Research Proposal Defense:

The Ecophysiological Effects of Water Temperature and Ibuprofen on *Hemigrapsus oregonensis*

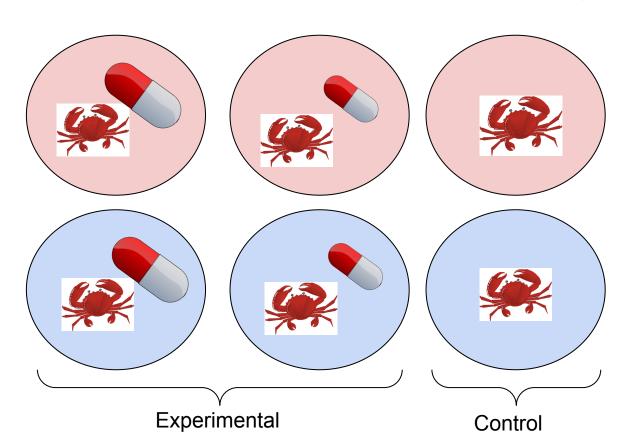
Background

- Hemigrapsus oregonensis as a species is known to have a broad physiological tolerance which
 makes it ideal for studying stress responses.
- Fernandes et al. (2021) explained that even though the presence of pharmaceuticals in aquatic environments has been known for years, only in the last 10-15 years have analytical methods had the capacity to test for environmentally relevant concentrations of the pharmaceuticals.
- Mezzelani et al. (2016) detected the presence of Non-Steroidal Anti-inflammatory drugs in wild mussel tissue in a coastal area not affected by industrial pollution.
- Berezina et al. (2021) found increased oxygen consumption in a freshwater amphipod exposed to environmentally relevant concentrations of NSAIDS.
- Blasco & Trombini (2023) found a marine invertebrate showed a decrease in respiratory rate when exposed to NSAIDS.
- Eshky et al. (1996) found that when a crab species was exposed to increased water temperature it had an increased oxygen consumption rate.
- Stress at the individual level could scale up and affect mortality, behavior and ecosystem structure.
- "If crabs can't respire or feed—What does that mean for the rest of the ecosystem?"

Research Questions

- 1. How will increasing concentrations of ibuprofen affect **glucose** and **respiration** rates in *H. oregonensis*?
- 2. How will an increase in temperature affect **glucose** and **respiration** rates in *H. oregonensis*?
- 3. How will the combined effects of ibuprofen concentrations and increased temperature affect **glucose** and **respiration** rates in *H. oregonensis*?

Experimental Design



High Temperature

Ambient Temperature

lbuprofen environmental dose (0.02 µg/L)

Ibuprofen high dose (0.1 µg/L)

> 3 replicates 6 crab in each

Hypotheses

Null Hypothesis(Ho):

Ho1: Ibuprofen has no effect on respiration and glucose levels

Ho2: Increased temperature has no effect on respiration and glucose level

Ho3: There is no interaction between ibuprofen and temperature on respiration and glucose levels

<u>Alternative Hypothesis(Ha):</u>

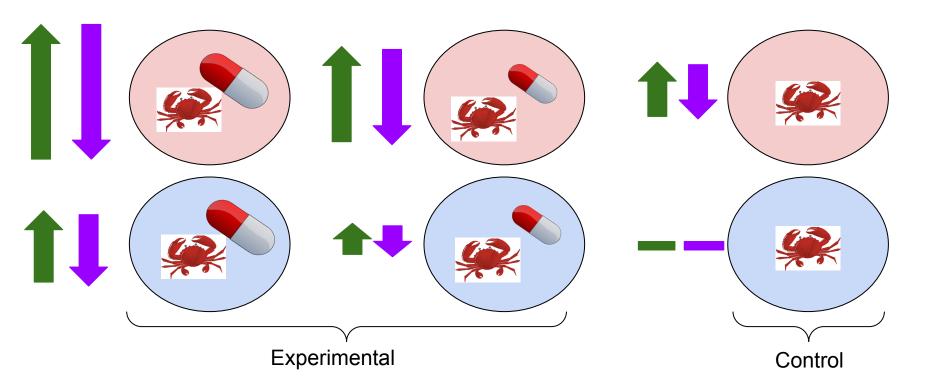
Ha1: Ibuprofen exposure causes increased respiration and a decrease in glucose levels

Ha2: Increased temperature causes increased respiration and a decrease in glucose levels

Ha3: Ibuprofen exposure and increased temperature have an additive effect on respiration and glucose levels

Glucose Respiration

Hypotheses



Justification

Ibuprofen

- Affordable and accessible
- Levels: 0.0217 μg/L (Salish Sea value) and 0.1 μg/L

Temperature

- Increase temperature due to climate change
- Levels: 8.5°C (Puget Sound SST) and 20°C

Respiration

○ Increased temperatures raise metabolic rate → higher oxygen consumption

Glucose

• Will indicate change in metabolic rate and energetic cost within the different environments

Timeline

- 3 weeks of data collection
- Glucose measurements:
 - Wait until the end to gather from hemolymph do not want to accidentally kill crabs before data gathering is done
 - Able to see difference between treatments
- Oxygen consumption/respiration:
 - Weekly data collection of water use respirometry with resazurin
 - Able to see not only difference between treatments but possibly the change of oxygen consumption overtime within treatments

Real World Relevance

Climate change

- Affecting all marine organisms
- Increasing temperature → increased metabolic demands and reduction of oxygen availability

Pharmaceutical pollution

- Water being put into the ocean are not being treated for NSAIDs that are affecting organisms
- Affecting marine organisms that live on/near the shore (like crabs!) and organisms that move near the shore (like fish)

Understanding how other organisms will be affected by NSAIDs

 Crabs are early signals to these challenges as they are in close contact with NSAIDs and are experiencing temperature changes the fastest by living on the shore

References

- 1. Berezina, N. A., Sharov, A. N., Chernova, E. N., & Malysheva, O. A. (2021). Effects of diclofenac on the reproductive health, respiratory rate, cardiac activity, and heat tolerance of aguatic animals. Environmental Toxicology and Chemistry, 41(3), 677–686. https://doi.org/10.1002/etc.5278
- 2. Blasco, J., & Trombini, C. (2023). Ibuprofen and diclofenac in the marine environment A critical review of their occurrence and potential risk for invertebrate species. *Water Emerging Contaminants & Amp; Nanoplastics*, 2(3), 14. https://doi.org/10.20517/wecn.2023.06
- Eshky A. A., Taylor A. A., & Atkinson R. J. A.. 1996. The effect of temperature on aspects of respiratory physiology of the semi terrestrial crabs, *Uca inversa* (Hoffmann) and *Metopograpsus messor* (Forskai) from the Red Sea. Comparative Biochemistry and Physiology Part A: Physiology, 114(4), 297-304.
- 4. Fernandes, J. P., Almeida, C. M., Salgado, M. A., Carvalho, M. F., & Mucha, A. P. (2021). Pharmaceutical compounds in aquatic environments—occurrence, fate and bioremediation prospective. *Toxics*, *9*(10), 257. https://doi.org/10.3390/toxics9100257
- 5. Mezzelani, M., Gorbi, S., Da Ros, Z., Fattorini, D., d'Errico, G., Milan, M., Bargelloni, L., & Regoli, F. (2016). Ecotoxicological potential of non-steroidal anti-inflammatory drugs (nsaids) in marine organisms: Bioavailability, biomarkers and natural occurrence in Mytilus galloprovincialis. Marine Environmental Research, 121, 31–39. https://doi.org/10.1016/j.marenvres.2016.03.005