I. Introduction

II. Methods

Experimental Set-up: A control crab population was maintained under temperature and salinity conditions similar to the Salish Sea. In three plastic 2L containers we placed three small glass jars to create partitions between crabs. The containers were filled with Instant Ocean® water to mimic natural conditions in the Salish Sea. We randomly selected 15 crabs from the control population to divide among the treatments. Five crabs were placed in each container, with one in each small jar and the other two placed in separate areas left open by the jar placement. We placed one container in a cold bath at 13°C and placed the other two containers in a warm bath at 27 °C. We added 1 g Nutricost® calcium carbonate powder to the cold-water treatment and one warm-water treatment to raise the calcium concentration from 400 ppm to 600 ppm. We set lids atop each treatment container and fixed a hose to oxygenate the water through a hole in each lid. This design resulted in cold-water calcium carbonate (CWCC), warm-water (WW), and warm-water calcium carbonate (WWCC) We repeated the calcium carbonate powder additions to the appropriate treatments at the nine-day mark.

Data Collection and Analysis: We collected hemolymph from one random crab in each treatment at the eight- and 15-day marks to determine changes in lactate levels. The hemolymph was prepared according to the Caymen Chemical® L-Lactate Assay Kit. Deceased crabs were collected and frozen for preservation. At the end of the treatment period, we opened the carapace of deceased crabs and examined them under a dissecting microscope for a visual assessment of their gill condition.

III. Results

We sought to quantify the relationship between water temperature, calcium carbonate saturation, and lactate production by measuring the lactate levels in crabs under various treatment conditions. Additionally, we sought to assess the physical condition of the crabs gills to identify potential salt buildup on the tissue.

Mortalities: On day eight we saw evidence of an apparent cannibalism event in the CWCC treatment, prompting a water replacement and additional calcium carbonate powder to maintain treatment conditions. We also had one mortality in the WW treatment. Between day eight and day 11 the entire WWCC treatment died. Between day 11 and day 15, there was another CWCC mortality as well as four WW mortalities. In total, we lost two CWCC, four WW, and five WWCC crabs.

Hemolymph Lactate Levels: Control crab lactate levels at the end of the treatment period average 333.7 µM/L (n = 8). In the WW treatment, lactate levels in the hemolymph dropped from 377.5 µM/L on day eight to 11.3 µM/L on day 15. The lactate level in the WWCC treatment on day eight is 100.9 µM/L and there is no day 15 measurement due to the total loss of the treatment population. The lactate level in the CWCC treatment on day 15 is 372.0 µM/L and there is no day eight measurement due to the sample being dropped during transport. Because

of the missing data, we are unable to make a definitive assessment of the relationship between water temperature, calcium carbonate saturation, and hemolymph lactate levels. The data do show unexpected results that may warrant further investigation, particularly the lower lactate levels in the WW treatment on day 15 compared to the control and CWCC treatment as well as the WWCC treatment showing lactate levels three times lower than the control group shortly before their mortality event (Fig. 1).

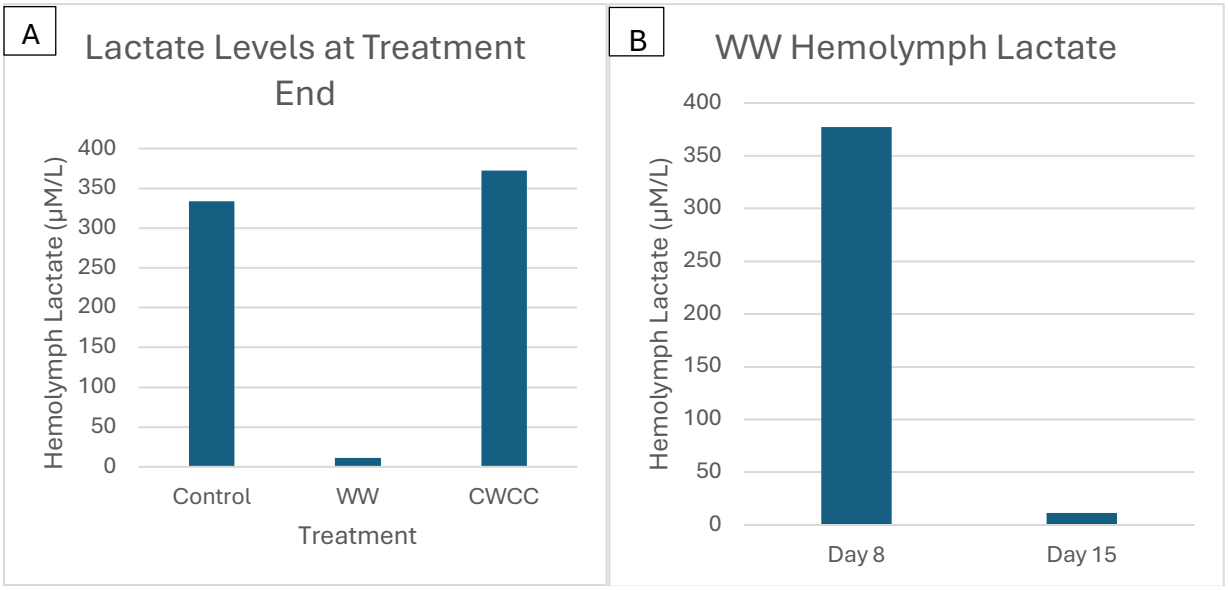

Figure 1: After 15 days, lactate levels in the control and cold-water calcium carbonate treatment are much higher than in the warm-water without calcium carbonate treatment (A). Between day 8 and day 15, lactate levels in the warm-water without calcium carbonate treatment dropped.

