Impacts of calcium buildup on *Hemigrapsus oregonensis* in recirculating aquaculture systems

Introduction

Calcifying invertebrates, namely crustaceans and mollusks, incorporate calcium carbonate ($CaCO_3$) into their physiology in order to construct their shells. Global harvests in 2018 alone resulted in 16.1 million tonnes of shellfish ($19 billion USD) and 6.9 million tonnes of crustaceans ($36.1 billion USD) according to NOAA reports (2021). Meanwhile, harvest methods have been developed to control the molting, or ecdysis, of these crustaceans. Recirculating aquaculture systems (RAS) are controlled tanks which typically continually pass water through a mechanical and/or biological filter and return the water back into the original tank, where farmed organisms are held in high-density. In RAS such manipulation has yielded a preference for crustaceans which have not finished hardening of the shell post-ecdysis, as these soft-shelled crabs are higher valued commercially (Perry *et al.,* 2000). Understanding of the control over ecdysis provides valuable information for optimal management of these systems.

The reliance of calcifying invertebrates on dissolved $CaCO_3$ has underscored the significance of monitoring its concentration in RAS. Furthermore, alkalinity is heavily derived from the deposition of bicarbonate (HCO^-) and carbonate (CO_3^{2-}) into water, originating from this $CaCO_3$ (Lindholm-Lehto, 2023). Accumulation of these nutrients, alongside trace minerals also suggests a proclivity for higher pH unlike what is found across the increasingly acidic seawater in nature. As a result of this close relationship between alkalinity and $CaCO_3$ crustaceans are sensitive to changes in pH and concentrations must be monitored to facilitate proper ecdysis and hence growth. Higher amounts of dissociated calcium ions (Ca^{2+}) have additionally been reported to enhance the rate of molting in the mud crab *Scylla paramamosain*, given the nature of ecdysis across crustaceans to regulate passive diffusion of the ion (Zhang *et al*., 2024). Even still, it is largely unknown which degree of calcium concentration hinders or even halts this process.

This study aimed to investigate the physiological and behavioral effects of elevated $CaCO_3$ concentrations in crustaceans housed in RAS. We hypothesized that if excessive Ca^{2+} concentrations were introduced to the environment, then it would result in an accumulation inside and out of the body, with initial increased ecdysis but ultimately an increase in physiological stress, impaired righting behavior, and possibly mortality.

Methods

Hairy shore crabs (*H.* oregonensis) were utilized and obtained from the Puget Sound region and maintained in recirculating tanks housed in the Fisheries and Teaching Building at the University of Washington, Seattle. Artificial seawater was prepared using the Instant Ocean commercial seawater kit. Tanks were set to two temperature regimes: a baseline 13^{o} C and an elevated 27^{o} C, controlled via a thermoregulator device. There were three 2-L tubs in total, with one in the former tank and two in the latter and all were isolated from one another to prevent water mixing with the tank. Five crabs were housed in each tub. Dissolved oxygen levels were maintained using air stones.

$CaCO_3$ lime was bought in a powder form and kept in a dry place. In order to simulate a high calcium environment, it was weighed to 1.000 g and dissolved into one 2-L tub of each temperature regime, to raise the Ca^{2+} concentration from the approximate baseline of 400 mg/L to 600 mg/L. Control tubs did not receive any further calcium. Calcium levels were verified in weeks two and three using a saltwater calcium concentration test kit. Molts or deceased crabs were removed prompt

Crabs were held from May 2 to May 16. Samples were taken and tests were conducted at weekly intervals (day 7 and 14), with hemolymph samples taken from one crab per tub to assess physiological stress as a result of Ca^{2+} accumulation. The hemolymph was analyzed using a lactate assay kit to determine its concentration. Righting response times were also conducted in the same weekly intervals, with a different crab whenever possible. The crab would be placed upside down and the time it took to return upright was recorded, serving as an indicator for locomotive burden resulting from Ca^{2+} accumulation.

