# 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

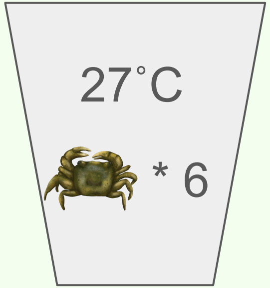
This treatment will help determine how heat and exposure to pathogens affects crab immune systems.


## Group 2: No Heat, Mud

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

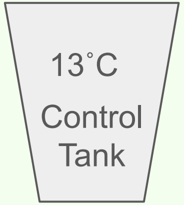

## Group 3: Control, No Mud

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 | Mud | Covers |
|---|---|---|
| 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. | 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. | 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:**<br>Haemolymph was collected using a needle from the coxa joints of the crabs. Haemocytes were then counted using a hemocytometer. |
| **Physiological assays** | **Stress:**<br>Righting time was measured by gently placing the crabs on their back and timing how long it took them to right themselves.<br>Haemolymph was used to measure Glucose. |
| **Respirometry** | **Stress:**<br>Measured resazurin levels at 30, 60, and 90 minutes using a Resazurin assay. |

## Replication:

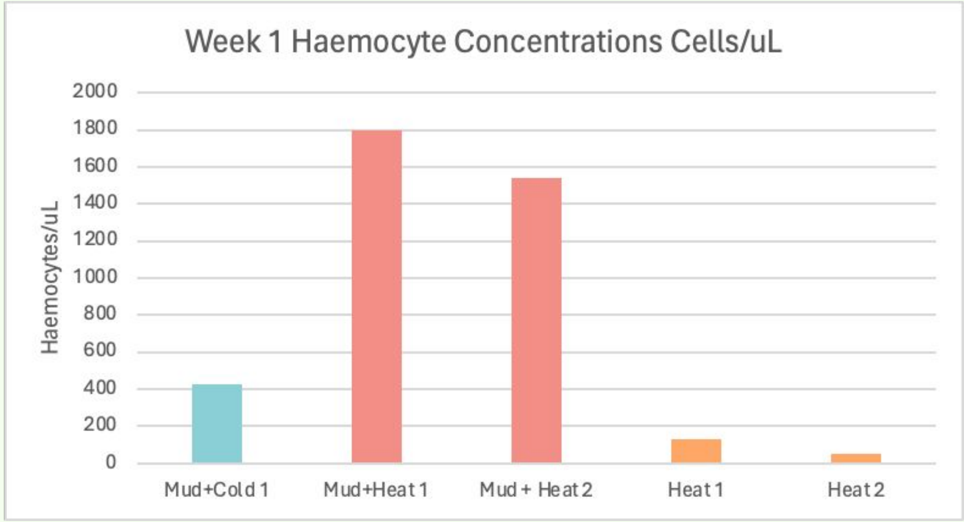
Six crabs allowed for three replicated tests for each treatment, three designated for resazurin and three for haemolymph extraction.

## 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.

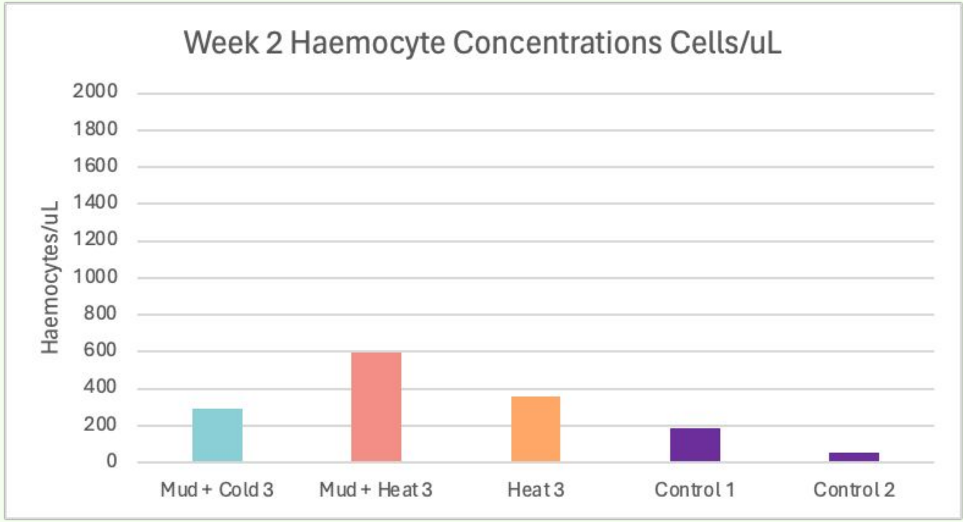
# Results-Haemocyte Concentrations Week 1

