

Caffeine Exposure in Shore Crabs

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Model Organism

Hemigrapsus oregonensis = Hairy Shore Crab

Range: Resurrection bay, Alaska to Baja California, Mexico

Intertidal Species (Often estuarine)

Appear in many colors and often misidentified
(Caldwell, 2022)

Effective osmoregulators (Cowles, 2005)



Caffeine in the Environment

- Representative pharmaceutically active compound pollutant (Li et al., 2020)
- Used as marker of anthropogenic activities (Hillebrand et al., 2012)
- Caffeine concentrations found in marine organism tissues after long-term exposure (Vieira et al., 2022)
- Negatively influences marine species' abundance, biomass, and fitness (Vieira et al., 2022)
 - Delayed hatch time and embryo development (Garcia et al., 2014)

Caffeine Effects on Crabs

- Decreases in the lysosomal membrane stability and depolarizes the membrane (Aguirre-Martínez et al., 2012; Hermann, 1981)
- Disrupts enzyme and nerve activity in the digestive tract (Baracchini et al., 2024)
- Induces tension in muscle cells by lowering threshold (Huddart, 1969)
- Effects on the Na^+/K^+ -ATPase transporter (Ortega et al., 2014; Sá & Zanotto, 2013)
 - Increased flux of intracellular Cd and Cu in gill cells (Ortega et al., 2014; Sá & Zanotto, 2013)

Question and Hypotheses

- **Q:** How do different environmental concentrations of caffeine facilitate physiological stress responses in *Hemigrapsus oregonensis*?
- **Null:** Different environmental concentrations of caffeine will have no effect on the physiological stress response in *Hemigrapsus oregonensis*.
- **Alt:** Higher environmental concentrations of caffeine will have an increased effect on the physiological stress response in *Hemigrapsus oregonensis*
 - Increased lactate, oxygen consumption and righting times

Experimental Design

Control



- 3L tanks, filled with 2.5 L of water
- Dosed with powdered capsules of caffeine

Low Dose



5 $\mu\text{g/L}$ of caffeine

Medium Dose



15 $\mu\text{g/L}$ of caffeine

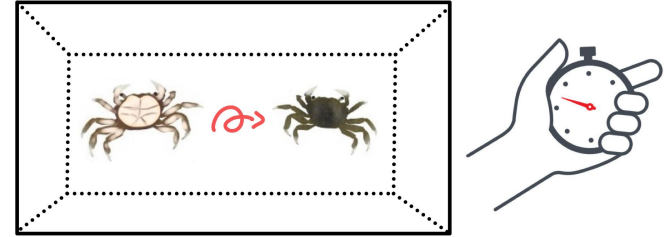
High Dose



20 $\mu\text{g/L}$ of caffeine

Data Collection

- Week 0:
 - Establish caffeine concentrations
- Week 1:
 - Re-dose tanks with caffeine
 - Respiratory and righting time
- Week 2:
 - Record hemolymph, respiratory and righting time
- Hemolymph Assay:
 - Lactate



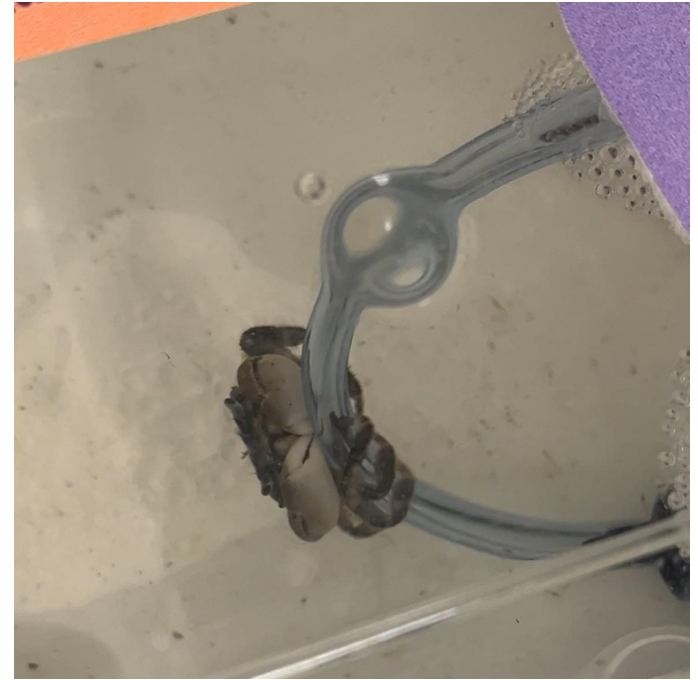
Behavioral Observations



Leg contractures (20 $\mu\text{g/L}$)



Death and possible cannibalization (20 $\mu\text{g/L}$)

