Wheaton Journal of
Developmental Biology Research

Issue 6, Winter 2021:

R.L. Morris Ph.D., Editor. Wheaton College, Norton, Massachusetts.

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BIO 298 / Research in Cell & Developmental Biology
Final Research Paper
29 January 2021

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Introduction

As of 2019, global emissions of carbon dioxide were higher than previous levels in the past 8,000 centuries (Lindsey & Dlugokencky, 2020). This unsustainable rise of carbon dioxide's prevalence in the air is likely to both directly and indirectly alter the quality of marine environments (Alto et al., 2011). An abundance of atmospheric carbon dioxide carries many detriments including, but not limited to, an increase in air and water (Trial et al., 2006) and lower pH values when dissolved in water (Adnan et al., 2020). Collectively, these problems contribute to ocean acidification, which plays a potentially fatal role in marine life.

Many organisms face the consequences of higher levels of carbon dioxide, one of them being *Artemia salina*, commonly known as brine shrimp. Despite their diminutive size, *Artemia salina* play an important role in the environment. Specifically, brine shrimp are integral in the structure and maintenance of simple food webs for marine environments, especially saline lakes (Wurtsbaugh 1992). Additionally, due to *Artemia's* advantageous characteristics of cyst banks and quick generation cycles, they serve as exemplars for scientific research, particularly that of resurrection ecology (Lenormand et al., 2018).

Ocean acidification plays a lethal role in the development of marine animal larvae, especially that of invertebrates (Ross et al., 2011). At decreased pH levels, *Artemia salina* may experience complications during growth and development phases and have reduced survival rates (Zheng et al., 2015).

Previous research in this area has been predominantly performed on the effects of ever-growing carbon dioxide rates on terrestrial ecosystems. However, much less research has been done on the effects of atmospheric carbon dioxide increase on marine and aquatic ecosystems (Alto et al., 2011). This preliminary research study aims to test the hypothesis that increases in dissolved carbon dioxide in water negatively affects hatching rates of *Artemia salina* cysts. Many prior experimentation efforts do not examine the degree to which carbon dioxide alters changes in hatching rates. Additionally, similar earlier research attempts to test many variables simultaneously, whereas this project solely focuses on dissolved carbon dioxide to test this very crucial issue with great focus.

Materials & Methods

Carbon dioxide production: To synthesize carbon dioxide, a 500 mL bottle, a 50 mL Falcon tube, baking soda ($NaHCO_3$), and distilled white vinegar ($HC_2H_3O_2$) were used. One tablespoon of baking soda and one tablespoon of distilled white vinegar were poured into the 500 mL bottle, generating the chemical reaction below:

$$NaHCO_3 + HC_2H_3O_2 \rightarrow NaC_2H_3O_2 + H_2O + CO_2$$

As seen above, gaseous carbon dioxide was released from the reaction. Once the reaction stopped (measured by the dissipation of bubbles), the uncapped 50 mL Falcon tube was inverted directly over the mouth of the bottle so that no gas can escape. Since carbon dioxide sinks, the bottle and tube were tipped so that the tube was the bottle. Relying on gravitational pull, the gas was then poured into the Falcon tube while the liquid remained in the bottle. The Falcon tube was capped to retain the gaseous carbon dioxide. This process was repeated one more time to obtain two carbon dioxide-filled 50 mL Falcon tubes.

Preparing experimental and control samples: To make the experimental and control samples, the materials necessary include *Artemia* cysts, petri dishes, 1% saltwater solution, and Falcon tubes with carbon dioxide (made in "carbon dioxide production" procedure). The preparation began with pouring 20mL of 1% saline solution into two Falcon tubes filled with carbon dioxide. The two tubes were then shaken for either 30 seconds or 75 seconds. The solutions were then poured into two different petri dishes. Another petri dish containing 20 mL 1% saltwater solution was made as the control. A consistent amount of *Artemia* cysts were added to each dish, around 14,000 per sample. Additionally, pH values of all solutions were taken using Micro Essential Laboratory Hydrion Paper test strips.

