

Final Presentation:

Impacts of temperature on immune response in *Hemigrapsus oregonensis*



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Introduction

Crabs are important!

Snow crab (~US\$200 million ex-vessel industry in East Bering Sea prior to collapse) and Dungeness crab fisheries (Close to US\$70 million ex-vessel industry in Washington state) are important to the economies of the northeast Pacific

Studying hairy shore crabs (*Hemigrapsus Oregonensis*) in lab might allow us to better understand how crabs interact with pathogens amidst temperature rise



Background

Warming Oceans: Anthropogenic driving is causing atmospheric warming, and oceans absorb this heat. The way this heat dissipates through marine systems may affect the physiology of many key species

2024 warmest sea surface temperatures on record (EPA)

Impacts to species distribution, reproductive success, and metabolic rates (Shields 2019; Dehnel 1960)

Thermal stress can reduce thresholds to infection (Dehnel 1960)

Pathogen Exposure: Within a changing climate, pathogen prevalence is shifted.

Bitter crab disease correlated with temperature increases and population density (Balstad et al. 2024)

Host-pathogen interactions are expected to change in several ways (Cohen et al. 2018)

- Host stress levels
- Pathogen transmission
- Pathogen habitat and range
- Host habitat and range

Research Question

What are the impacts of temperature on the immune response of *H. oregonensis*?

H0: There is no resulting difference in immune response between crabs treated with and without heat treatments

Ha: Immune response is displayed in crabs treated with heat by decreases in haemocyte count and increases in the stress response

Haemocyte count ↓

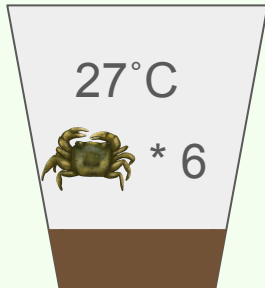
Glucose ↑

Respiration ↑

Righting Time ↑

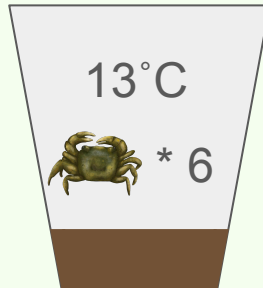
Group 1: Heat, Mud

This treatment will help determine how heat and exposure to pathogens affects crab immune systems.



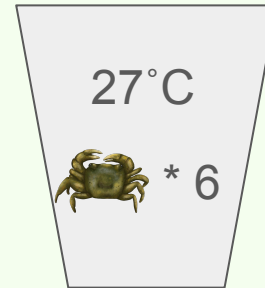
Group 2: No Heat, Mud

This control will help determine the impact the pathogens have so we can hopefully pinpoint the heat impact .

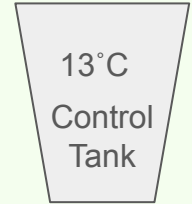


Group 3: Control, No Mud

This treatment will help determine the impact heat have on the crabs, so we can hopefully separate the heat impact from the mud impact.



Control



Temperature

A temperature increase to 27 C was chosen to simulate possible temperature changes due to climate change. Temperature was lowered to 27 C, as suggested post proposal.

Mud

A 1 ½ inch layer of mud was chosen to simulate bacteria and infectants crabs may be exposed to in their natural habitat. Depth was chosen to provide significant hiding space, while allowing the same volume of water in all 3 tanks.

Covers

Two oyster shells were placed on opposing sides of the tank to simulate rocks and other natural habitat structures to help prevent additional stress due to being exposed.

Haemocyte Concentration

Immune Response:

Haemolymph was collected using a needle from the coxa joints of the crabs. Haemocytes were then counted using a hemocytometer.

Physiological assays

Stress:

Righting time was measured by gently placing the crabs on their back and timing how long it took them to right themselves.

Haemolymph was used to measure Glucose.

Respirometry

Stress:

Measured resazurin levels at 30, 60, and 90 minutes using a Resazurin assay.

