogen storage in an organism's muscle or hepatopancreas (Hui, 1958). Though in crustacean decapods, their metabolism is controlled by stomatogastric ganglion neurons, which control transit time that varies by species and environmental factors (McGaw, 2012). In a fed state, decapods have energy readily available to fuel energetically intensive processes such as protein synthesis. In a fasted state, mobilization of stored energy is crucial, as food availability is now unpredictable (McGaw & Curtis, 2012).

Known for its hairy, setae-covered legs, *Hemigrapsus oregonensis*, or the hairy shore crab, is a native intertidal species to the Puget Sound. Due to its intertidal environment, they are known to withstand various levels of thermal stress. But how glucose levels affect them under different feeding states is yet to be known. Using two different levels of thermal stress, coupled with groups of fed and fasted crabs, we hypothesize that crabs under thermal stress will display altered glucose levels within different feeding states. Seeing how glucose levels change over time and under different temperature conditions will reflect their metabolic processes and determine how much glucose is being absorbed, and how temperature works as a stressor. Understanding how thermal stress impacts glucose metabolism is key for future conservation.

Innovation of medical instruments for use in the field is a growing topic, due to expensive and non-portable equipment. A study using a glucometer for the reading of glucose in crayfish *Orconectes virilis* was deemed successful (Caldari-Torres et. al

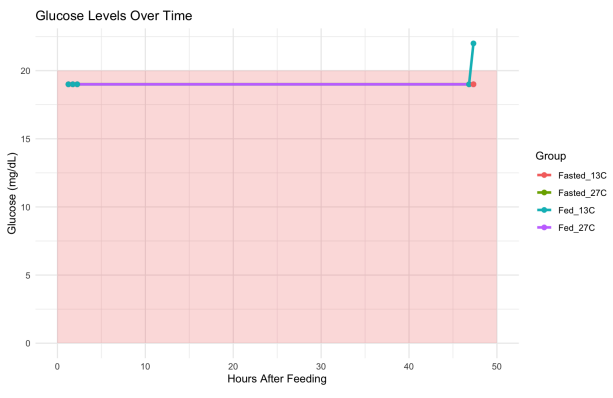
2018). We will also asses if a glucometer can be used as an efficient resource for monitoring glucose even in crustaceans.

Methods:

A total of 15 individuals of *Hemigrapsus oregonensis* were divided into 3 groups. 5 individuals in a tank with a baseline temperature of 13°C who were fed, 5 individuals in elevated temperatures at 27°C who were fed, as well as 5 at 27°C who were fasted. A control tank was used for the fourth group, at 13°C, and individuals were fasted. The fed groups received a forcep-pinch of a carbohydrate-rich food once a week, consisting of bullkelp, *Nereocystis luetkeana,* a total of 2 times. Fasted groups did not receive food for the entirety of the project. Hemolymph was extracted 1.25 hours after the first feeding in trial 1, then 4 hours after feeding in trial 2, to allow for a greater time for digestion and metabolization. Hemolymph was extracted every 30 minutes, 5 times for each group, for a total of 25 readings. After each reading, the crabs were placed back in their respective tanks, and a different crab was used for future readings. The extracted hemolymph was read for glucose using the ReliOn Premier Classic Glucose meter and Test Strips. Only 0.5 µL of hemolymph was needed per reading, but extra hemolymph was saved in each reading for subsequent glucose assays. A reading of “Lo” on the glucometer meant that glucose levels were less than 20 mg/dL, while an “Er4” message indicated insufficient volume or abnormal viscosity. Hemolymph assays were later performed using a Cayman Chemical Glucose Colorimetric Assay Kit. Glucose curves for the two trials were then generated using R for both the values obtained from the glucometer as well as data from the glucose assay.

Results:

For the glucometer glucose curve, we did not obtain usable values except for one reading of 22 mg/dL in the Fed 13°C in trial 1. Except for that reading, all other ones displayed “Lo” when tested. The assay results showed more conclusive results. Both the Fed groups at 13°C and 27°C showed the highest levels of glucose at the last extraction time, in both trials. Though these values differed between the glucometer and the assay (20 vs 0.31 mg/dL). Some values were lost in the glucose assay in the Fed 27°C group in trial 2, due to having too low of hemolymph leftover for a successful and accurate run. Both fasted groups showed the lowest glucose levels in both trials. 2 crab mortalities were experienced in the Fed 13°C group, in the first trial, with a third mortality in the Fasted 27°C group.



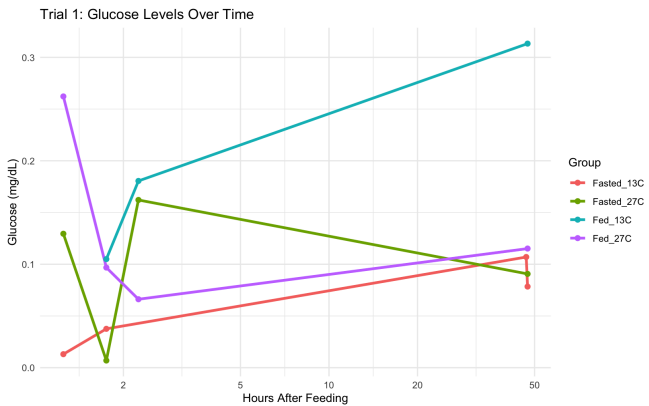
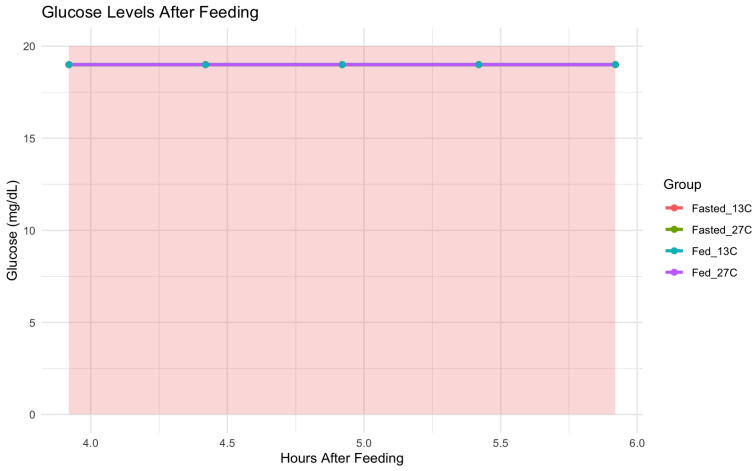


