



A Hint of Lyme



The Effects of CaCO_3 on *Hemigrapsus oregonensis*

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Introduction

- Global harvest in 2018: 6.9 million tonnes of crustaceans (\$36.1 b USD) (NOAA, 2021)
- Between 2018 and 2020, aquaculture live weight globally jumped from 115.9 million tons to 122.6 million tons (FAO, 2022)

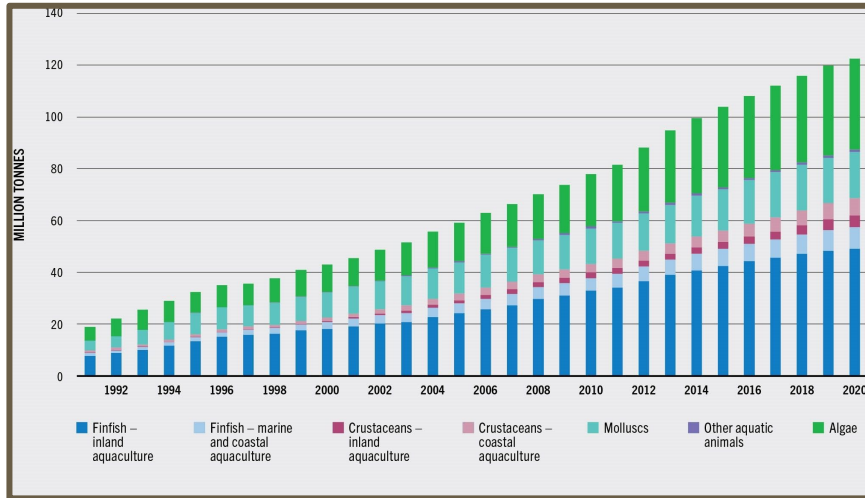


Fig 1). World Aquaculture Production 1991-2020 (Source FAO)

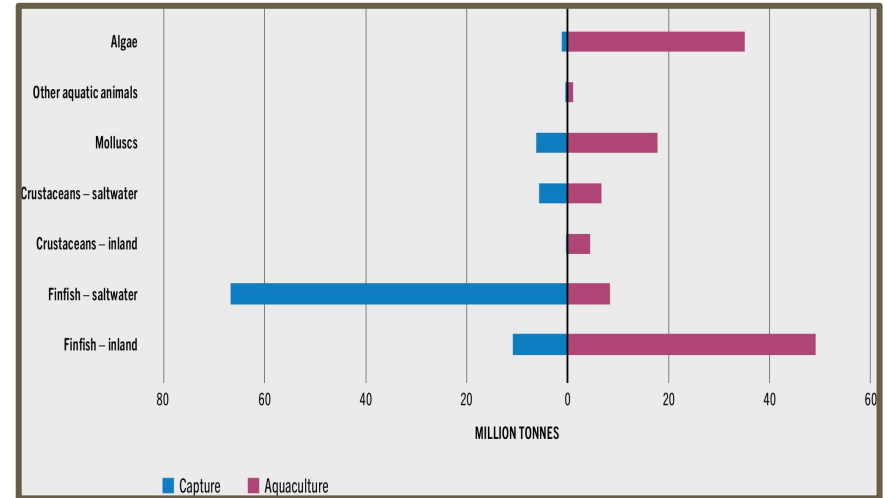


Fig 2). Aquaculture Composition by Functional Species Group 2020 (Source FAO)

Background

- Mineral accumulation of heavy metals have been observed in RAS (Recirculating Aquaculture Systems), and have been shown to have effects on other aquaculture relevant species such as the Nile tilapia (*Oreochromis niloticus*) (Martins et al. 2010)
- Alkalinity increases can result from increased CaCO_3 levels, and alkaline conditions can have adverse effects on internal physiological functions
- Higher $[\text{Ca}^{2+}]$ levels were found to facilitate molt in *S. paramamosain* (Zhang *et al.*, 2024), and build up within the exoskeleton and the gills was observed

Research Question

Research Question: How will dissolved calcium carbonate affect Hairy Shore Crab (*Hemigrapsus oregonensis*)?

