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

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Background - Chemistry

- Shelled invertebrates incorporate CaCO_3
 - Molting rate is tied to $[\text{Ca}^+]$ (Zanotto 2002; Perry *et al.* 2000); increasing $[\text{Ca}^+]$ 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

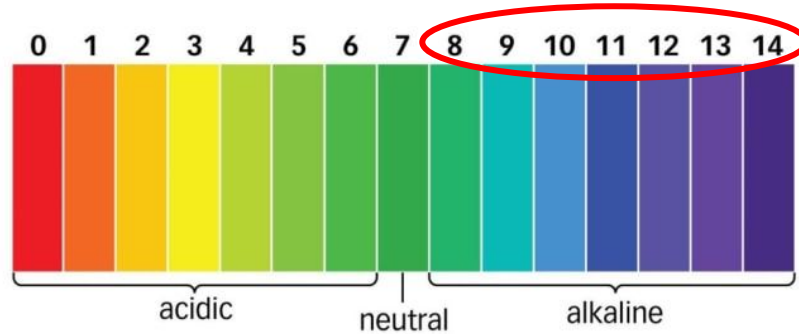
- Hairy Shore Crab (30-60)
- Tank (2-4)
- Temperature Manipulation Mechanism
- Lactate and BCA Protein Physiology Assays

➤ 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?



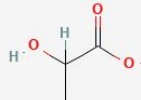
- Sub Question: Will the dissolved calcium carbonate in the water buildup on the crab's shells?



Hypotheses

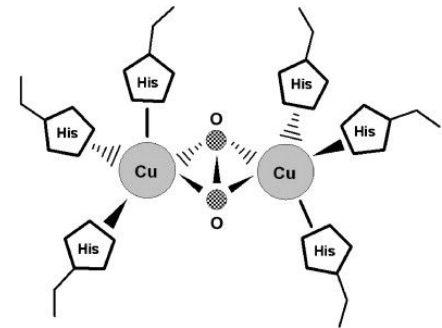
Research Question 1: Alkalinity changes

- 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.



Lactate

& -



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)
- Calcium Carbonate Hypersaturation
 - Average Temperature (7.6 °C)
 - Augmented Calcium Concentration (800 ppm)
- Heat Stress
 - Augmented Temperature (17.6 °C)
 - Average Calcium Concentration (400 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 CaCO₃ = 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

- Averina M., Bjorke-Monsen A.L., Bolann B.J., Brox J., Eggesbo M., Hokstad I., Huber S., and Orebech P. (2022, September 5) High level of heavy metals in crab meat. *Tidsskriftet*. <https://tidsskriftet.no/en/2022/09/perspectives/high-level-heavy-metals-crab-meat>
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