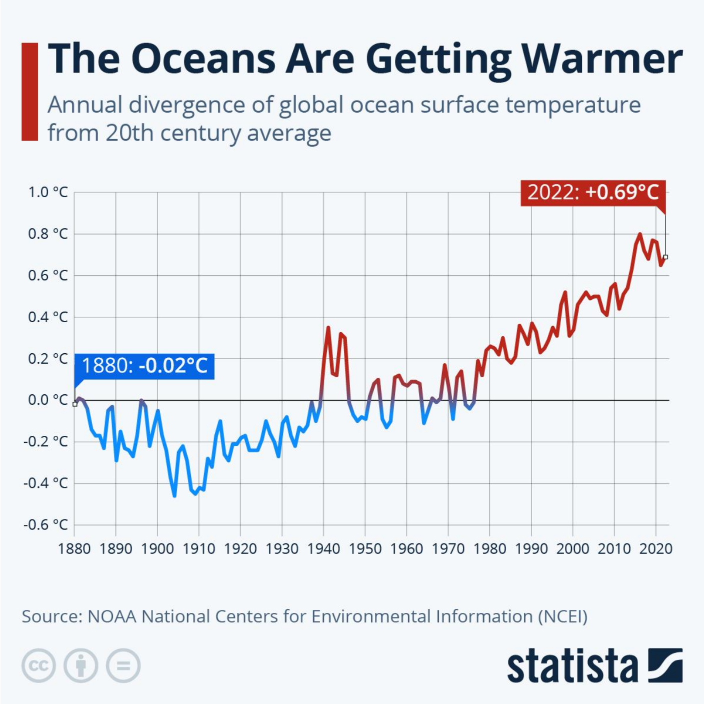
Impacts of temperature on immune response in *Hemigrapsus oregonensis*

Emily, Isaac, Kea, Luke

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



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

Climate change indicators: Sea surface temperature. (2023, July 21). US EPA. https://www.epa.gov/climate-indicators/climate-change-indicators-sea-surface-temperature

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

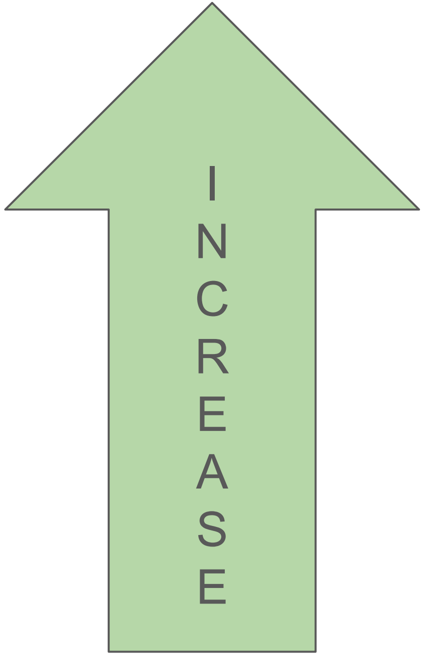
Under heat-stress conditions…

Righting time ↑	BCA Protein ↓
Glucose ↑	Osmolarity ↑
Lactate ↑	Oxygen consumption ↑
Triglycerides ↓	Haemocyte concentration ↓

compared to control conditions.

Sub-Alternative Hypothesis

Under heat-stress conditions…



Righting time

Glucose

Lactate

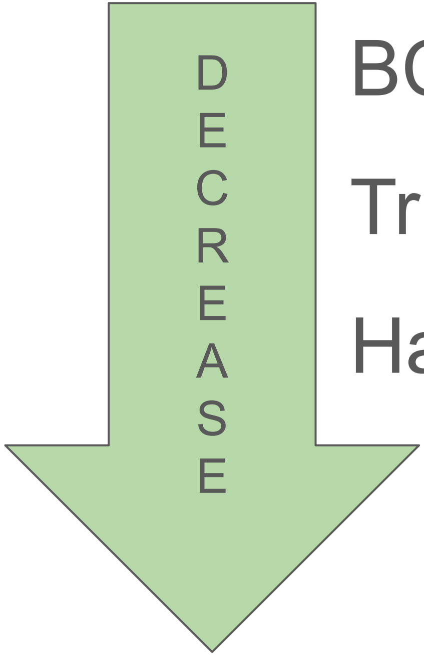
Osmolarity

Oxygen consumption

compared to control conditions.

