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Preliminary Findings: Physiological Effects of Ocean Acidification on Two Species of Intertidal Snails

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Abstract

*Ocean acidification (OA) has been shown to negatively affect organisms that use calcium and carbonate (CO_3^{2-}) to build their shells, such as marine snails, because of the change in water chemistry. This creates an issue with carbonate availability as the organisms are depleted in a necessary ion for shell building. Related to OA, saturation state (**Ω**) is a measurement of how saturated the seawater is with a certain ion or compound. The lower the saturation state the less calcium carbonate is in the water, and if it goes below one it can dissolve the calcium carbonate in the snail's shell. This can cause decreased shell strength, reproductive issues, and more. If these effects are severe enough it could damage whole populations of calcifying organisms. Saturation state relies on both ions in the compound, so decreasing either one will bring the saturation state down. To change the saturation state, calcium concentration will be manipulated by adding a specific amount of liquid calcium to each tank. Calcium carbonate in the ocean is in the form of either calcite or aragonite, aragonite is the dissolved form in the water column, while calcite is in the sediment. I will investigate the effect of calcium carbonate and aragonite saturation state on two marine snails: Littorina littorea and Ilyanassa obsoleta collected from Chincoteague Bay, Virginia. These snail species play an important role in the bay ecosystem. L. littorea is prey for many bay organisms such as birds and crabs, and I. obsoleta is a predator to barnacles and mussels. To determine the physiological effects of saturation state on these two species, live snails and empty shells will be exposed to different levels of saturation state. Data will be recorded on weight, size, density, morphology, shell composition, and shell strength over the course of about six months. Results of this study will showcase how the data might differ between species, as well as examine the likelihood of survival of these marine snails at different saturation states. This presentation highlights the experimental process to find the final methodology to measure the impact of OA on the snails.*

Introduction

The ocean is a carbon sink, absorbing about one third of the carbon dioxide (CO_2) in the atmosphere. The carbon dioxide reacts with water through a series of reactions that change the water chemistry, the more carbon dioxide the lower the pH and vice versa. Due to the increase of carbon dioxide in the atmosphere from burning fossil fuels, more carbon dioxide is being absorbed by the ocean, lowering the pH. With bicarbonate (HCO_3^-) being the most abundant species of dissolved inorganic carbon in the ocean, the carbonate ions are more likely to bond with the extra hydrogen ions to form bicarbonate. This creates a problem of carbonate availability, as there are more hydrogen ions from the lower pH. Certain calcifying organisms are unable to extract the carbonate from bicarbonate which enhances the problem of carbonate availability.

Originally, the goal of the final method was to determine how the two snail species would react to different pH levels. However, after many trials with different methods, the pH could not be kept steady for the experiment. Now, since the previous methods could not be maintained, the saturation state of calcium carbonate and aragonite will be measured instead. Calcium will be monitored and manipulated to show how the snails react when they are depleted in calcium instead of looking exclusively at the carbonate and hydrogen ion concentrations. It takes both calcium and carbonate to build and maintain shells, so the results are predicted to be similar to how the snails would react with a lower concentration of carbonate caused by ocean acidification (OA).

Methodology

Diffuser Method: A CO_2 diffuser is a large canister that dispenses carbon dioxide over time made by mixing a ratio of baking soda and citric acid to create carbon dioxide. It was used with the intention to bring the pH to three specific experimental levels. However, the tanks were not a closed system which is necessary to monitor and maintain the partial pressure of carbon dioxide (pCO_2) that determines how much is needed. Additionally, there was not a feasible method that could be used to determine pCO_2. The pH did not remain steady enough to determine how the snails would be affected at different pH levels long-term, so a new method was needed.

SeaChem Acid Buffer™ Method: SeaChem Acid Buffer™ contains a solid sodium bisulfate salt that reacts with the calcium carbonate in the water to create carbon dioxide and start the OA reaction process shown in **Figure 1.**

$$2NaHSO_4 + CaCO_3 \rightarrow CaSO_4 + Na_2SO_4 + H_2O + \mathbf{CO_2}$$

$$\mathbf{CO_2} + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \rightarrow H^+ + CO_3^{2-}$$

Figure 1. How Acid Buffer™ reacts with calcium carbonate in seawater (upper) and creates carbon dioxide that starts the ocean acidification process (lower).