- 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



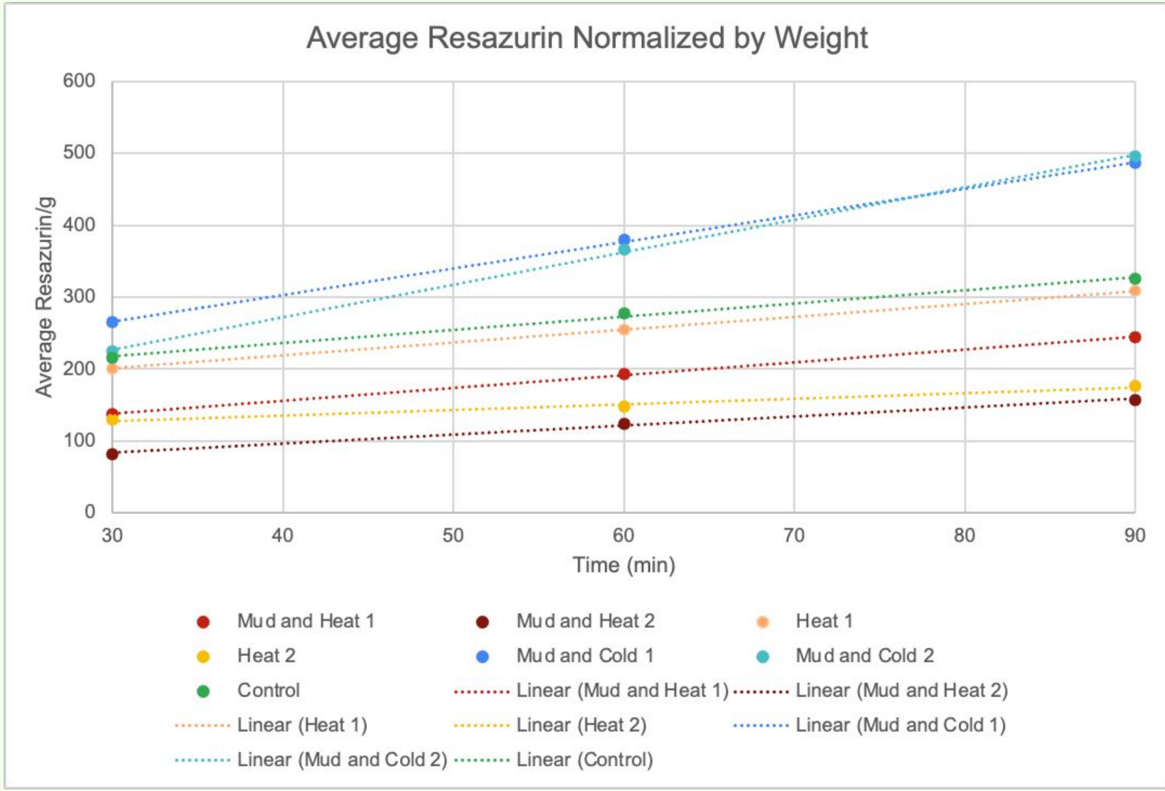
# Results-Haemocyte Concentrations Week 2

- 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.