Replication:

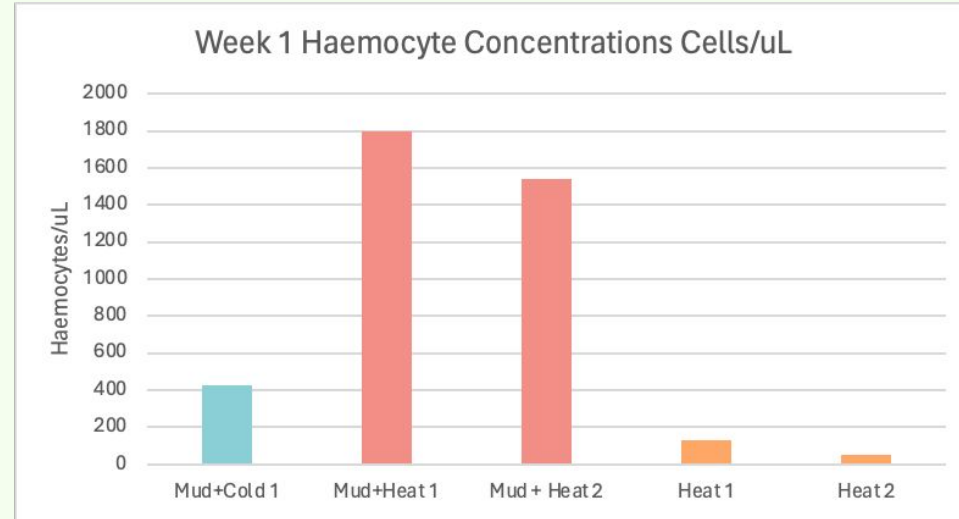
Six crabs allowed for three replicated tests for each treatment, three designated for resazurin and three for haemolymph extraction.

Sample Size:

Six crabs/treatment and using lab control crabs for no heat and no mud control treatment allowed for the highest replication options given the small sample size.

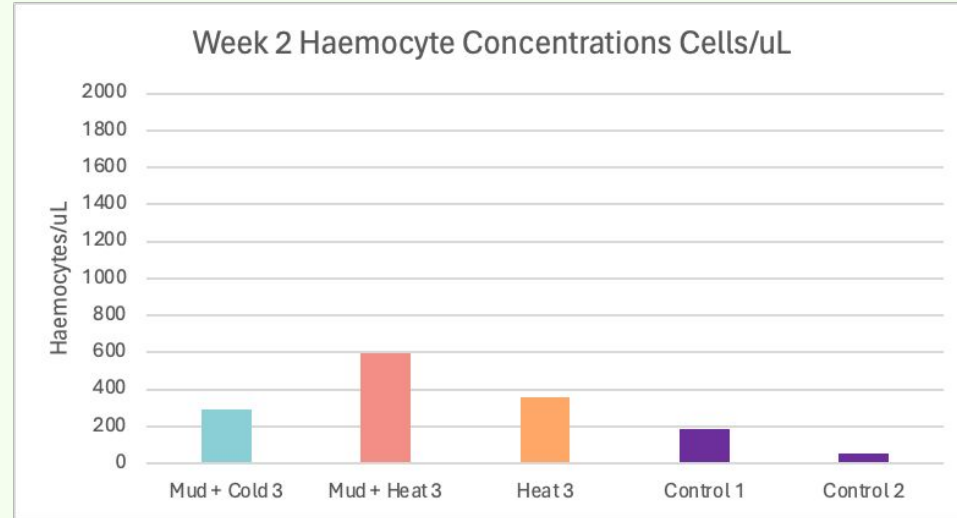
Results-Haemocyte Concentrations Week 1

- Week 1, haemocyte concentrations were highest in the mud+heat treatment groups, ranging from ~1800/uL to ~1500/uL .
- Second highest Haemocyte count was seen in the mud+cold group, ~400/uL.
- This suggests the mud was successful at simulating pathogens.
- The 27C group without mud had counts below 200.
- Suggests immune response in mud tanks, with a higher response under elevated temperatures