Sub-Alternative Hypothesis

Under heat-stress conditions…



BCA Protein

Triglycerides

Haemocyte concentration

compared to control conditions.

Sub-Alternative Hypothesis

A: Righting time - Crabs treated with warmer temperatures and immune challenge presence (mud) will take longer to right themselves than control crabs.

B: Glucose - Crabs treated with warmer temperatures and immune challenge presence will experience higher haemolymph glucose levels than control crabs.

C: Lactate - Crabs treated with warmer temperatures and immune challenge presence will experience higher haemolymph lactate levels.

D: Triglycerides - Crabs treated with warmer temperatures and immune challenge presence will experience decreased levels of triglycerides compared to control crabs.

E: BCA Protein - Crabs treated with warmer temperatures and immune challenge presence will experience decreased levels of BCA proteins compared to control crabs.

F: Osmolarity - Crabs treated with warmer temperatures

G: Respirometry - Crabs treated with warmer temperatures and immune challenge presence will experience increased respiratory rates compared to control crabs.

H: Haemocyte Concentration - Crabs treated with warmer temperatures will experience decreased haemocyte concentrations compared to control crabs.

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

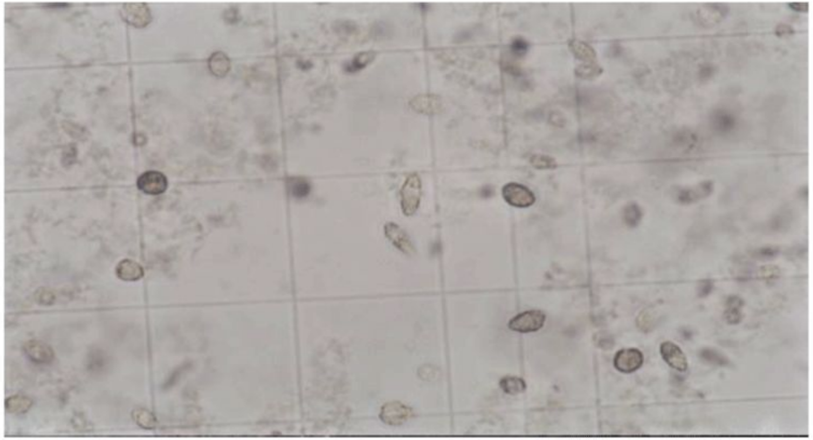
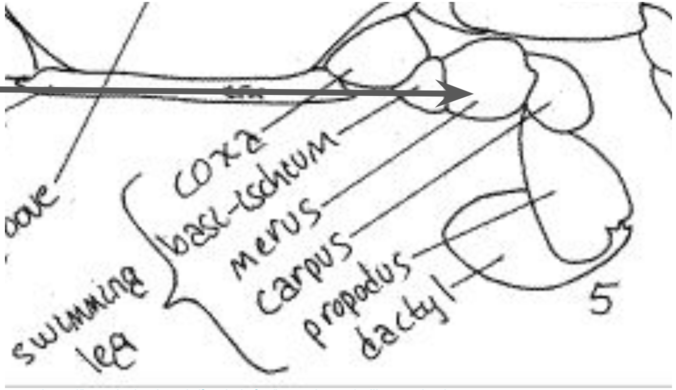


Figure 3. Semigranule haemocyte in the haemolymph of male blue crab (x400).

https://www.itmedicalteam.pl/articles/determination-of-haemocytes-amount-and-haemocytes-type-in-mature-blue-crab-callinectes-sapidus-rathbun-1896-captured-in--105084.html



https://lanwebs.lander.edu/faculty/rsfox/invertebrates/callinectes.html

Haemocyte Concentration

Using a syringing needle to break through the arthrodial membrane, we can extract haemolymph from the merus of the swimming leg of the crab.

We can then use a haemocytometer to count the haemocyte concentration within the crab’s haemolymph.