Results

The low sample size of treatments made it difficult to determine significance of results, as well as losing week one Cold-Ca^{2+} hemolymph extractions due to a laboratory mishap. Significance was further diminished by the total mortality of crabs in the Hot-Ca^{2+} regime.

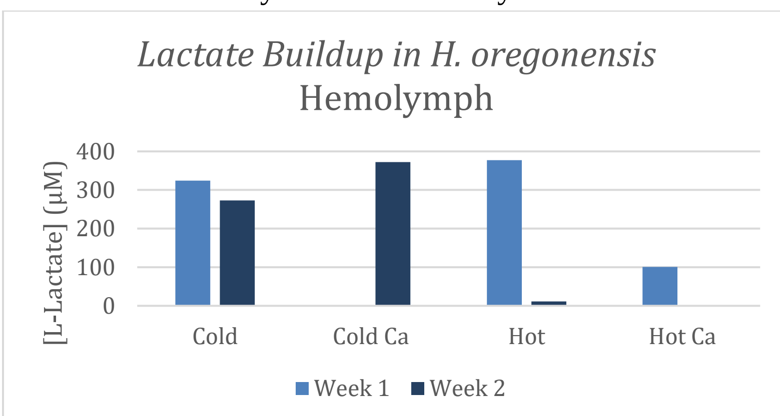

Figure 1 L-Lactate concentrations in the hemolymph of crabs under each experimental regime

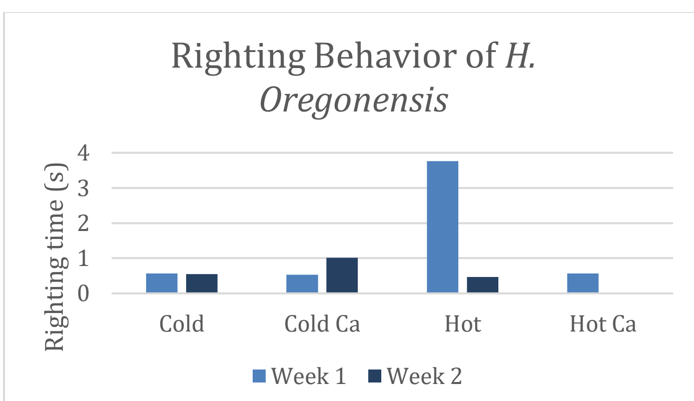

Figure 2. Recorded righting times of crabs under each experimental regime.

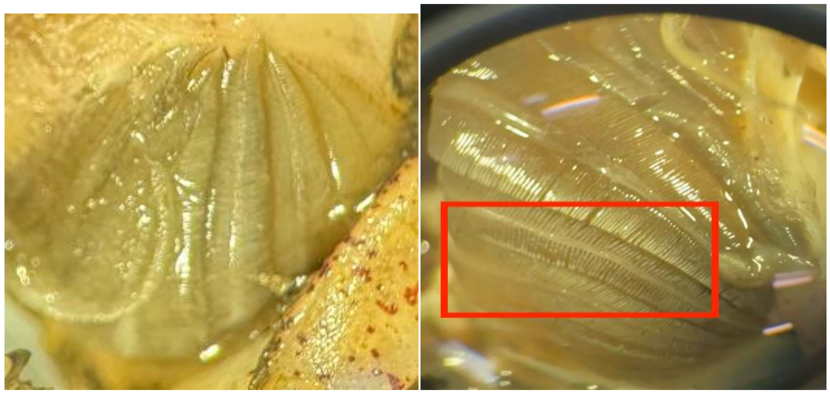

Figure 3. Left:Typical gill of *H. oregonensis* taken from mortality under hot temperature regime, with no elevated $CaCO_3$**.**

Right: Discoloration of gills resulting from calcium buildup, found on the first mortality of the Cold-Ca^{2+} experimental regime. Red rectangle indicates site of most notable buildup

Figure 4 Calcium buildup apparent on *H. oregonensis* from Cold-Ca^{2+} regime mortality during week 1 of experiment