Data collection of hatched *Artemia*: Research shows elevation of carbon dioxide does not impair phototaxis in marine animal larvae (Munday et al., 2016) and may in fact increase sensory responses (Forsgren et al., 2013). Hence, *Artemia's* phototactic behavior was employed to gather the data in the current study. To identify the amount of *Artemia* that hatched, an iPhone 11, petri dish, transfer pipette, and the experimental and control samples were utilized. An iPhone 11 flashlight was shined upon one side of all the samples. In three different, empty, petri dishes, 9.5 mL of 1% saltwater solution were added to each one. After 10 minutes of the samples being exposed to the light, 0.5 mL from the center of the cluster of *Artemia* (attracted to the light source) were extracted using a transfer pipette and transferred into the 9.5 mL of 1% saltwater to make a 10 mL solution for all three samples. Using the iPhone 11, images of each of the petri dishes were taken with the camera lens being 70 mm away from the dish. All photos were taken at the default zoom level. This procedure was kept constant so that digital images would have a similar view field and magnification for data quantification. All images were taken on a dark table to get rid of shadows of *Artemia* in each sample. Overhead lighting was kept constant throughout all photographs of the samples. One round of experimentation was performed for each sampled condition and all data are based on those results. Conducting multiple trials of each study was beyond the scope of the current experiment.

Data quantification: Materials needed to quantitate hatched *Artemia* included an HP Touch-Screen Laptop Intel® Core™ i5 with Microsoft application Paint 3D (version 6.2009.30067.0) and images of petri dishes taken in "Data collection of hatched *Artemia*" section. One at a time, images displaying data were uploaded onto the Paint 3D software. For each picture, the dots, which are the *Artemia*, were traced over with a blue pixel pen tool and counted to determine how many hatched *Artemia* there were in each sample. Air bubbles and other externalities were not included in the counting process. An example of the method is depicted in Figure 1.

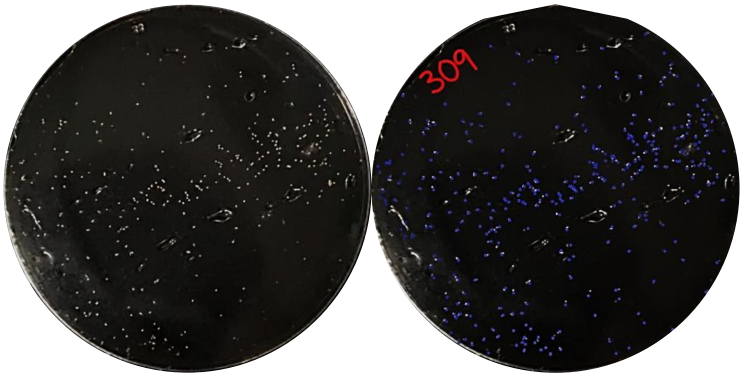

Figure 1. Sample of method utilized to count hatched *Artemia*. A digital blue pixel pen was used to trace over *Artemia* in the digital images. The raw photo is on the left and the edited image is on the right. The blue pen was used to dot the *Artemia* and at the end of quantification, the amount of *Artemia* was digitally written on the side of each dish. In this sample, n=309 *Artemia*.

Results

The *Artemia* themselves were observed under the Foldscope and were photographed at 2x magnification on an iPhone 11 camera. These specimens pictured in Figure 2 are the dots seen in Figures 1 & 3. Before the measurement of data, there was conspicuous difference between the number of *Artemia* in the varying samples. As seen in Figure 3, the control sample contains more *Artemia* than the sample with high levels of dissolved carbon dioxide.

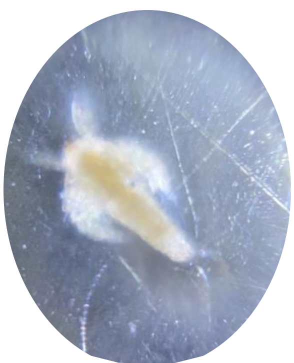

Figure 2. *Artemia salina* under Foldscope of control sample. This is a zoomed-in image of the *Artemia* in the control sample of the study. The image was taken on an iPhone 11 camera with 2x magnification through the Foldscope lens.

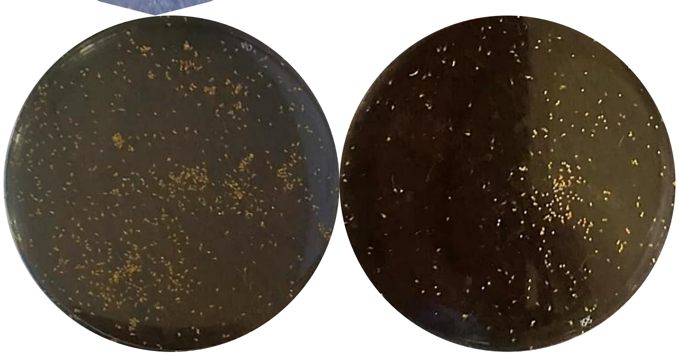

Figure 3. Comparison of control sample (left) and high carbon dioxide sample (right). The dots in the image are the hatched *Artemia* illustrate the data in Figure 4. Notice how many more hatched Artemia are in the control sample than in the dish with additional carbon dioxide.