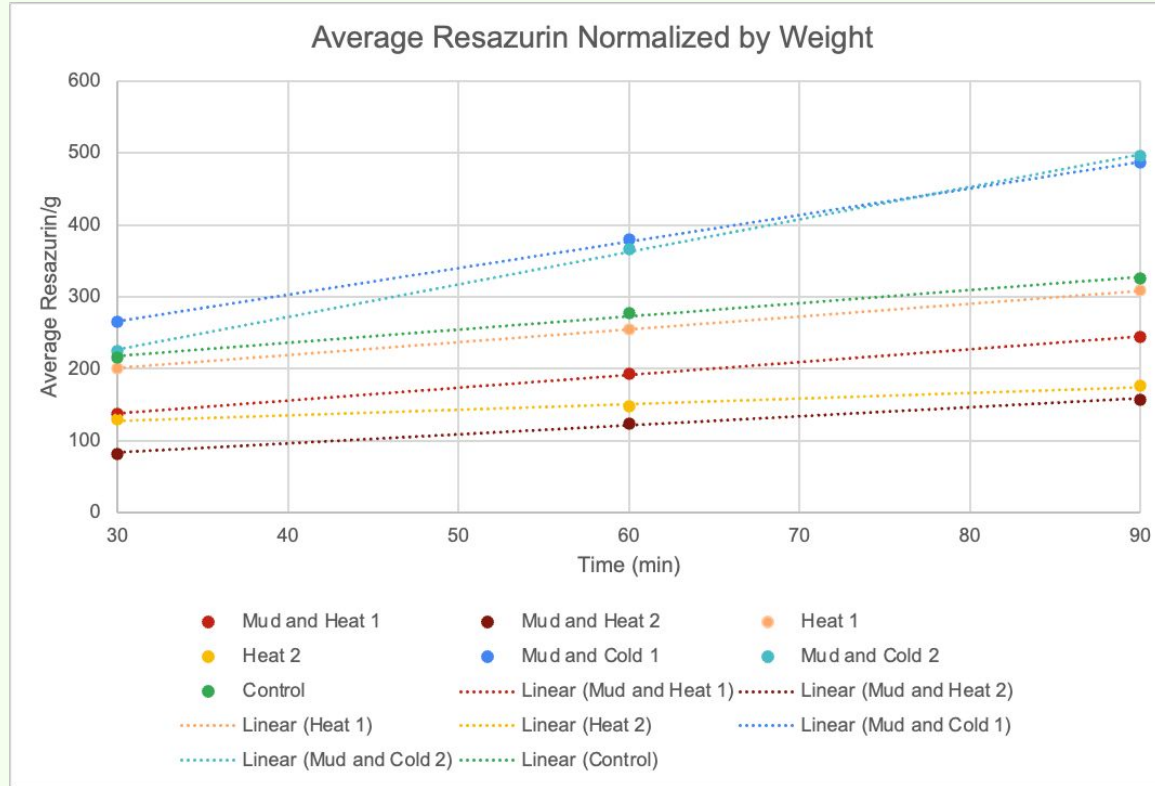
Results-Haemocyte Concentrations Week 2

- Week 2 saw a decrease in haemocyte concentrations in both mud treatment groups.
- Mud+heat dropped to ~600/uL and mud+cold to ~300/uL.
- The 27C treatment saw an increase to ~400
- Control haemocyte concentrations were 200/uL and <100/uL, suggesting an elevated hemocyte concentration in mud groups.