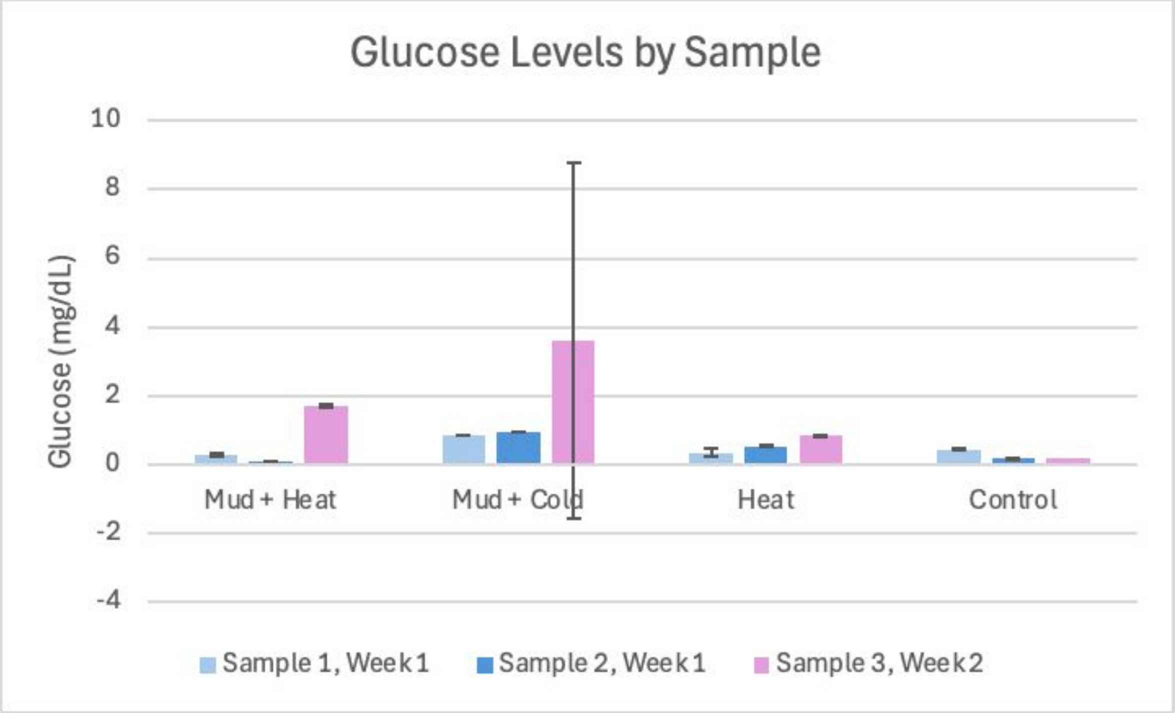
# Results- Resazurin

- 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

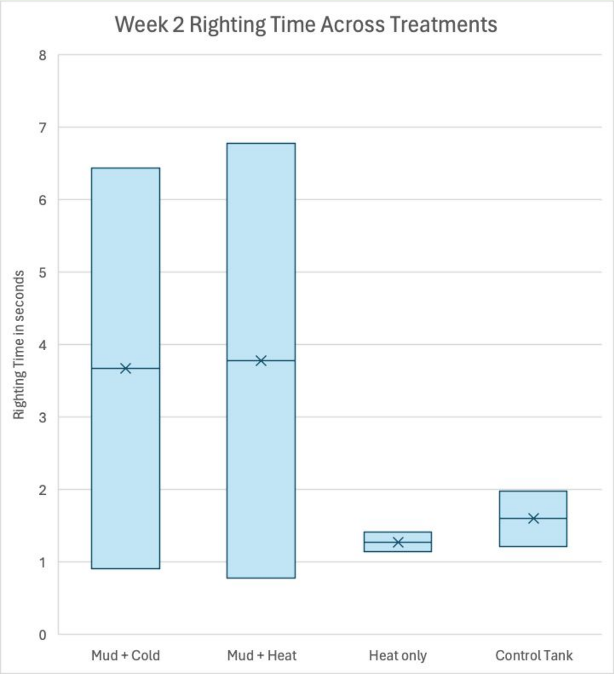


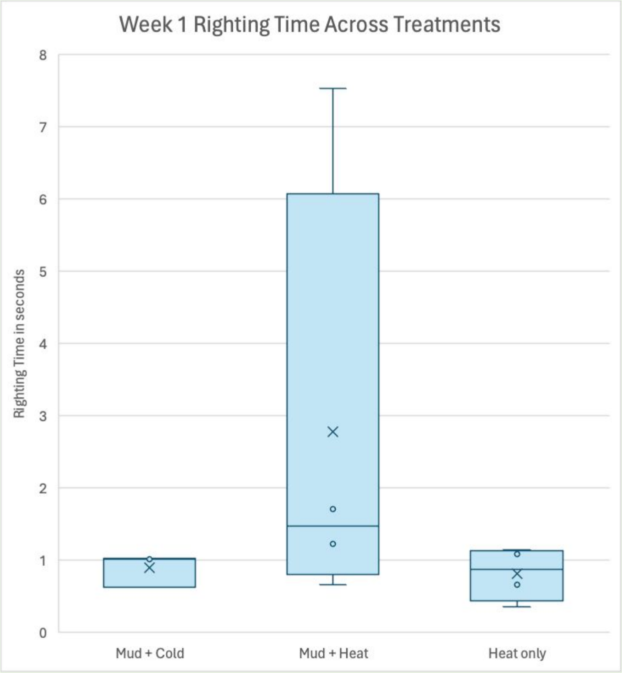
# Results-Glucose levels

- 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

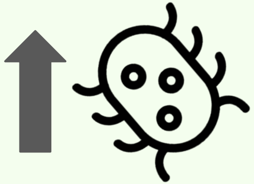

Handling stress

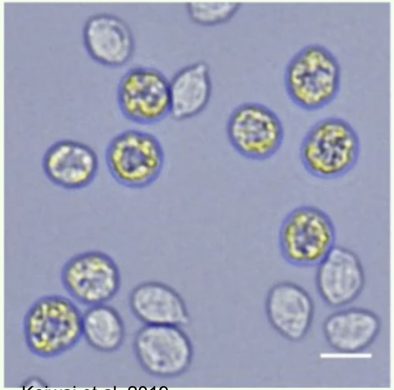
# Conclusion - Haemocyte and Glucose Concentrations

**In mud + 27˚C treatment we hypothesized…**

**Haemocyte concentration ↓** **Glucose Concentration ↓**

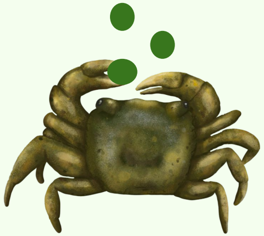
