



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)

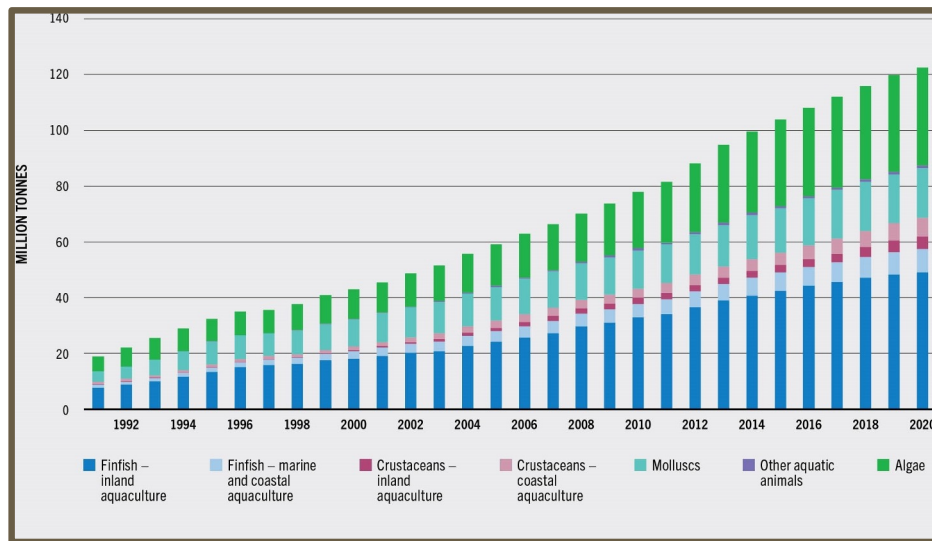


Fig 1). World Aquaculture Production 1991-2020 (Source FAO)

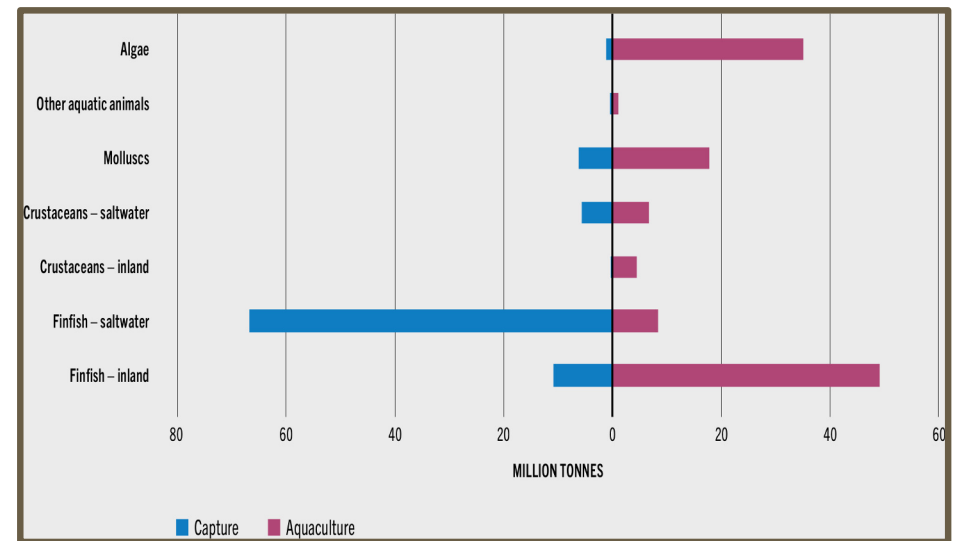


Fig 2). Aquaculture Composition by Functional Species Group 2020 (Source FAO)

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
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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 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
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Results - Calcium accumulation