The pH levels of the water samples declined somewhat linearly as the amount of carbon dioxide increased. The control sample had a pH of 8, the sample with low carbon dioxide resulted in a pH of 7.5 and the high carbon dioxide sample had a pH of 7. Thus, higher levels of dissolved carbon dioxide resulted in a lower pH value. This trend within these data was fully anticipated before the experiment. When water dissolves carbon dioxide, carbonic acid is formed and will dissociate into bicarbonate and hydrogen ions, which ultimately lower pH values of the water, making it more acidic. This higher hydrogen ion concentration may cause confirmational changes of proteins within organisms, impairing their effectiveness.

The results revealed an inverse correlation: as the amount of dissolved carbon dioxide increased, the amount of hatched *Artemia* decreased. Data show that the 10 mL petri dish of the specimens not subjected to carbon dioxide was twice as populated as the low sample and 2.59 times greater than the high dish (Figure 4).

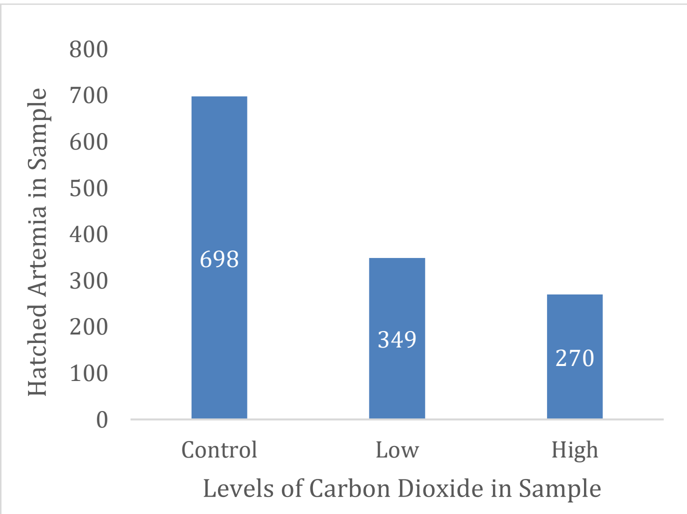

Figure 4. Quantities of hatched *Artemia* in each of the experimental and the control samples. The bar graph portrays the decline in the amount of hatched *Artemia* in each sample based on higher carbon dioxide levels. The data are derived from the results of one experimentation of each condition.

Discussion

The data drawn from the experiment support the hypothesis that higher dissolved carbon dioxide levels will decrease hatching rates in *Artemia*. The results from this data suggest that lowered pH values (induced by dissolved carbon dioxide) reduce hatching rates of *Artemia* nauplii. *Artemia* hatching enzymes are at optimal performance at a pH range of 8-9 (Sui et al., 2014). One specific developmental enzyme, trehalase, may be suppressed at pH values ≤ 7.4 and subsequently inhibit respiration and trehalose-fueled metabolic processes (Hand & Carpenter, 1986). Trehalose is also a critical component of the cysts' longevity by preserving membranes and proteins during desiccation (MacRae, 2016).

On a larger scale, the data may suggest that perhaps *Artemia salina* populations are in danger to the ongoing issue ocean acidification. The current anthropogenic rise of atmospheric carbon dioxide will ultimately lower pH values of oceans, maybe as drastically as 0.7 units (Caldeira & Wickett, 2003). Consequently, lower pH levels will negatively affect marine life, especially calcifying species, including *Artemia salina* (Kibria, 2015).

An improvement to the methods in the current study is to include more data from different points of development. In this research project, data were only collected 48 hours after the rehydration of the cysts. Extending the developmental window will add an extra dimension to the current study allowing better understanding of hatching rates and impediments of development in environments with lower pH values. At these later stages, there will be other enzymes and molecules that may be vitally affected due to pH changes.