**Haemocyte concentration ↑**





Koiwai et al. 2019

**Glucose Concentration =**

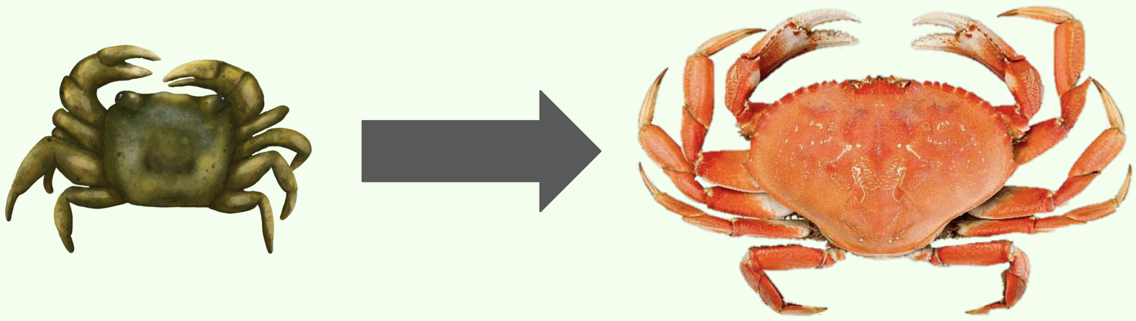


Feeding in mud treatments

Conspecific consumption in mud + 27˚C

# Conclusion - Implications and Context

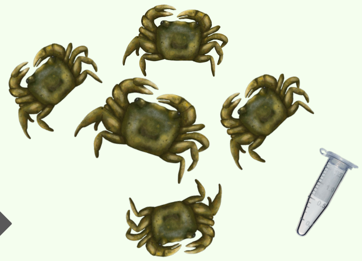
- Increased immune response is energetically taxing
  - Risk of coupled impacts of heat stress and increased immune stress
- Risk of pathogen range expansion
- Threat for Dungeness crab fisheries
  - Decreased growth and reproduction