Many trials were conducted with this salt. The most successful method was a slow drip acclimation system. The salt was mixed into freshwater then poured into a bag with a tube and flow control switch where it would slowly drip into the tank. The mL/hour of solution could be changed per tank which allowed for more control over the system. This system was the most successful in keeping the pH steady because the amount of salt and solution entering the tank were able to be controlled. The sister component, SeaChem Alkaline Buffer™ was also added in a ratio as it was built to work together with the Acid Buffer™. Testing was done to determine the Acid:Alk ratio that would work for a specific pH. However, while the

pH was steadier than before, it could not be kept constant for more than a few days.

Saturation State Method: This is the final method that will be measuring the saturation state of both calcium carbonate and aragonite using the equations in **Figure 2.**

$$\Omega_{CaCO_3} = \frac{([Ca^{2+}]\gamma_{Ca}\frac{\text{free } Ca^{2+}}{\text{total } Ca^{2+}})([CO_3^{2-}]\gamma_{CO_3^{2-}}\frac{\text{free } CO_3^{2-}}{\text{total } CO_3^{2-}})}{K_{sp\ (in-situ)}}$$

$$\Omega_{arag} = \frac{([Ca^{2+}])([CO_3^{2-}])}{K_{sp,\ arag\ (in-situ)}}$$

Ω - saturation state

[ ] - ion concentration

γ - activity coefficients

$K_{sp\ (in-situ)}$ - solubility product constant, measured in-situ

Figure 2. Calcium carbonate saturation state formula and the condensed aragonite saturation state formula (Pilson, 2013; Reibesell, 2011).

To change the saturation state to be constant, liquid calcium will be added in specific ratios in each experimental tank. Carbonate and calcium concentrations will be monitored and recorded to determine how they are being used by the snails. By looking at the carbonate and calcium concentrations and the physiological measurements of the snails, the effects of different saturation states will be determined.

$$TA = \frac{(-[H^+]_T V_{SA} + M_A V_A)}{V_S}$$

$[H^+]_T$ – total excess hydrogen ion concentration

V_{SA} – acid titrant and seawater volume

M_A – molality of the acid titrant

V_A – volume of acid titrant added

V_S – initial seawater volume

Figure 3. Total alkalinity equation (Roche & Millero, 1998).

The CO2SYS Excel program (Pierrot & Lewis, 2006) is a useful tool that will calculate many parameters of seawater with just two variables. For this experiment the pH and total alkalinity (TA) will be calculated to be input into the program. The pH will be found using a YSI Multiparameter Water Quality Meter and TA will be calculated using the equation in **Figure 3.** The total excess hydrogen ion concentration in the equation will need to be calculated separately by using a HCl and spectrophotometry technique (Roche & Millero, 1998). The program will be used along with the equation in Figure 2 to consider the calcium concentration. Free calcium concentration in parts per million will be determined with a calcium colorimeter. This method is successful because it can maintain saturation state long-term, and all of the parameters can be measured.

Conclusion

The methods that did not work for this experiment still gave valuable insight on how to determine a feasible method for this experiment. The expected results related to the two species of snails are: a decrease in mass, little to no growth, a change in density, a visual indication of shell dissolution, a difference in calcium carbonate composition compared to the control, and a difference in shell strength compared to the control. These expected results are based on previous studies done on calcifying organisms and their reaction to low pH and low saturation state (Barclay et al., 2019; Coleman et al., 2014; Kurihara, 2008; Orr, 2005). The final results will show how severely these snails are affected by saturation state and if they are at high risk for harm or extinction related to ocean acidification. Throughout the research process I gained valuable insight on how to determine and manipulate methodology to

work for my exact experiment while enhancing my knowledge of ocean chemistry.

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Recommended Citation
Reynolds, S. (2023). Preliminary Findings: Physiological Effects of Ocean Acidification on Two Species of Intertidal Snails. *Made in Millersville Journal*, 2023. Retrieved from https://www.mimjournal.com/earth-environmental-&-ocean-sciences-2023