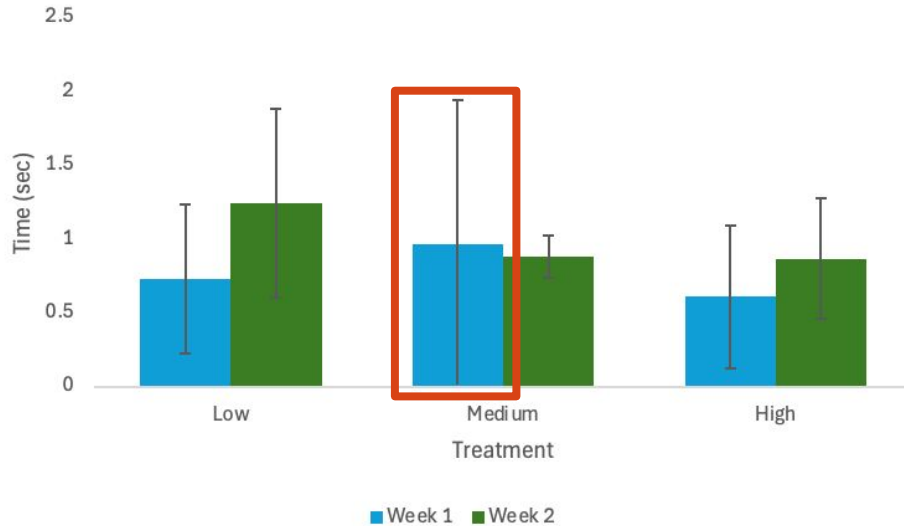


Crab climbing aeration tubing (15 $\mu\text{g/L}$)

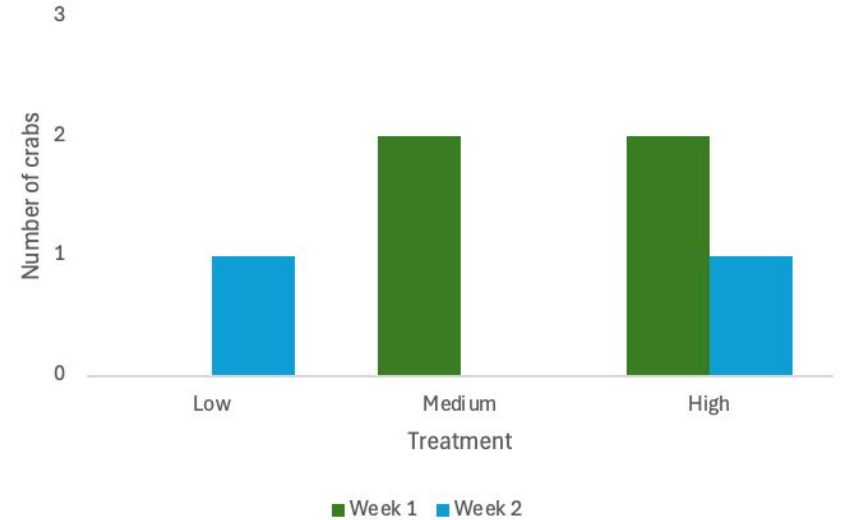
- A low-dose and a high-dose crab escaped