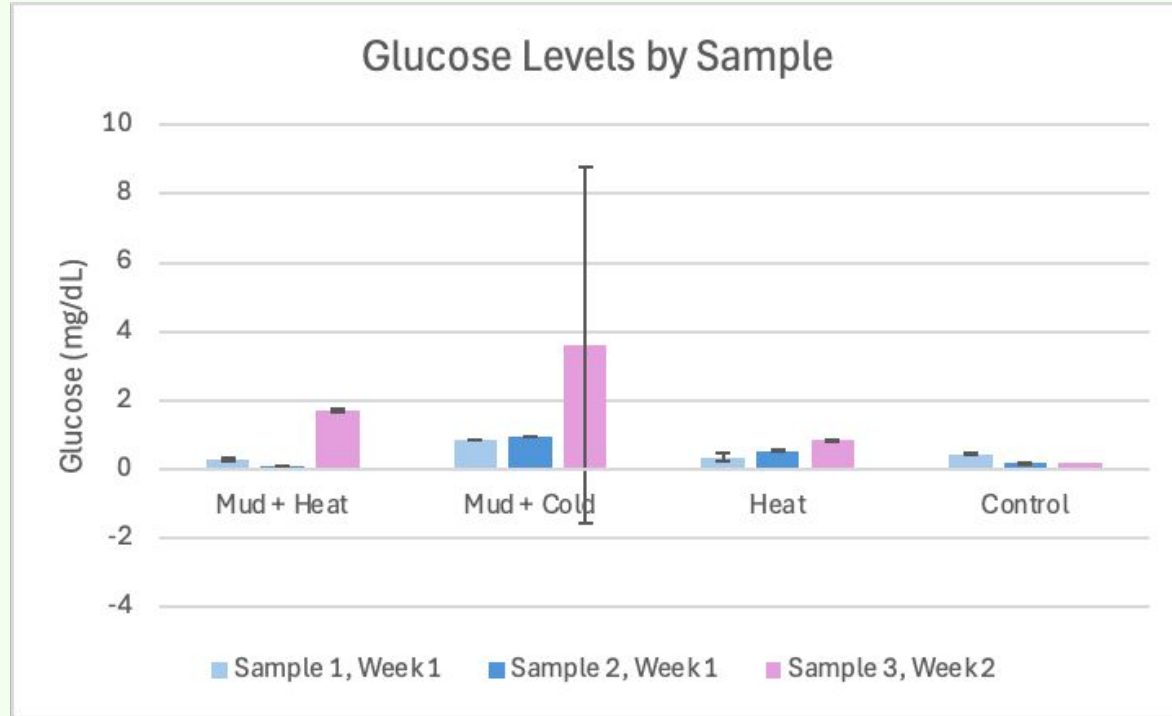
Results- Resazurin

- RFU was the highest in the mud treatment group at 13C.
- The mud+heat and heat groups has little statistical difference
- Mud+heat group had the lowest RFU
- Control had second highest RFU

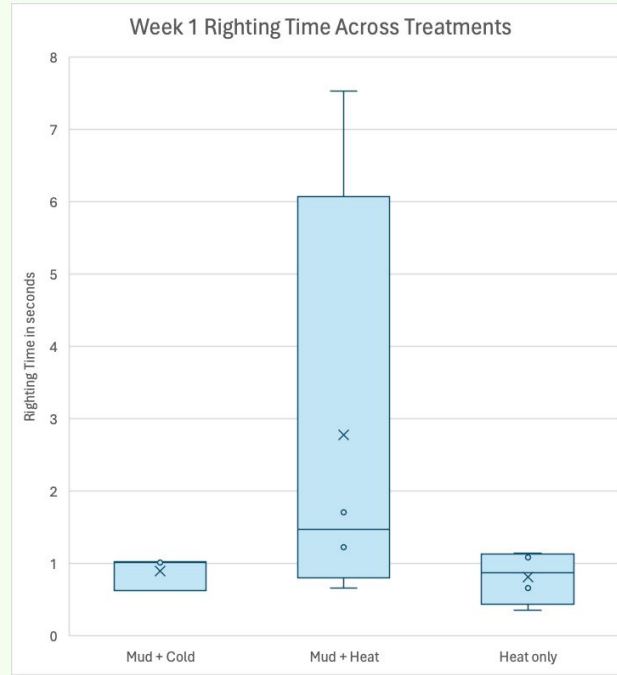
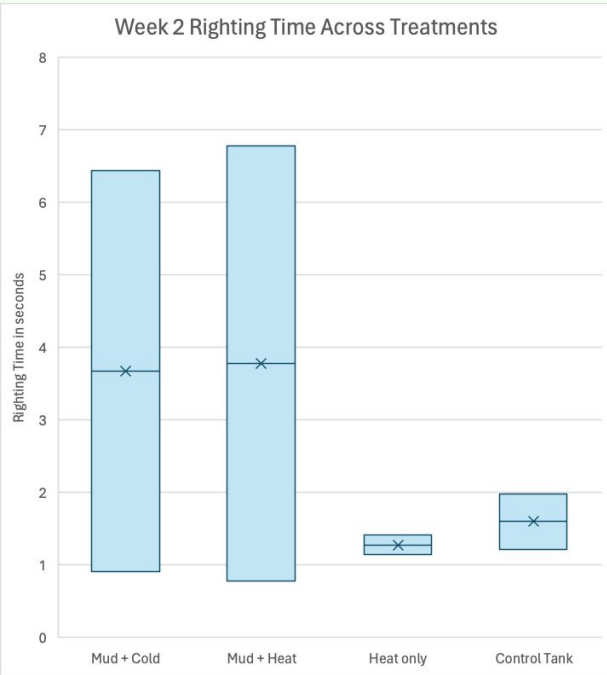


Results-Glucose levels

- Mud+heat had low levels of glucose in week 1 with an increase in week 2
- Similar pattern in mud+cold but with higher glucose levels, large standard deviation in week 2
- Glucose levels increase slightly in week 2 of heat treatment but less so than mud treatments
- Control glucose levels decreased over time



Results-Righting Time



- Righting time was the highest in mud+heat group
- Control righting time average ~1.5 seconds
- Mud+cold had very much different righting time in week 2

Conclusion - Righting time and Resazurin

In mud + 27°C treatment we hypothesized...

