

Impacts of temperature on immune response in *Hemigrapsus oregonensis*



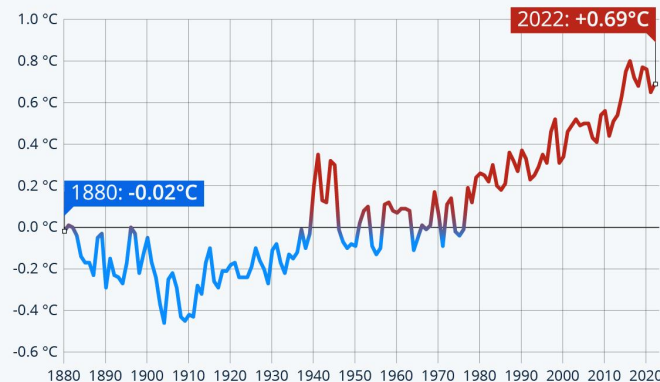
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Background - Warming Oceans

- 2024 was the warmest recorded year for sea surface temperature
- Marine heat waves are increasing in frequency
- Warmer ocean temperatures impact species distributions, reproductive success, and metabolic rates
- Fisheries and economic impacts

The Oceans Are Getting Warmer

Annual divergence of global ocean surface temperature from 20th century average



Source: NOAA National Centers for Environmental Information (NCEI)



statista

<https://www.statista.com/chart/19418/divergence-of-ocean-temperatures-from-20th-century-average/>

Background - Snow Crab Fishery Collapse

- In 2018-2019 the Bering Sea snow crab population declined by 90%
- Snow crab fishery value before the collapse was estimated to be \$227 million.
- Potential Causes:
 - Range shift
 - Starvation due to increased metabolism
 - Increase in bitter crab disease

How does heat stress affect crab's immune systems?

Background - Thermal Stress in Invertebrates

- Ectothermic organisms' physiology can be significantly impacted by temperature changes (Shields 2019)
- *H. oregonensis* is tolerant of temperatures from ~3 – 27°C (Dehnel 1960)
- Increases in body temperature generally lead to increases in oxygen consumption (Dehnel 1960)
- Thermal stress results in lower threshold barrier to infection (Shields 2019)

Background - Invertebrate Immune Response

- Invertebrates under additional stressors exhibit a decrease in immune response (Adamo 2012)
- The innate immune response of invertebrates depends on haemocytes to phagocytize pathogens (Adamo 2012)
- High and low temperature extremes heavily impact crab immune systems (Truscott and White 1990)

Background - Pathogens and Climate Change

- Host-pathogen dynamics are changing in four key ways (Cohen 2018):
 - Host stress levels
 - Pathogen transmission
 - Pathogen habitat and range
 - Host habitat and range
 - Habitat preference plays an important role (Dittmer 2011)



Research Gap

- Understood susceptibility of crabs to parasitism under stress, but a lacking understanding exists of how locally present parasites interact with *H. Oregonensis*
- Host - pathogen interactions in commercially important hosts (*H. Oregonensis* as a proxy)

Hypothesis:

Null hypothesis: Haemocyte concentrations and stress does not change in *Hemigrapsus oregonensis* treatments with higher temperatures.

Alternative hypothesis: Higher temperatures result in increased stress levels and decreased haemocyte concentrations in *Hemigrapsus oregonensis* when exposed to pathogens.

Sub-Alternative Hypothesis

Under heat-stress conditions...

Righting time ↑

BCA Protein ↓

Glucose ↑

Osmolarity ↑

Lactate ↑

Oxygen consumption ↑

Triglycerides ↓

Haemocyte concentration ↓

compared to control conditions.

Treatment Groups and Experimental Design

Group 1: Control, no Mud

- Five Crabs
- ~13°C.
- No Mud
- 1-2 covers

Group 2: Control, Mud

- Five Crabs
- ~13°C.
- 1 ½ inches of mud
- 1-2 covers

Group 3: Heat, Mud

- Five Crabs
- 30°C.
- 1 ½ inches of mud
- 1-2 covers

Group 4: Heat, No Mud

- Five Crabs
- 30°C.
- No Mud
- 1-2 covers

Treatment Groups and Experimental Design

Temperature

A temperature increase to 30 C was chosen to simulate possible temperature changes due to climate change.