Hypothesis 1 (Calcium Carbonate): Excess dissolved calcium carbonate in the water will bind to both their shells and to their gills internally, limiting their ability to respire

Null Hypothesis 1 (Calcium Carbonate): Excess calcium carbonate will have no effect on the crabs

Hypothesis 2 (Temperature): Increased temperature stress will further exacerbate the effects of the calcium carbonate and lead to higher mortality rates

Null Hypothesis 2 (Temperature): Temperature will have no effect on the physiological impacts of the calcium carbonate

Materials

- Hairy shore crabs (*H. oregonensis*) n=15; control population
- Three 2-L tubs: n=5/ea.
 - Heat controlling device
 - Air stone
 - CaCO_3 lime powder
 - Instant Ocean mix
- Saltwater calcium concentration test kit
- Disposable syringe
- Cayman chemical L-Lactate assay kit



Fig. 3) The hairy shore crab, *Hemigrapsus oregonensis*

Methods

- CaCO_3 lime weighed out to 1.000g and dissolved into 2-L tubs
 - 1 mg CaCO_3 = 0.4 mg Ca^{2+}
 - Raised $[\text{CaCO}_3]$ from ~400 mg/L to 600 mg/L
- Placed into one of two tanks: 13° C or 27° C
 - Air stone maintained dissolved O_2
- Samples and tests conducted at weekly intervals (day 7 and 14)
 - One hemolymph extraction per tub when possible
 - One to two righting tests per tub
- Hemolymph processed with L-Lactate assay kit and results recorded