Future experiments in this field of study should more accurately mirror the conditions of the current environment. Many studies researching crustaceans and carbon dioxide do not accurately simulate the real environment; instead, crustaceans are exposed to twice the amount of carbon dioxide that is expected at the end of the century (Ross et al., 2011). Understanding how current carbon dioxide levels can affect species will produce accurate and appliable data that are crucial in understanding the implications of the perpetually changing environment.

References

Adnan, A. I., Ong, M. Y., Nomanbhay, S., & Show, P. L. (2020). Determination of dissolved CO2 concentration in culture media: Evaluation of pH value and mathematical data. *Processes*, *8*(11), 1–15. https://doi.org/10.3390/pr8111373

Alto, B., Yanoviak, S., Lounibos, P., & Drake, B. (2011). Effects of Elevated Atmospheric CO2 on Water Chemistry and Mosquito (Diptera: Culidae) Growth Under Competitive Conditions in Container Habitats. *Bone*, *23*(1), 1–7. https://doi.org/10.1161/CIRCULATIONAHA.110.956839

Caldeira, K., & Wickett, M. (2003). Anthropogenic carbon and ocean pH. *Nature, 425*(September), 2003.

Forsgren, E., Dupont, S., Jutfelt, F., & Amundsen, T. (2013). Elevated CO2 affects embryonic development and larval phototaxis in a temperate marine fish. *Ecology and Evolution*, *3*(11), 3637–3646. https://doi.org/10.1002/ece3.709

Hand, S. C., & Carpenter, J. F. (1986). H-Induced Metabolic Transitions in Artemia Embryos

Mediated by a Novel Hysteretic Trehalase. *Science*, *232*(4757), 1535 LP – 1537. https://doi.org/10.1126/science.232.4757.1535

Kibria, G. (2015). *Ocean Acidification and Its Impact on Marine Biodiversity , Seafood Security & Livelihoods- A Short Review*. *2100*(October), 1–7. https://doi.org/10.13140/RG.2.1.5138.4808

Lenormand, T., Nougué, O., Fabien, R. J., Dezileau, L., & Marta, L. C. (2018). *Resurrection ecology in Artemia*. *April 2017*, 76–87. https://doi.org/10.1111/eva.12522

Lindsey, R., & Dlugokencky, E. (2020). *Climate Change: Atmospheric Carbon Dioxide*.

MacRae, T. H. (2016). Stress tolerance during diapause and quiescence of the brine shrimp, Artemia. *Cell Stress and Chaperones*, *21*(1), 9–18. https://doi.org/10.1007/s12192-015-0635-7

Munday, P. L., Watson, S., Parsons, D. M., King, A., Barr, N. G., Mcleod, I. M., Allan, B. J. M., & Pether, S. M. J. (2016). Effects of elevated CO2 on early life history development of the yellowtail kingfish, Seriola lalandi, a large pelagic fis. *ICES Journal of Marine Science*, *73*, 641–649.

Ross, P. M., Parker, L., O'Connor, W. A., & Bailey, E. A. (2011). The impact of ocean acidification on reproduction, early development and settlement of marine organisms. *Water (Switzerland)*, *3*(4), 1005–1030. https://doi.org/10.3390/w3041005

Sui, L., Deng, Y., Wang, J., Sorgeloos, P., & Van Stappen, G. (2014). Impact of brine acidification on hatchability, survival and reproduction of Artemia parthenogenetica and Artemia franciscana in salt ponds, Bohai Bay, China. *Chinese Journal of Oceanology and Limnology*, *32*(1), 81–87. https://doi.org/10.1007/s00343-014-3107-5

Trial, S. T., Practice, I., Journal, L., Donald, J. W., & Tor, L. G. (2006). *CLIMATE CHANGE AND THE D & O POLLUTION EXCLUSION Author (s): J . Wylie Donald and Loly Garcia Tor Published by : American Bar Association Stable URL : https://www.jstor.org/stable/25763814 CLIMATE CHANGE AND THE D & O POLLUTION EXCLUSION*. *41*(4), 1033–1047.

Wurtsbaugh, W.A. Food-web modification by an invertebrate predator in the Great Salt Lake (USA). Oecologia 89, 168–175 (1992). https://doi.org/10.1007/BF00317215

Zheng, C. qun, Jeswin, J., Shen, K. li, Lablche, M., Wang, K. jian, & Liu, H. peng. (2015). Detrimental effect of CO2-driven seawater acidification on a crustacean brine shrimp, Artemia sinica. *Fish and Shellfish Immunology*, *43*(1), 181–190. https://doi.org/10.1016/j.fsi.2014.12.027