Results: Righting Time and Mortality

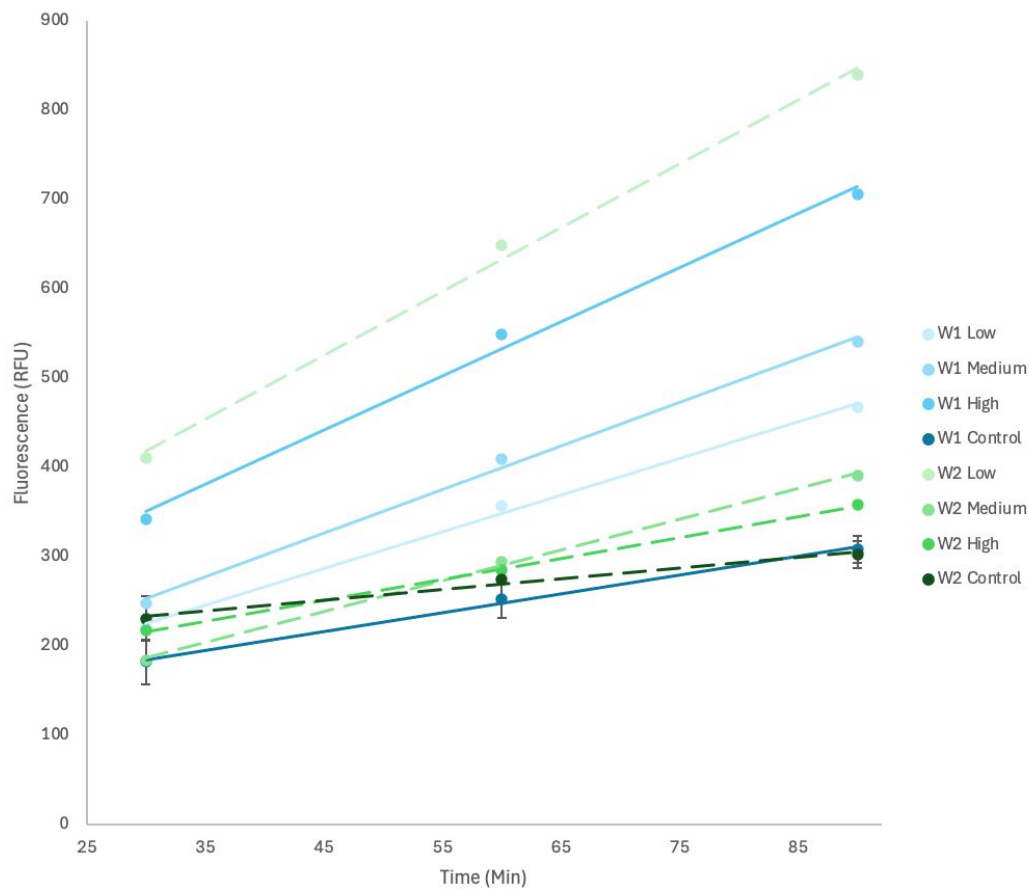
Average Righting Time



Mortality



Week 1 and Week 2 Resezurin results

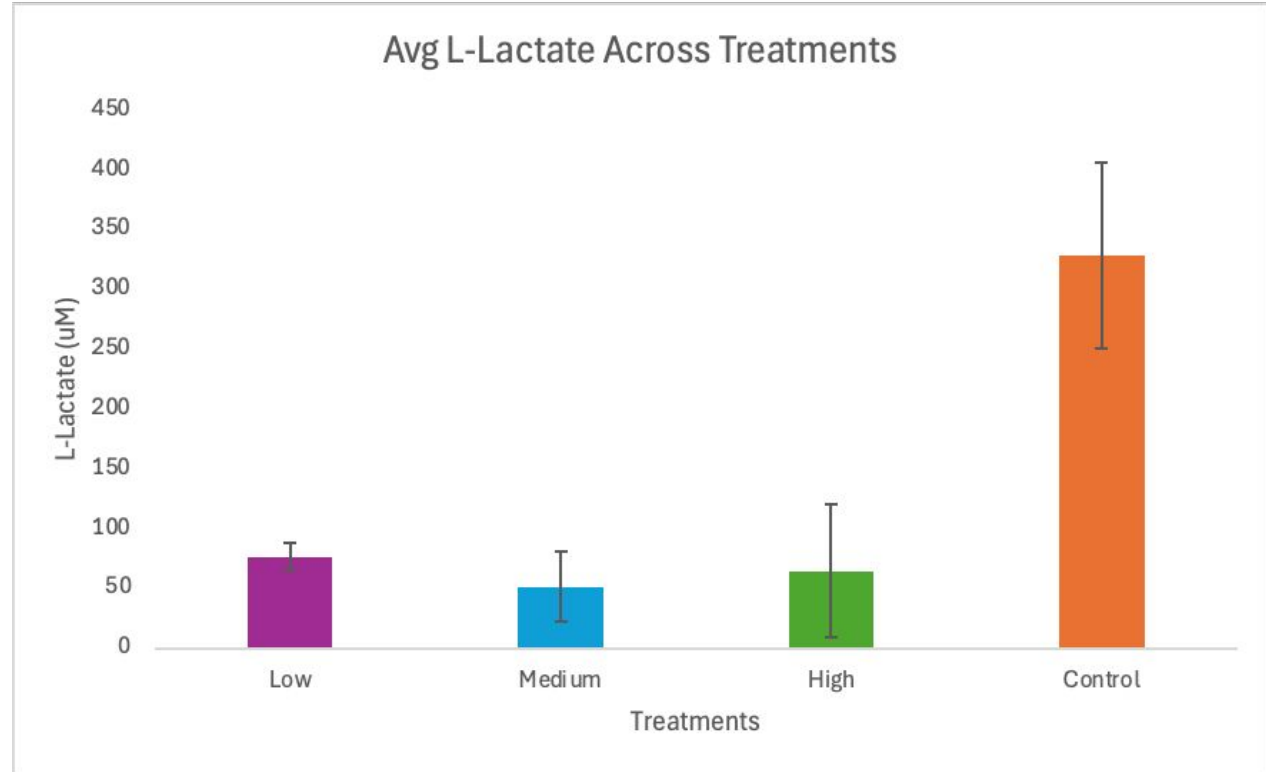


Results: Resazurin

- Rate of O₂ consumption as a proxy for cellular respiration
- W1: Highest rate from High Dose
- Reverse trend seen in Week 2
 - W2: Highest rate from Low Dose

Results: Hemolymph

- Statistically significant differences between treatments
- Higher rates of fermentation in control group



Conclusion/Interpretation

The results state that caffeine can induce behavioral, metabolic and physiological stress effects in *H. oregonensis* over varied concentrations.

Future questions

- How will more closer concentrations of caffeine (5 $\mu\text{g/L}$, 7 $\mu\text{g/L}$, 9 $\mu\text{g/L}$) affect crab physiology and behavior?
 - Critical tissue level threshold
- How much did other environmental stressors affect the results collected?
- How would long term exposure (30+days) affect crab physiology and stress response?

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