NOAA Fisheries

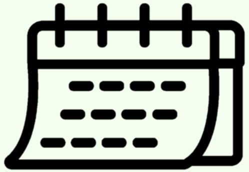
# Future Directions

**Further hemolymph assays and stress assessment**



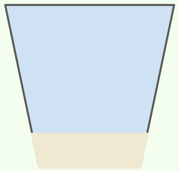
Replication

**Does immune suppression occur?**



Longer treatment exposure

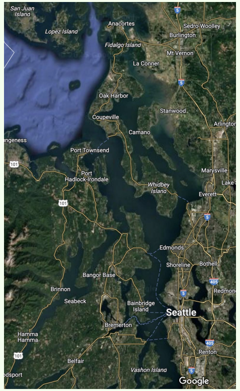
**Do different mud treatments have different pathogen impacts?**



Sterile Substrate

Known pathogen load

**and**




Different mud collection locations

# References

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# Thank you!

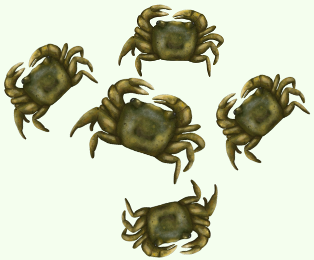
Any questions?

| Criteria | Ratings | Pts |
|---|---|---|
| **This criterion is linked to a Learning Outcome**<br>**Introduction**<br>**Clearly states the topic, its importance, and relevance to the broader field** | **1 pts**<br>**Full Marks**<br>**0 pts**<br>**No Marks** | **1 pts** |
| **This criterion is linked to a Learning Outcome**<br>**Background**<br>**Summarizes relevant literature or prior research and sets up the study's context** | **3 pts**<br>**Full Marks**<br>**0 pts**<br>**No Marks** | **3 pts** |
| **This criterion is linked to a Learning Outcome**<br>**Research Questions**<br>**Articulates focused, testable research questions or hypotheses** | **2 pts**<br>**Full Marks**<br>**0 pts**<br>**No Marks** | **2 pts** |
| **This criterion is linked to a Learning Outcome**<br>**Experimental Design & Methods**<br>**Describes procedures with clarity and justifies methods used; appropriate controls, replication, and sample sizes are addressed** | **3 pts**<br>**Full Marks**<br>**0 pts**<br>**No Marks** | **3 pts** |
| **This criterion is linked to a Learning Outcome** | **4 pts** | **4** |

**Final presentation**

Total Points: 20

# Future Directions



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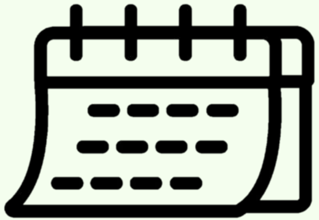
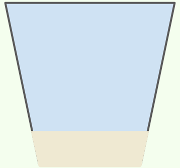


Longer treatment exposure

**Immune suppression?**



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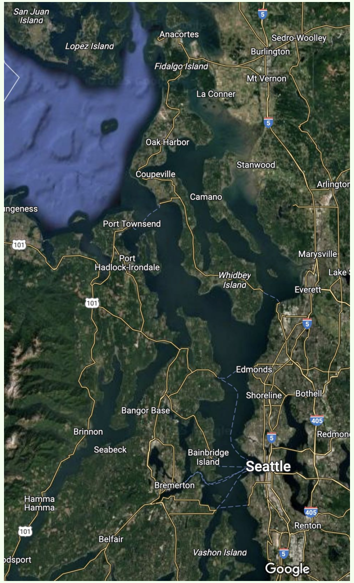


Different mud collection locations

**Pathogen impacts?**