Needed a temperature high enough to trigger changes without heating beyond crab's ability to adapt. This temperature was chosen based off of papers performing similar research.

Mud

A layer of mud, roughly 1 ½ inches deep was chosen to simulate bacteria and infectants crabs may be exposed to in their natural habitat.

Covers

Covers, numbering 1-2, will be placed in the tanks to simulate rocks and other natural habitat structures crabs can use to hide or for protection.

These will be placed in all tanks to help prevent additional stress due to being exposed.

Stress and Immune Response Measurements

Haemocyte Concentration

Immune Response:

Will be measured by: counting haemocytes in a hemocytometer

Physiological assays

Stress:

Will be measured by: Righting time, extracting haemolymph to measure Glucose, Lactate, Triglycerides, BCA protein, Osmolarity

Respirometry

Stress:

Will be measured by: Resazurin assay

Haemocyte Concentration

- Using a needle to break through the membrane, we can extract haemolymph from the merus of the leg of the crab.
- We can then use a haemocytometer

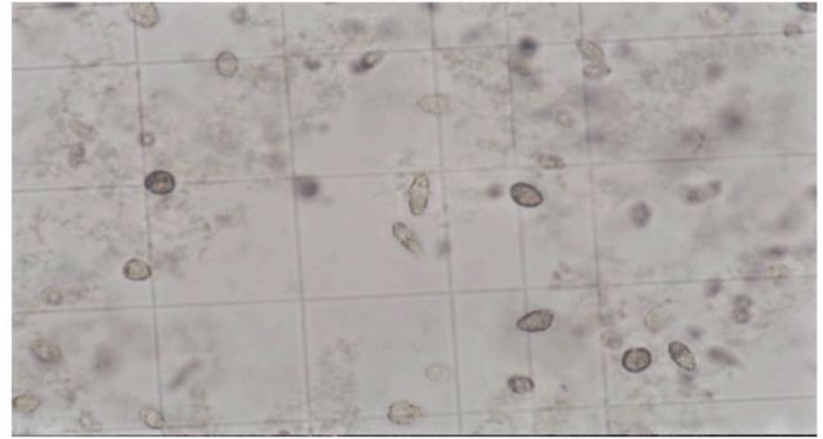
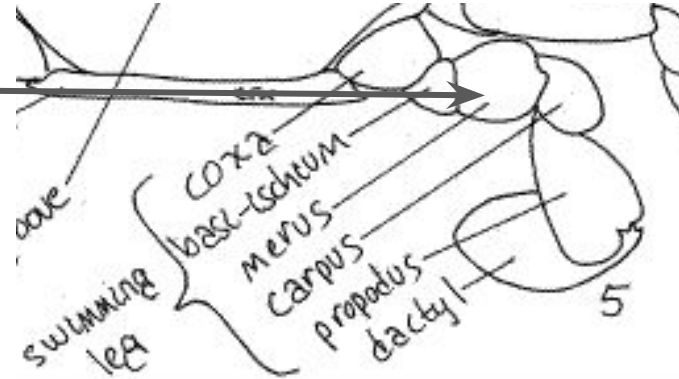
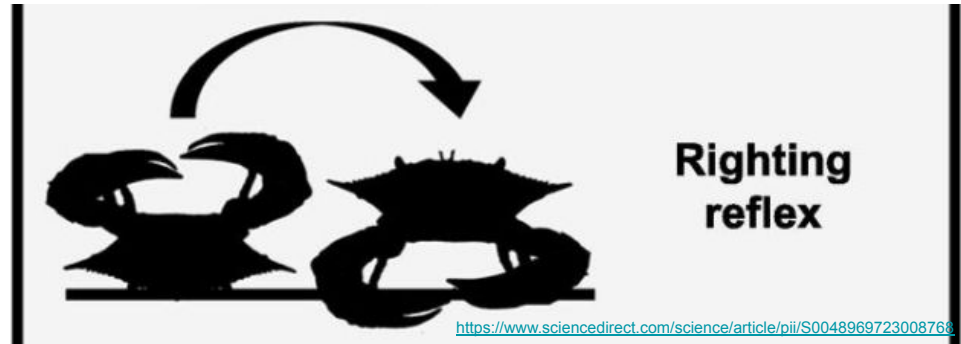