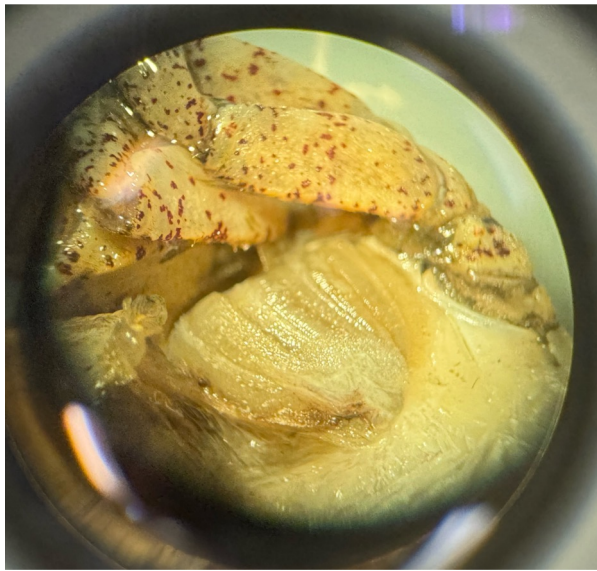


Fig. 4) Typical gill of *H. oregonensis* under baseline regime

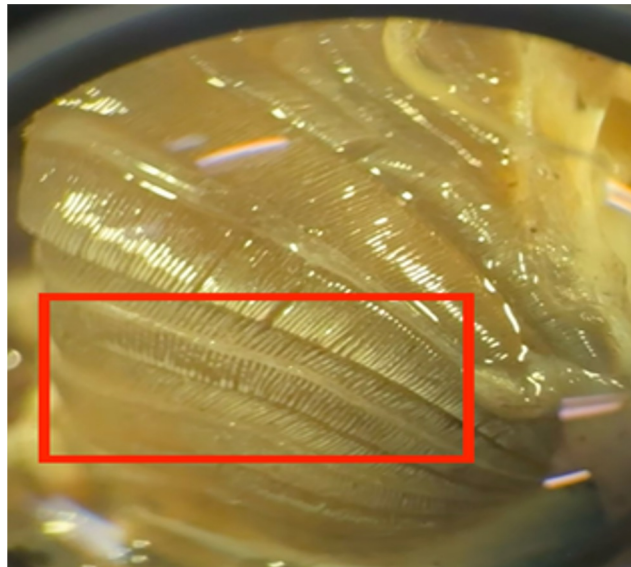
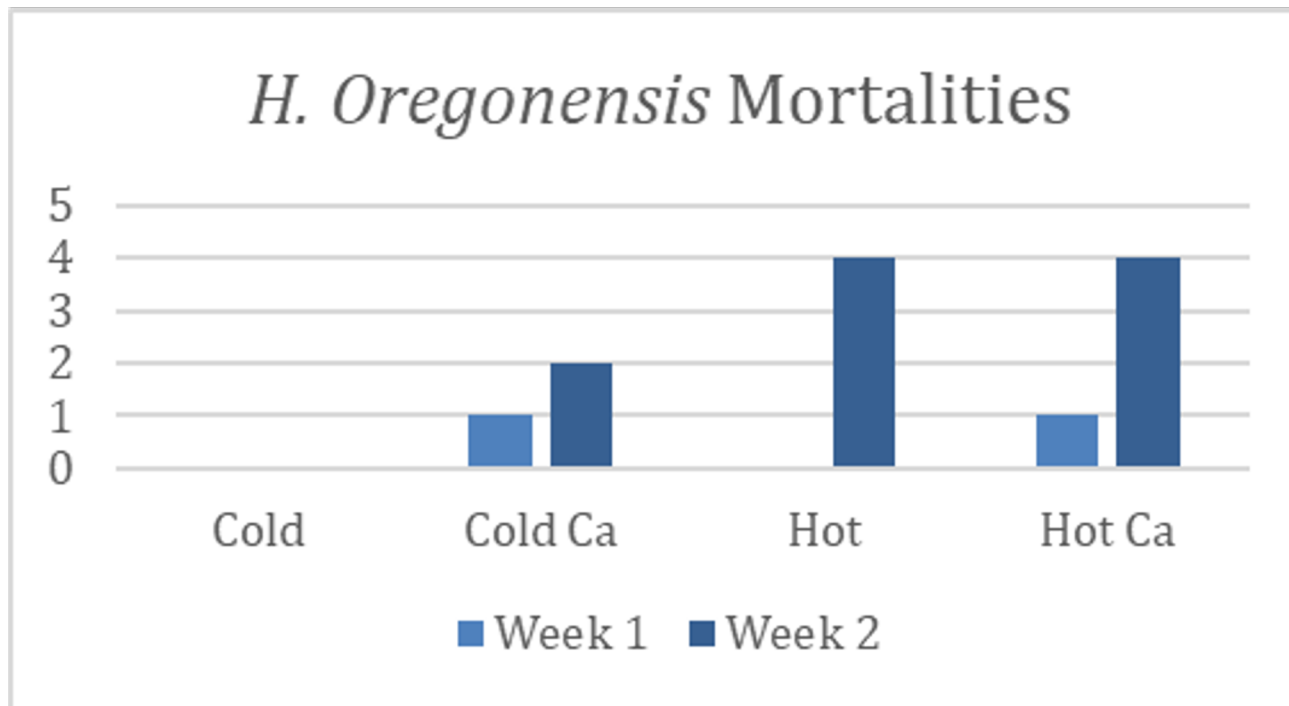


