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FISH 460 Final Paper
6/1/2025

Effects of Calcium Carbonate buildup on *Hemigrapsus oregonensis* physiology

Introduction:

As the global population increases, aquaculture has proven to be one of the most important global trade industries. This is because as populations grow, pressure to feed them increases alongside creating further demand for aquaculture products such as crabs and other arthropods (FAO, 2024). Wild stocks of economically desirable species such as the Giant Mud Crab (S*cylla serrata*) or more locally the Dungeness Crab (*Metacarcinus magister*) are limited by nature and as a result vulnerable to overfishing from these increased pressures. As a result, interest has been expressed in culturing these species domestically to avoid overexploiting the wild populations. Inland aquaculture has been a subject of interest for areas where seafood may not be naturally available or expensive to import either as supplemental to local protein sources or as a potential economic opportunity.

That said, often the only source of water for these aquaculture projects is sourced from local aquafers or similar water sources. As these water sources often have different mineral compositions from the natural environments of these species, even aside from the obvious differences of salt opposed to freshwater, it can lead to unintended and sometimes unaccounted for levels of dissolved minerals such as heavy metals. Recirculating aquaculture systems can be even more vulnerable to this type of buildup as when water is evaporated off and replaced with more water from local sources, heavy metals from these sources can build up within the system if not accounted for as they stay behind when the water evaporates off. A study by (Martins et al. 2010) looked at the effects of RAS (Recirculating Aquaculture Systems) heavy metal concentrations, and the effects of those concentrations on Nile tilapia (*Oreochromis niloticus*). They found that high levels of buildup were experienced within the fish, particularly within the liver. While the levels they found were considerably lower than permissible safety levels for human consumption, further research towards other species may provide better insight towards what levels prove to be deleterious for consumption and if they vary by species. Another study by (Emenike et al. 2022) served to illustrate that while a lot of research looks at the potential bioaccumulation of heavy metals in aquaculture species, there are research gaps surrounding other aspects such as embryonic development and effects metals can have on their early development. Calcium carbonate is particularly well known for building up in aquaculture systems, and several studies such as one done by (Sá et al. 2017) serve to illustrate how that can heavily impact inland systems.

Our experiment's objective is to determine if there is an influence of calcium carbonate ($CaCO_3$) built up in a closed water system on crabs as a target aquaculture group. This study aims to look at the physiological impacts around mobility and internal buildup through righting times, hemolymph analysis and autopsy results. In terms of local interests, running an experiment on Dungeness crabs would be ideal. That said, the species is difficult to work with in context of our experiment due to size constraints and the species having greater legal protection to avoid overexploitation. As a result, the Shore Crab (*Hemigrapsus oregonensis*) was chosen as a model organism for the experiment due to their high population density for ease of capture, small size, and low population concern. In addition, as members of the true crab family (Brachyura) they are physiologically similar and usable as a replacement for Dungeness. To determine the physiological impacts of increased calcium carbonate levels on the crabs, hemolymph was extracted, and physiological assays were run and analyzed to compare against baseline levels. Secondarily, righting time data was collected to determine if it was impaired by the calcium carbonate introduction. Lastly, visual autopsy results were recorded under a microscope and considered to be compared against prior morphological descriptions of the Brachyura infraorder to determine if any impact was observed on their internal anatomy.

Methods:

Experimental Design:

InstantOcean aquarium mix was utilized to create water consistent with seawater conditions. The crabs were separated into four different groups, both to account for the effects temperature and for the effects of the introduced calcium carbonate. Groups of five crabs each were submerged in two-liter containers of water. The ambient temperature groups were set at a temperature of 13°C, and the other two warm water groups were set at a temperature of 27°C. Within each temperature treatment, one group was left within unaltered conditions and the other was increased by 200PPM through adding 1g of calcium carbonate powder to the water. Hemolymph and righting time data were collected twice, each after a one-week period after the experiment start date. During each one-week period, crabs were not fed or otherwise disturbed, barring data collection.