Righting time  Respiration Rate (RFU/min) 

Result: fail to reject the null hypothesis, lots of variability



Small sample
size



Handling stress

Conclusion - Haemocyte and Glucose Concentrations

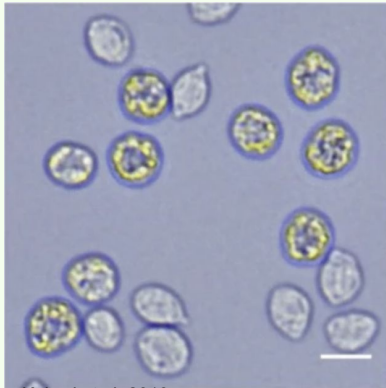
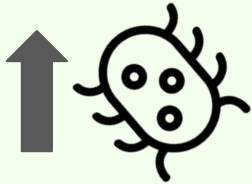
In mud + 27°C treatment we hypothesized...

Haemocyte concentration ↓

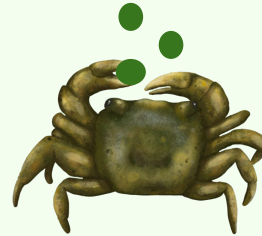
Glucose Concentration ↓

Haemocyte concentration ↑

Glucose Concentration =



Koiwai et al. 2019



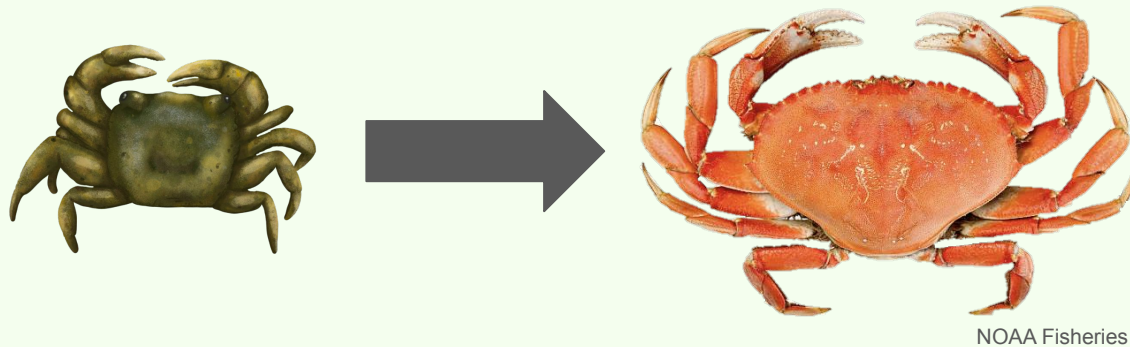
Feeding in mud
treatments



Conspecific
consumption in
mud + 27°C

Conclusion - Implications and Context

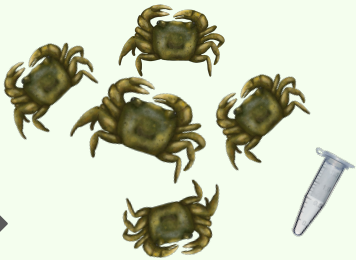
- Increased immune response is energetically taxing
 - Risk of coupled impacts of heat stress and increased immune stress
- Risk of pathogen range expansion
- Threat for Dungeness crab fisheries
 - Decreased growth and reproduction



NOAA Fisheries

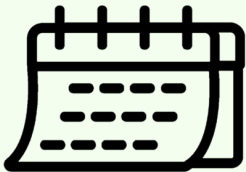
Future Directions

Further hemolymph
assays and stress
assessment



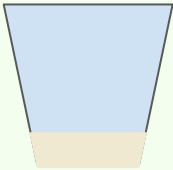
Replication

Does immune
suppression occur?



Longer treatment
exposure

Do different mud
treatments have
different pathogen
impacts?

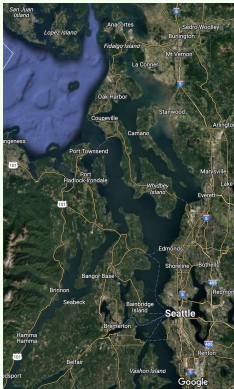


Sterile Substrate



Known
pathogen
load

and



Different mud
collection locations

References

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Thank you!

Any questions?