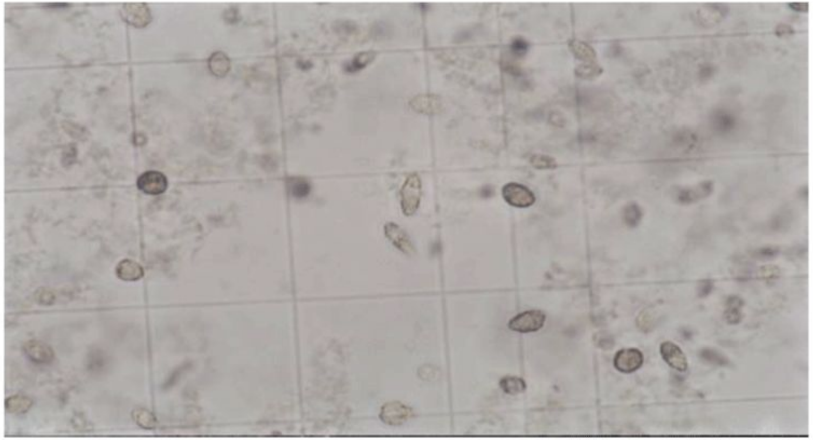


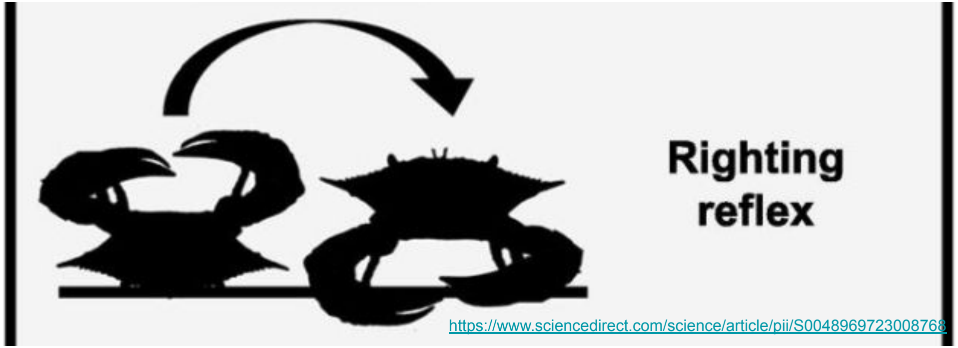
Figure 3. Semigranule haemocyte in the haemolymph of male blue crab (x400).

https://www.itmedicalteam.pl/articles/determination-of-haemocytes-amount-and-haemocytes-type-in-mature-blue-crab-callinectes-sapidus-rathbun-1896-captured-in--105084.html

https://lanwebs.lander.edu/faculty/rsfox/invertebrates/callinectes.html

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.



https://www.sciencedirect.com/science/article/pii/S0048969723008768

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. This will be done multiple times and the average righting time will be taken into consideration.

To measure the glucose level of the crabs’ haemolymph, we will be using the glucose oxidase method. Glucose reacts with glucose oxidase forming hydrogen peroxide and gluconic acid. The hydrogen peroxide can then be measured using peroxidase and a chromogen (such as 2-amino-4-hydroxybenzenesulfonic acid (AHBS)).

Lactate can be extracted from haemolymph and measured using an enzymatic assay. One assay is very similar to the glucose oxidase assay, using lactate oxidase to produce pyruvate, which then produces hydrogen peroxide which can be measured using a peroxidase and chromogen, as discussed above.

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

Physiological Assays

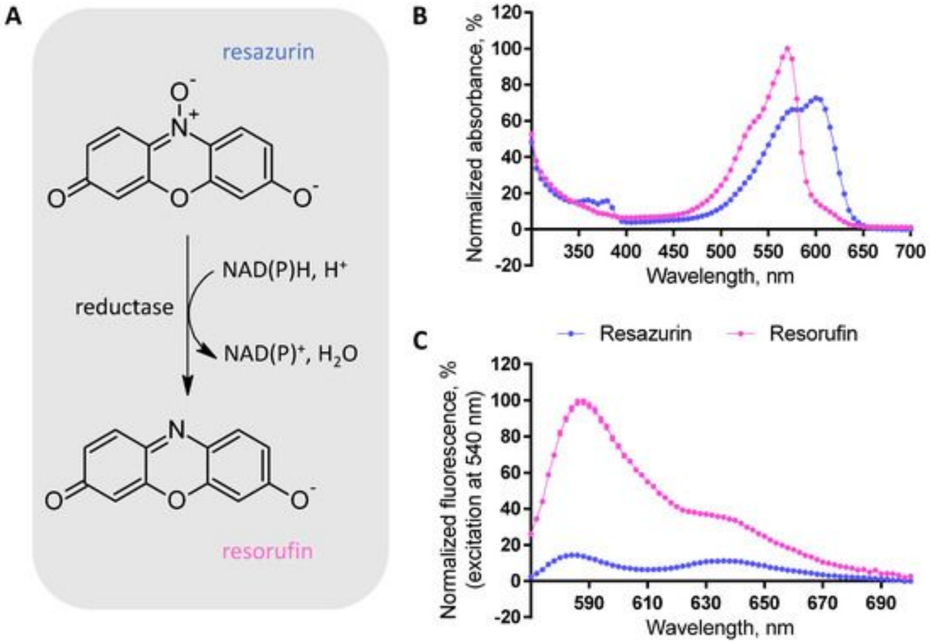
Triglycerides can be measured by taking crab tissue and homogenizing it. They can then be extracted using methanol/chloroform and measured using methods such as enzymatic assays.