Righting Time:

Righting times were determined by removing a crab from the water and placing it mantle down on a paper towel while a stopwatch ran to determine time needed to flip over. The same crab was used and tested in repeated tests without a break period three times if possible.

Hemolymph Extraction and Analysis:

The hemolymph extraction was performed through utilizing a syringe to penetrate the crab's joints through the legs, before extracting an inexact amount during each extraction event. This is due to the small size of the crabs making extraction difficult, and as a result, as much as possible was extracted whatever that may have been. Physiological assays on the hemolymph were performed by the section TA to test for glucose and lactate levels. Glucose levels were recorded in units of mg/dL, and lactate levels were recorded in units of μM.

Morphology Results:

Morphology was analyzed by cutting a crab open using a scalpel from the backmost seem of the exoskeleton that the crabs utilize for ecdysis to ensure minimal tissue damage during autopsy. Pictures were taken through a microscope and compared both between treatment groups, and against prior scientific descriptions of Brachyura morphology.

Results:

Morphology Results:

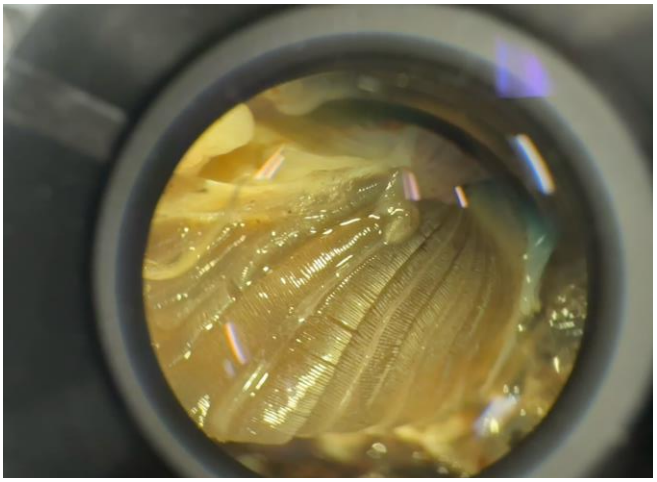

Fig. 1). Hot calcium carbonate autopsy gill results showed white aggregations on the ends of the gills, and the striations were more closely grouped together than the baseline.

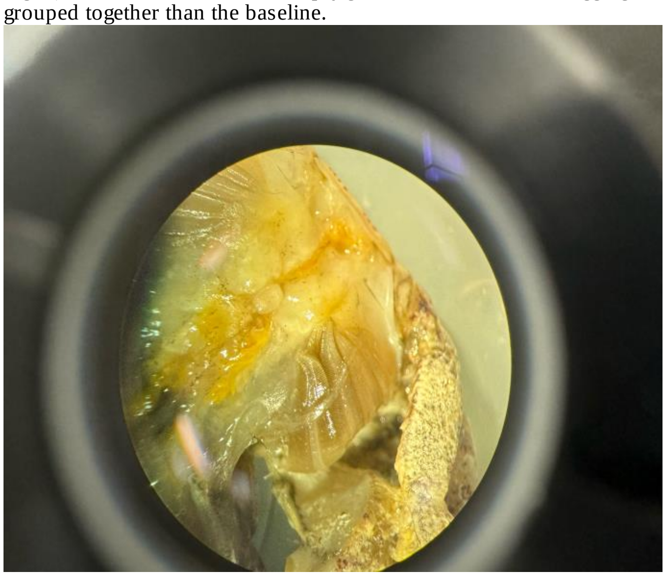

Fig. 2). Cold calcium carbonate treatment crabs had similar shading of whiter aggregations if less extreme on the ends of the gills but closeness of the striations was less observable.