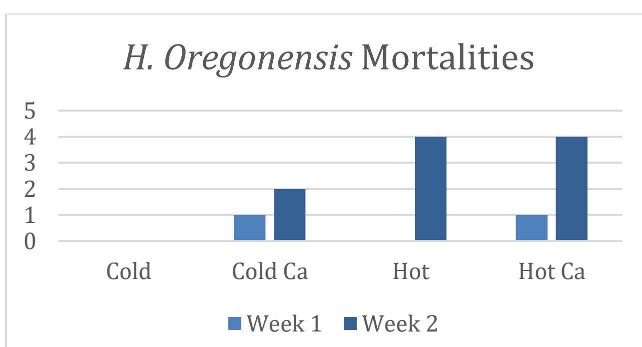

Figure 5 Mortalities of *H. oregonensis* as a result of each experimental regime

Discussion

This study supports the claim that increased $CaCO_3$ in RAS could negatively impact crab physiology and survival. While lactate levels and righting time did not prove to be significant physiological and behavioral markers, the accumulation visible on the gills of deceased crabs indicated a detriment to their homeostasis. This corroborates the findings of calcium buildup on the gills of *S. paramamosain* 6 hr and 3 days post-molt (Zhang *et al*. 2024); notably, that experiment elevated calcium at regimes of 200 mg/L, 300 mg/L, and 450 mg/L, all of which are far below this current regime of 600 mg/L. Such findings may allow for a more accurate window of optimal calcium concentration to be formed in order to stimulate ecdysis.

While we did not experience an increase in ecdysis for elevated Ca^{2+} regimes as expected in the work from Perry *et al*. (2000) and Zhang *et al*. (2024), this is likely due to the resulting mortalities and short time frame of the experiment. Calcium deposition and accumulation was readily apparent across the carapaces of all *H. oregonensis* under such regimes (**Fig. 5)**, suggesting that uptake of Ca^{2+} and subsequent ecdysis may not be able to consistently compensate for these concentrations and adequately remove buildup. Even still, these findings partially support the idea that mineral uptake requires precise timing relative to ecdysis, which is normally behaviorally regulated through a decrease in consumption (Perry *et al*. 2000). Further work may be done to more accurately follow these specifications as there are differing calcium demands across each stage of pre- and post-molt mineralization. This is further supported by findings of significantly diminished $[Ca^{2+}]$ in water post-molt, as this is when the greatest amount of mineralization occurs (Zhang *et al*. 2024). Perhaps this is the window when uptake is typically regulated, and maintaining increased levels in this experiment resulted in the overaccumulation found in Fig.4 and Fig.5.

The indications of accumulation and mortality point to a threshold beyond which increased calcium does not promote ecdysis, but rather causes physiological failure. Subsequently, it has been reported that nitrification in RAS is most optimal at alkalinity levels ranging from 50 to 300 mg $CaCO_3$/L (Lindholm-Lehto). While not crucial to promoting ecdysis, this still yields important information for management of RAS.

This experiment demonstrated the detrimental result of excessive calcium carbonate concentrations on the physiology of *H. oregonensis* which may be extrapolated to other crustaceans who regulate Ca^{2+} via ecdysis. The findings of mass mortalities, likely resulting from accumulation on the gills, highlight the importance of sustaining a mineral balance in RAS. Future studies should more closely follow dissolved calcium concentrations across the molting process, as well as include a wider array of $CaCO_3$ regimes. Water parameters of pH, as well as dissolved oxygen (O_2) and carbon dioxide (CO_2) would also better provide effects on alkalinity and respiration impairment. Additionally, in order to better predict behavioral detriments the reflex action mortality predictor (RAMP) method outlined by Stoner (2009) should be utilized over righting times. Furthermore, with a larger sample size more robust data may be yielded, notably recording mass of the crabs before, during, and after treatment. This would also allow for recording of the mass of the hepatopancreas and gastroliths of the cardiac stomach, both of which resulting in mortality yet providing proxies for calcium uptake as these are typical sites of deposition in crustaceans (Zanotto, 2002). This paper serves as a framework for identifying sites and issues of calcium buildup within the physiology of crabs and the implications this may have for managing recirculating aquaculture systems.

References

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