To measure protein concentration, a BCA (Bicinchoninic Acid) assay can be used by mixing the sample with a BCA reagent and using a spectrophotometer to measure colour absorbance. The more purple the final solution is, the higher the protein concentration from the sample.

Measuring osmolarity in crabs can be done by collecting haemolymph and using a vapour pressure osmometer or cryoscopic osmometer

Respirometry

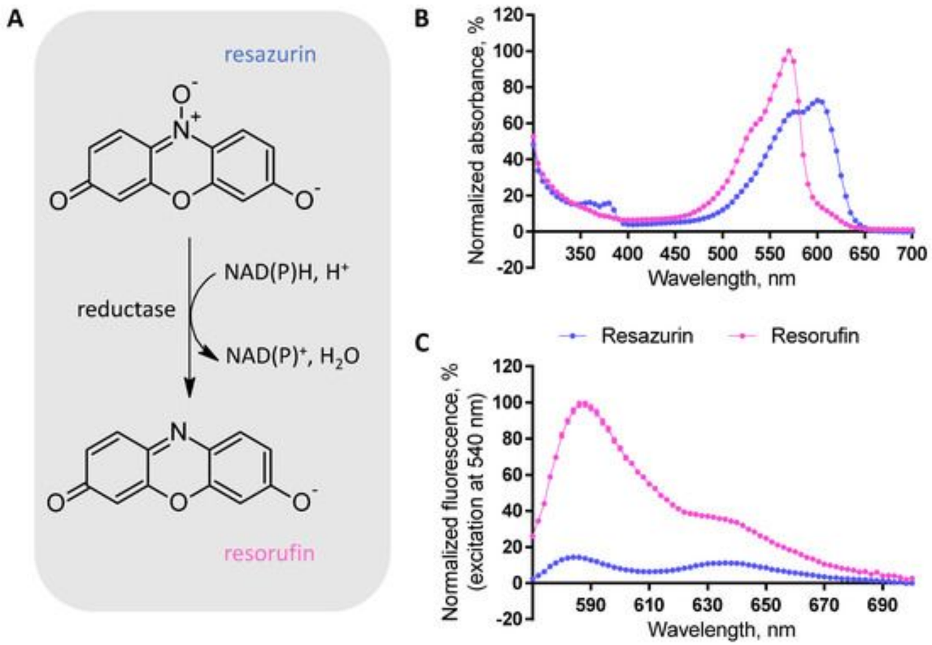
- The Resazurin Assay can be used as an indirect measurement of respiration rate.
- Fluorescence spectrometry can measure the concentration of resorufin using the red colour.



Respirometry

The Resazurin Assay, is a downstream indicator of cellular metabolic activity, and while not a direct indicator of respiration metabolic rate has been closely linked to respiration rate.

Measurement through the reduction of blue resazurin to red resorufin, which can be determined using fluorescence spectrometry or a colorimeter.



Our experiment will use the yellow shore crab (*H. oregonensis)* as a proxy for the Dungeness crab *(M. magister).*

The aim of this experiment is to study how rising temperatures due to climate change may affect disease and infection rates in Dungeness crab. Bitter crab disease has big impacts on fisheries, costing millions of dollars. An increase of bitter crab disease and others could be greatly detrimental to Washington fisheries.