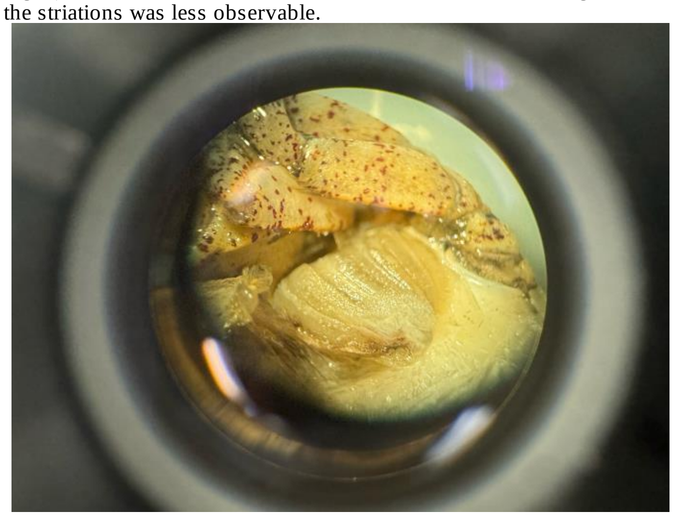

Fig. 3). Baseline treatment crabs showed no differences in coloration along the gill lengths and similar striation distances to the cold calcium carbonate.

Between each treatment, crabs who experienced calcium carbonate exhibited yellow to white shading from the gill shaft to the exterior lamellae. In contrast, crabs who were in baseline conditions had a consistent yellow coloration from the gill shaft to the gill lamellae lacking the white coloration that could potentially indicate buildup. In terms of the initial research goals, a distinct morphological differentiation between groups exposed to calcium carbonate and those who were not was determined through postmortem autopsy.

Discussion:

Regarding our data choices and study objectives, the righting times and physiological assay results proved to be inconclusive and were excluded for lack of any relevant statistical significance. Issues with righting time data were largely linked to the same crab being used for replicates during each data collection event. When crabs failed to right themselves past a period of twenty to thirty seconds, the result was considered an outlier and results were not recorded leading to a lacking number of data points to find anything conclusive. In a future study, either different crabs from the same treatment should be utilized, or the same crab should be used, and data should be recorded for all events regardless of length. By doing so, the rate at which they slow down their righting times could be compared between treatment groups. Issues with physiological assay results include the data for the cold calcium carbonate treatment for week one was lost due to laboratory events unrelated to our procedure, and we experienced a full mortality event leading to it being impossible to collect hemolymph from the week two warm calcium carbonate conditions. In addition, while analysis of both BCA protein and lactate levels was intended, due to unforeseen limitations only lactate assay results was received. Lastly, while morphological differences were clear and potentially indicative of calcium carbonate buildup in the gills, tissue analysis would be useful to determine if $CaCO_3$ buildup is the cause of the discoloration in future research. In addition, if it is determined to be calcium carbonate gill buildup, resazurin results could help to indicate if the crab's respiration capabilities were impaired.

Relating back to our initial study goals, while the other two objectives proved insubstantial, the potential link between gill discoloration and $CaCO_3$ presence serves as a platform for future research. In a comprehensive piece on the anatomy and functional morphology of Brachyura by (Davie et al. 2015), it is detailed both that the gills are usually uniform in color, and that that are responsible for several osmoregulatory processes including that of calcium. This serves to help support the assumption that the white discoloration was calcium buildup within the gill tissue, potentially due to an inability to excrete it quickly enough. In another study by (Zhang et al. 2024), the impacts of calcium accumulation were studied on soft-shell mud crab (*Scylla paramamosain*), which is another commercially important aquaculture species. It was found that calcium buildup over longer periods of time accelerated molting intervals and that gill and exoskeletal tissues were identified as where most of the calcium buildup occurred. While introducing just calcium is not the same as calcium carbonate, due to water chemistry calcium carbonate will break down to bio-accessible calcium leading to this study potentially supporting our results as well. If given the option for future research, it would be dedicated to testing the results of calcium carbonate buildup in a closed water aquaculture system at different levels over a longer timescale on a more commercially important species. Analysis would be dedicated to better analyzing potential mobility limitations through shell buildup, alongside tissue and respiration tests. This would potentially allow aquaculture producers to better optimize growth rates of farmed decapods alongside better understanding of the impacts of calcium oversaturation, and at what levels buildup becomes harmful to the organism.

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