Fig. 5) Accumulation on gills of *H. Oregonensis* in H-Ca⁺



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

Results - Mortalities



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

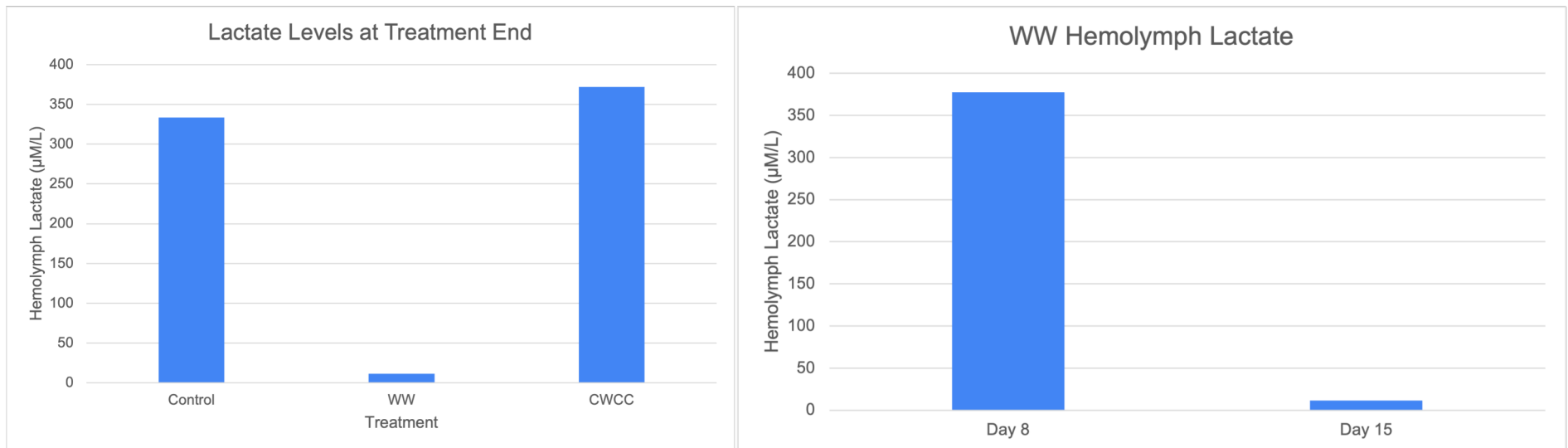
Fig. 7) Mortalities of *H. oregonensis* under each experimental regime

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
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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
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References

NOAA (2021, June 8). *Global Aquaculture*. NOAA.

<https://www.fisheries.noaa.gov/national/aquaculture/global-aquaculture>

Perry, H., Trigg, C., Larsen, K., Freeman, J., Erickson, M., & Henry, R. (2001). Calcium concentration in seawater and exoskeletal calcification in the blue crab, *Callinectes sapidus*. *Aquaculture*, 198(3), 197–208.

[https://doi.org/10.1016/S0044-8486\(00\)00603-7](https://doi.org/10.1016/S0044-8486(00)00603-7)

Zanotto F.P. and Wheatly M.G. (2002) Calcium balance in crustaceans: nutritional aspects of physiological regulation. *Comp Biochem and Phys*, 133(2003), 645-660.

Zhang, Y., Gao, W., Yuan, Y., Cui, W., Xiang, Z., Ye, S., Ikhwanuddin, M., & Ma, H. (2024). Impact and accumulation of calcium on soft-shell mud crab *Scylla paramamosain* in recirculating aquaculture system.

Aquaculture, 593, 741323. <https://doi.org/10.1016/j.aquaculture.2024.741323>