Body Condition and Gill Assessment: Each crab in the CWCC and WWCC treatments had a visible particulate coating on their shells by day eight. The coating would rub away when touched and had a consistency like wet chalk. We examined deceased crabs from all four groups under a dissecting microscope to determine the relative conditions of their gills. The gills of the control crab are a gray/brown color throughout, with no visual indication of buildup in individual filaments or particulate deposits on the gill surface. The WW treatment crab was likewise free from filament buildup or surface deposits, though the color of the gills was lighter and tanner than the control crab. In the CWCC treatment, we saw a buildup of a white substance in the filaments along with a slight discoloration, though not as light as observed in the WW treatment. The WWCC treatment showed the most drastic effects, showing buildup in individual filaments as well as small surface deposits.

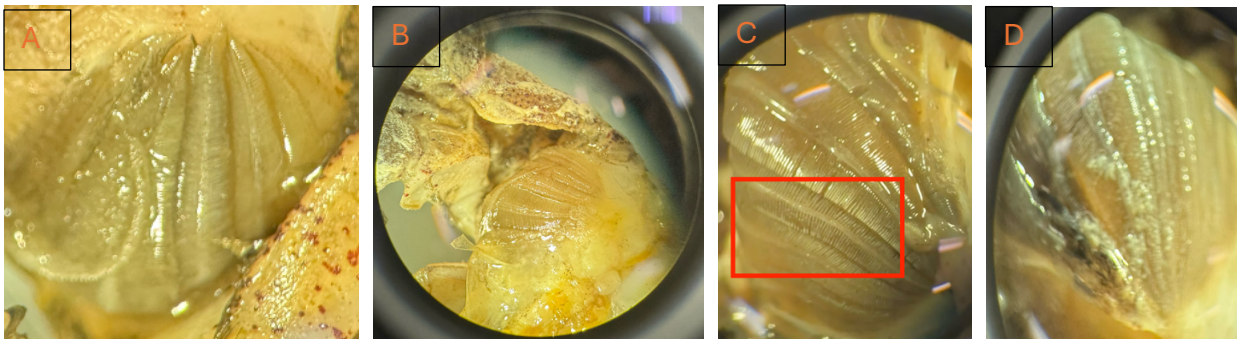

Figure 2: Control crab gills (A), cold-water calcium carbonate treatment gill filament buildup (B), warm-water calcium carbonate treatment gill filament buildup (C), and warm-water calcium carbonate treatment gill surface particulate deposit (D)

IV. Discussion

Calcium carbonate is a common buffer used in closed aquaculture systems to help regulate pH and alkalinity levels (Martins et al., 2017; Queiroz et al., 2004). While the effects of a reduction in available calcium carbonate for shelled marine invertebrates are well documented, there is no existing literature on the effects of exposure to high concentrations of calcium carbonate (Beniash et al., 2010; Kroeker et al., 2010; Talmage & Gobler, 2010). As a foundational effort, our study sought to identify physiological and physical indicators of stress in hairy shore crabs due to oversaturation of calcium carbonate. We also sought to determine the role of temperature in predicting the severity of those indicators.

Based on the visual assessment of the gill conditions in deceased crabs from all four treatment groups (control, CWCC, WW, and WWCC), we determined that exposure to 600 ppm calcium carbonate concentrations results in a visible build up of particulate in the gill filaments. When the temperature increases, the amount of visible filament buildup increases as well. There are also gill surface deposits visible at the higher temperature and higher calcium carbonate concentration. We saw higher mortality in the WWCC treatment than the CWCC treatment, indicating that temperature plays a role in a crab's ability to tolerate buildup in the gills. This evidence supports our hypothesis that increased calcium carbonate concentrations will result in internal particulate buildup and that increased temperature will exacerbate that effect

We were unable to draw meaningful conclusions about the impact of calcium carbonate saturation and temperature on physiological indicators for several reasons. First, to reduce mortality risks in our crabs, we chose to take limited hemolymph samples in each treatment which resulted in a data pool too small to evaluate properly. In retrospect, taking samples from each individual in the treatment instead of singular data points would have resulted in a more robust analysis. Second, the lack of week-to-week data in the CWCC treatment precluded a comparison with the control and WW treatment. Again, this could have been mitigated by a larger collection of samples so that the dropped sample did not derail analysis. Finally, the lack of a secondary physiological assay to corroborate or refute the lactate findings leaves us with little context for the values we got.

This foundational study supports continuing research to explore several applications of our findings. These considerations are aimed at closed aquaculture systems where proper water ventilation and temperature control are managed by humans instead of through natural systems. Understanding organismal responses to a variety of potential human errors, including poor temperature management or excessive applications of buffer, could help reduce risk to stocks and increase profits through reduced waste. Based on the differing mortality between the CWCC and WWCC treatments, future studies may test intermediate temperatures to create a more accurate risk assessment for aquaculture managers. This experiment could also be repeated to explore the effects of oversaturation with other popular aquaculture alkalinity and pH buffers like potassium bicarbonate and calcium hydroxide (Lennard, 2021). Determining the physiological effects of these salt buildups would be another avenue of continued research. Associating physiological changes like metabolic rate fluctuations helps us better understand the long-term impacts of exposure to salt saturated environments. By knowing the physical thresholds of a healthy population and identifying the most effective buffer with the least cost, managers can reduce mortality and increase earnings.

References:

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