Results - Calcium accumulation

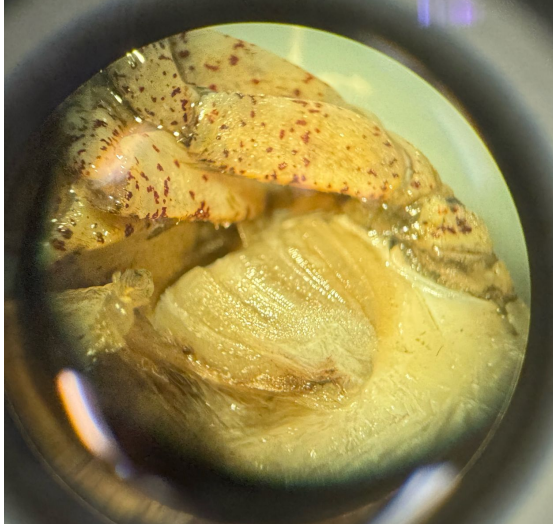


Fig. 4) Typical gill of *H. oregonensis* under baseline regime

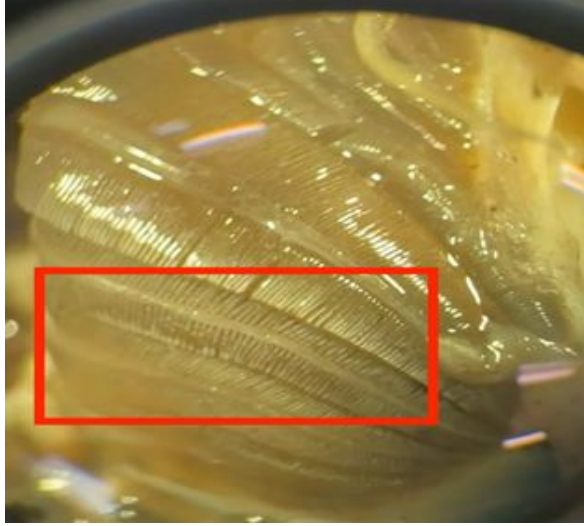


Fig. 5) Accumulation on gills of *H. Oregonensis* in H-Ca⁺



Fig. 6) Accumulation on carapace of *H. Oregonensis* in C-Ca

Results - Mortalities

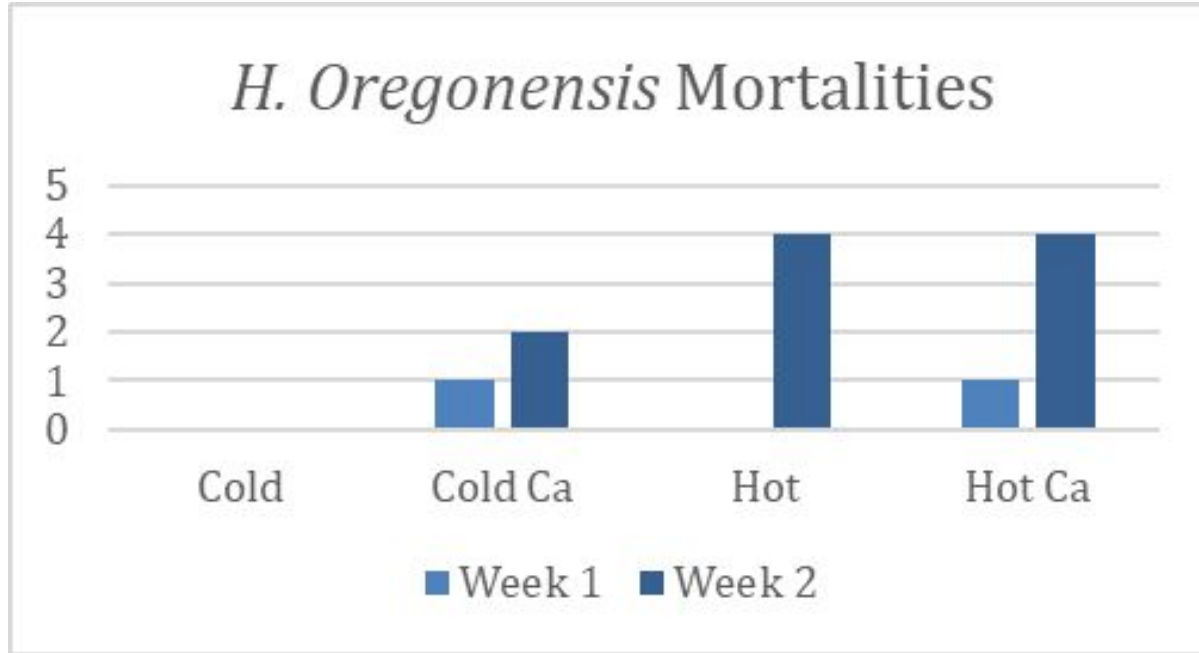


Fig. 7) Mortalities of *H. oregonensis* under each experimental regime

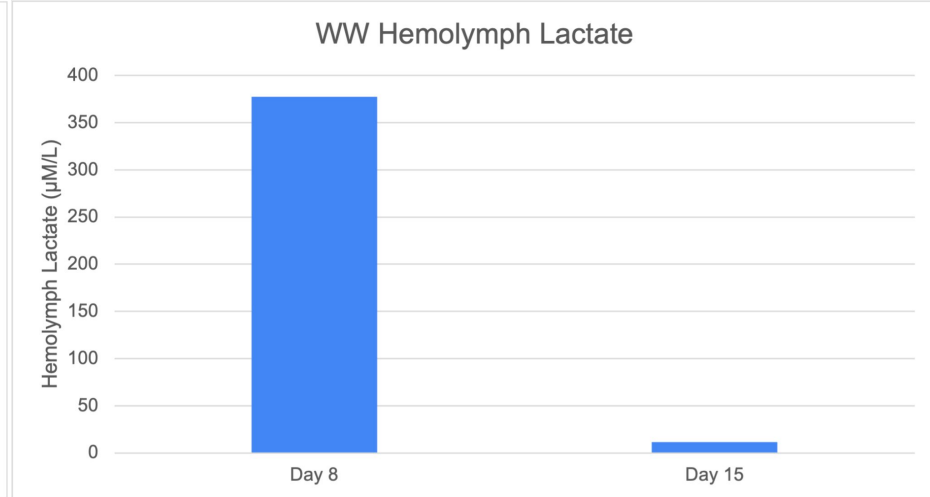
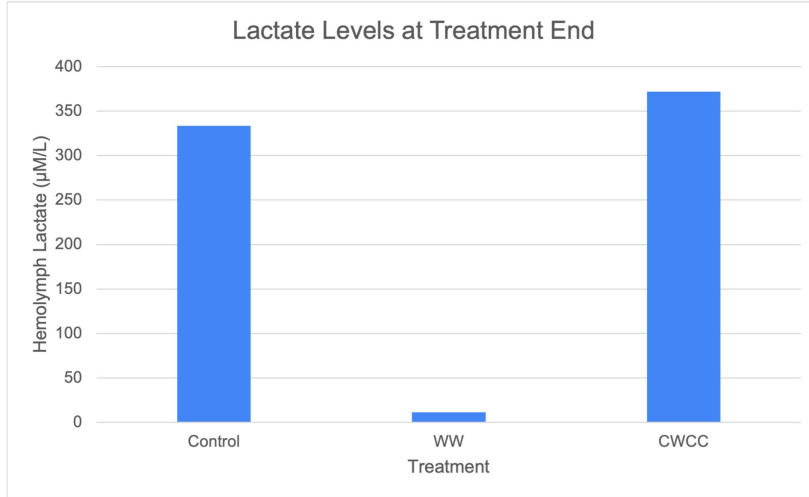
- Impact on respiration may have been the driving factor in resulting mortalities
- Overaccumulation may have occurred and homeostatic process hindered

What does it mean?

- Foundational study
- Evidence of particulate build up in gills
- Temperature increases may accelerate build up
- No connection between lactate and treatment
- Methodology hindered physiological assessment

What's next?

- Resolve temperature impacts on particulate accumulation
- Repeat experiment with other popular ph buffers
- Investigate unexpected lactate assay results



Who cares?

Aquaculture Managers!

- Most valuable stock for least expensive conditions
- Industry expansion
- More humans = more potential human error

References

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