Effect of Mineral Buildup in Closed Aquaculture Systems on Hairy Shore Crab (*Hemigrapsus oregonensis*)

Snodgrass, Bailey
Plinka, John Bradley
Berry, Sean

Background - Chemistry

- Shelled invertebrates incorporate $CaCO_3$
  - Molting rate is tied to [Ca^+] (Zanotto 2002; Perry *et al*. 2000); increasing [Ca^+] sped up molt cycle (Zhang *et al*. 2024)
  - Crabs absorb the minerals and molt for homeostasis
  - Unknown to which degree this process “shuts down”--if at all–or cannot remove sufficient calcium
- Heavy metal uptake (Cd, Hg, Pb) facilitated through calcium incorporation (Averina *et al*. 2022)
  - Heavy metals can be detrimental for organism development and human consumption through aquaculture (Martins *et al*. 2009)
- In closed systems, this buildup is more prominent due to lack of removal methods

Background - Aquaculture

- Aquaculture systems are important for farming shelled invertebrates
- NOAA (2021) reported that global harvests in 2018 amounted to:
  - 16.1 million tonnes of shellfish ($19 billion USD)
  - 6.9 million tonnes of crustaceans ($36.1 billion USD)

Typical research usually centers around ocean acidification, but often do not address alkaline conditions

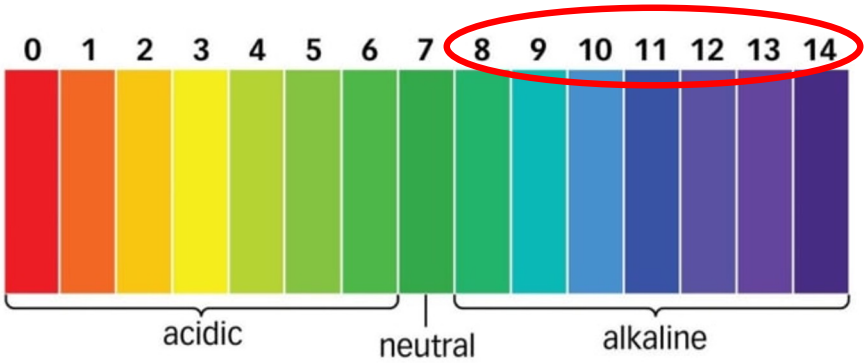
Resources

- Lab Provided
  - Hairy Shore Crab (30-60)
  - Tank (2-4)
  - Temperature Manipulation Mechanism
  - Lactate and BCA Protein Physiology Assays

- Independently Sourced
  - Calcium Carbonate Powder
  - Salt Water Calcium Concentration Test Kit

Research Question: What impact will increased dissolved calcium carbonate levels have?

- Sub Question: How will increased pH/Alkalinity affect our crab's physiology?



- Sub Question: Will the dissolved calcium carbonate in the water buildup on the crab's shell

Hypotheses

Research Question 1: Alkalinity changes

- Null Hypothesis: Increased pH levels from calcium carbonate will lead to no impact on the crab's physiological functions

- Alternative Hypothesis: Increased pH levels from calcium carbonate will lead to increased hemolymph lactate and decreased hemolymph protein levels.

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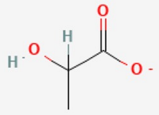

Lactate

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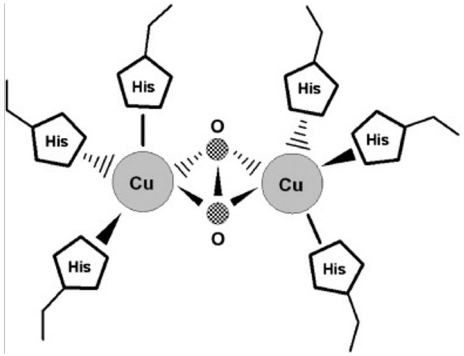

Hemolymph Protein (Hemocyanin: One of three main arthropod hemolymph proteins)

Hypotheses (Cont.)

Research Question 2: Calcification

- Null Hypothesis: Increased calcium carbonate levels in the water will not affect the crab's shells

- Alternative Hypothesis: Increased calcium carbonate levels in the water will lead to calcification on the crab's shells
  - Null Sub-Hypothesis: Calcification of the crab's shells will lead to no physiological responses
  - Alternative Sub-Hypothesis: Calcification of the crab's shells will cause negative physiological effects such as increased molting, increased response to heat stress, and longer righting times.

Experimental Design

- Tank Setup
  - 15 replicates per treatment
  - Sand substrate
  - Treatment Options
    - Control
    - Calcium Carbonate Supersaturation
    - Heat stress (Optional)
    - Heat Stress With Calcium Carbonate Hyper Saturation (Optional)

Experimental Design (continued)

- Control
  - Average Temperature (7.6 °C)
  - Average Calcium Concentration (400 ppm)
- Heat Stress
  - Augmented Temperature (17.6 °C)
  - Average Calcium Concentration (400 ppm)
- Calcium Carbonate Hypersaturation
  - Average Temperature (7.6 °C)
  - Augmented Calcium Concentration (800 ppm)
- Heat Stress with Calcium Carbonate Hypersaturation
  - Augmented Temperature (17.6 °C)
  - Augmented Calcium Concentration (800 ppm)

Experimental Design (continued)

- Calcium Carbonate Application
  - 1 mg Ca/L = 1 ppm
  - 1mg $CaCO_3$ = 0.4 mg Ca
  - Weekly addition to maintain concentration (daily if possible)
  - Weekly calcium concentration test

- Heat Application
  - Set to 17.6 °C (if digital) or apply heat lamp and take temperature reading

Assessment

- Acute Stress (Open Systems)
  - Lactate Physiology Assay
  - Indicative of anaerobic respiration
  - Within 24 hours of final calcium carbonate application

- Chronic Stress (Closed Systems)
  - BCA Protein Physiology Assay
  - Indicative of protein consumption

- Behavior
  - Righting
  - Indicative of overall health

References

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