Figure 1: (Left) Line plot showing H. oregonensis glucometer glucose levels in 30-minute intervals after feeding for trial 1. (Right) Line plot showing glucose assay levels at the same intervals for trial 1.



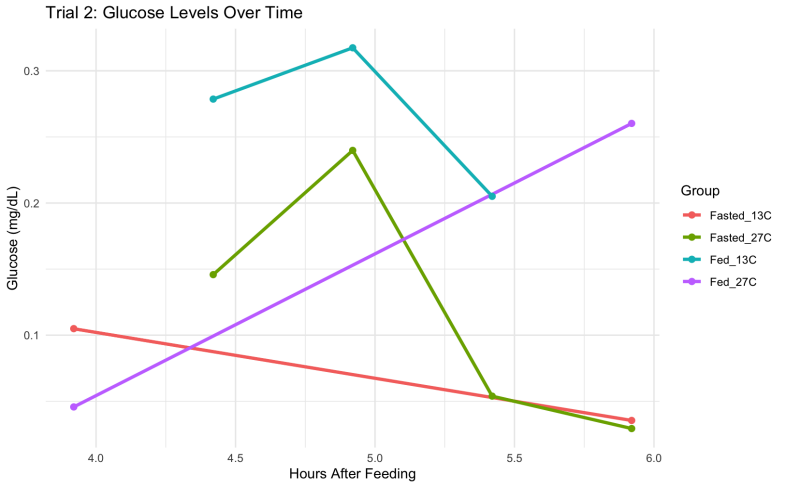


Figure 2: (Left) line plot showing H. oregonensis glucometer glucose levels in 30-minute intervals after feeding for trial 2. (Right) Line plot showing glucose assay levels at the same intervals for trial 2. Some groups are missing data points due to some samples having too small hemolymph levels for glucose assays.

Discussion:

In Figure 1, there is a sharp decrease in the Fed 27°C group directly after the first reading, while there is an increase in the 13°C Fed group. This may indicate that the decapod transit time, as mentioned in McGaw & Curtis (2012), depleted glucose due to the increased temperature environment. Since some data was lost in the Fed 27°C group as well as others, this makes it difficult to interpret its trend throughout the second trial. Though its endpoint is still the highest value at the end of the trial. Altered feeding and temperature states did lead to a difference in glucose levels, with the fed groups having a higher glucose level after the end of each trial, compared to the fasted groups.

The Crustacean Hyperglycemic Hormone (CHH) is also something that directly impacts the levels of glucose in hemolymph. When a stressful event occurs, such as warming, this hormone is excreted through a sinus gland, which then breaks down glycogen. This allows a mobilization of glucose to travel within the hemolymph to other tissues (Miller, 2023). A study involving the blue swimmer crab, *Portunus pelagicus,* found that after experiencing a heat shock, the crabs demonstrated an increase in CHH and glucose, with a depletion in hepatopancreas glycogen. Our results may have indirectly shown this process. In Figure 1, there is a higher level of glucose in the Fasted 27°C group than in the Fasted 13°C group at the second reading. This is also seen in trial 2, with the peak in the Fasted 27°C group. This may indicate that the CHH is being released due to a stress response, leading to higher glucose levels. These

preliminary results, coupled with further research, could help the conservation of invertebrates in the face of marine heatwaves and warming temperatures. As starvation and increased metabolism have been shown to be byproducts of these anthropogenic impacts.

The use of a glucometer to measure glucose is a promising innovation of technology in the field of marine biology. Most successful studies, however, have used dilution methods with the hemolymph, then read them with human-use glucometers. A high glucose solution was mixed in with hemolymph before being read by a glucometer in the crayfish study (Caldari-Torres et. al, 2018). After calculating the difference in glucose levels, this resulted in an accurate reading. Similar methods were used with honeybees, *Apis mellifera,* where a NaCl solution was used to bypass the glucometers' enzymatic assay and to maintain osmotic balance when using a hemolymph sample (Cournoyer et. al, 2022). Unsuccessful results were seen when glucose meters were used directly on the mussel *Perna perna,* due to their very low hemolymph levels. When used on the mangrove fiddler crab Leptuca thayeri, results were obtained, but were higher when compared to a laboratory-level glucose assay (Principe et al. 2019). Since our results with the glucometer reached a similar conclusion, it seems diluting the hemolymph would have made for more accurate and successful readings. Research on this topic is still limited, so further studies could be conducted on what dilution methods would be best for readings, or even for the innovation of glucose monitors for invertebrates.

References:

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