Figure 3. Semigranule haemocyte in the haemolymph of male blue crab (x400).
<https://www.imedicalteam.pl/articles/determination-of-haemocytes-amount-and-haemocytes-type-in-mature-blue-crab-callinectes-sapidus-rathbun-1896-captured-in-105084.htm>



Physiological Assays

- In order to measure righting time we will flip the crabs onto their back and time how long it takes them to get back into an upright position again.
- To measure the glucose level of the crabs' haemolymph, we will be using the glucose oxidase method.
- Lactate can be extracted from haemolymph and measured using an enzymatic assay.

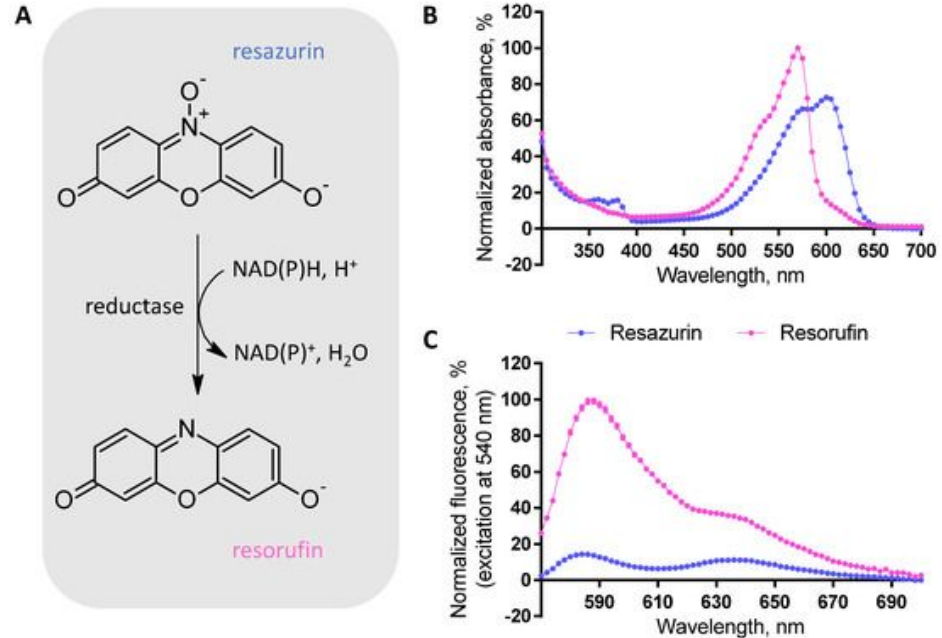


Physiological Assays

- Triglycerides extracted from homogenized crab tissue can be measured using enzymatic assays.
- To measure protein concentration, a BCA (Bicinchoninic Acid) assay can be used, the more purple the final solution is, the higher the protein concentration.
- Measuring osmolarity in crabs can be done by collecting haemolymph and using a vapour pressure osmometer or cryoscopic osmometer

Respirometry

- The Resazurin Assay can be used as an indirect measurement of respiration rate.
- Fluorescence spectrometry can measure the concentration of resorufin using the red colour.



Limitations

- Mud sample is highly variable factor with little control on our end
- Simple one host scenarios involving just *H. oregonensis* and lacks definitive hosts
- Confounding aggregators of stress forcing on crabs
 - Temperature
 - # of crabs confined to space
 - Peripheral movement
- Incapacity to observe specific parasites

Relevance to real-world ecological or management outcomes

- Our experiment will use the yellow shore crab (*H. oregonensis*) as a proxy for the Dungeness crab (*M. magister*).
- The 2023-2024 Dungeness crab season ex-vessel value in Washington state was \$66.8 million
- Dungeness crabs are harvested by the Hoh, Quinault, Makah, and Quileute tribes
- Will rising ocean temperatures affect the Dungeness crab's ability to fight diseases like BCB?

Project Timeline and Milestones

- Today (project proposal)
- April 29th - Study setup
- April 29th - Data collection
- May 6th - Data collection
- May 13th - Data collection
- Hypothesis defense and initial results
- Preliminary paper
- Final presentation



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