-find out value of dungeness fishery

-talk about importance of dungeness to economy, culture/tourism/tribes

-bitter crab disease

-investigate income lost from bcb and other crab diseases.

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

Works Cited:

Adamo, S. A. (2012). The effects of the stress response on immune function in invertebrates: An evolutionary perspective on an ancient connection. *Hormones and Behavior*, *62*(3), 324–330. https://doi.org/10.1016/j.yhbeh.2012.02.012

Alaska Fisheries Science Center. (2025, March 3). *Snow crab collapse due to ecological shift in the Bering Sea*. NOAA Fisheries. https://www.fisheries.noaa.gov/feature-story/snow-crab-collapse-due-ecological-shift-bering-sea

Climate change indicators: Sea surface temperature. (2023, July 21). US EPA. https://www.epa.gov/climate-indicators/climate-change-indicators-sea-surface-temperature

Cohen, R.E., C.C. James, A. Lee, M.M. Martinelli, W.T. Muraoka, M. Ortega, R. Sadowski, L. Starkey, A.R. Szesciorka, S.E. Timko, E.L. Weiss, and P.J.S. Franks. 2018. Marine host-pathogen dynamics: Influences of global climate change. *Oceanography* 31(2):182–193, https://doi.org/10.5670/oceanog.2018.201.

Dehnel L, P. A. (1960). Effect of temperature and salinity on the oxygen consumption of two intertidal crabs. *The Biological Bulletin*, *118*(2), 215–249. https://doi.org/10.2307/1538998

Dittmer, J., Koehler, A. V., Richard, F.-J., Poulin, R., & Sicard, M. (2011). Variation of parasite load and immune parameters in two species of New Zealand shore crabs. *Parasitology Research*, *109*(3), 759–767. https://doi.org/10.1007/s00436-011-2319-2

Shields, Jeffery, Climate change enhances disease processes in crustaceans: case studies in lobsters, crabs, and shrimps, *Journal of Crustacean Biology*, Volume 39, Issue 6, November 2019, Pages 673–683, https://doi.org/10.1093/jcbiol/ruz072

Works Cited:

Snow crab collapse due to ecological shift in the Bering Sea. (2024, August, 21). NOAA. https://www.fisheries.noaa.gov/feature-story/snow-crab-collapse-due-ecological-shift-bering-sea

Truscott, R., & White, K. N. (1990). The Influence of Metal and Temperature Stress on the Immune System of Crabs. *Functional Ecology*, *4*(3), 455–461. https://doi.org/10.2307/2389609

Washington Coastal Dungeness Crab Fishery Newsletter. (2024). Washington Department of Fish & Wildlife. https://wdfw.wa.gov/sites/default/files/2025-01/newsletter-dungeness-crab-fishery-2024-5.pdf

Proposal assignment requirements

From the ROP

- Project Title and Summary (max 300 words)
- Research Objectives and Background
- Methodology, including stressor types and physiological metrics
- Relevance to real-world ecological or management outcomes
- Timeline and Milestones
- Experimental Design and Justification

From the slides assignment:

- We expect you to prepare a 10-15 min group presentation using PowerPoint slides.
- You must include a background (using relevant literature) leading to a general research question, and a set of specific hypothesis (framed as null and alternative hypotheses)
- You must include a very specific experimental design, making sure that results generated therein will answer the hypothesis you propose. Don't forget the use replicates.
- Be ready to receive feedback and to answer questions. You will be graded on both the presentation of your project, and the defense of it (your response to questions from the audience, including the teaching team). Expect to use the whiteboard to answer some of the questions.
- Every member of the research team must participate in the presentation and defense of the project, otherwise members that do not engage will be penalized with a 20% penalty in their grade.

Summary

The collapse of the Bering Sea snow crab fishery and the mystery surrounding it necessitates a cultivation of knowledge surrounding crab physiology and ecology. Marine heatwaves, proximal to global temperature forcing upon oceans, is the culprit behind a population decline of over 10 billion individuals (Szuwalski et al. 2023;) in

Week 5	Apr28	Apr29	Collect Data I
Week 6	Apr29	Apr30	Collect Data II
Week 7	May5	May6	Collect Data III
Week 8	May12	May13	Present initial results; hypotheses defense (google slides)
Week 9	May19	May20	Mini paper due (use lab for peer-feedback)
Week 10	Jun2	Jun3	Final project presentation