current THE JOURNAL OF MARINE EDUCATION

Will Climate Change Alter the Swimming Behavior of Larval Stone Crabs?: A Guided-Inquiry Lesson

ACTIVITIES AND PROGRAM MODEL

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ABSTRACT

The ocean has absorbed ~one third of the excess atmospheric carbon dioxide (CO_2) released since the Industrial Revolution. When the ocean absorbs excess CO_2, a series of chemical reactions occur that result in a reduction in seawater pH, a process called ocean acidification. The excess atmospheric CO_2 is also resulting in warmer seawater temperatures. These stressors pose a threat to marine organisms, especially during earlier life stages (i.e., larvae). The larvae of species like the Florida stone crab (*Menippe mercenaria*) are free swimming, allowing a population to disperse and recruit into new habitats. After release, stone crab larvae undergo vertical swimming excursions in response to abiotic stimuli (gravity, light, pressure) allowing them to control their depth. Typically, newly hatched larvae respond to abiotic cues that would promote a shallower depth distribution, where surface currents can transport them offshore to complete development. As larvae develop offshore, they become less sensitive to certain abiotic stimuli, which promotes a deeper depth distribution that may expose them to variable current speeds, thus influencing the direction of advection (horizontal movement). Environmental stressors like ocean acidification and elevated seawater temperatures may also impact the larvae's natural response to these abiotic stimuli throughout ontogeny (development). Changes in their natural swimming behavior due to climate stressors could, therefore, influence the transport and dispersal of the species. This guided-inquiry lesson challenges introductory marine biology and oceanography students to determine how future ocean pH and temperature projections could impact the swimming behavior of Florida stone crab larvae.

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KEYWORDS:
Crusteacean; Ocean Acidification; Larvae; Stone Crab

TO CITE THIS ARTICLE:
Smith, A. L., LaBadie, J., Busse, A., Solomon, E., Farrell, C, Holstein, D. M., Xue, Z., & Gravinese, P. M. (2024). Will Climate Change Alter the Swimming Behavior of Larval Stone Crabs?: A Guided-Inquiry Lesson. *Current: The Journal of Marine Education*, 39(2), pp. 3–12. DOI: https://doi.org/10.5334/cjme.117

DOI: 10.5334/cjme.117

CLIMATE THREATS TO THE OCEAN: WARMING AND OCEAN ACIDIFICATION

The ocean serves as an important reservoir for CO_2 and has absorbed about 31% of the carbon emissions from the atmosphere as the partial pressure of CO_2 gas equilibrates with the seawater (Gruber et al. 2019; Sabine et al. 2004). The partial pressure of CO_2 is the amount of pressure exerted by CO_2 when it is mixed with other gasses. As seawater absorbs CO_2, a series of chemical reactions occur within the ocean. These reactions lead to an increase in the partial pressure of CO_2, causing the water to become more acidic in a process known as ocean acidification (Feely, Doney & Cooley 2009). At the current emissions rate, the pH of the ocean is predicted to decrease by 0.10–0.41 units by the end of this century. Increases in atmospheric CO_2 concentrations have led to warming in our atmosphere and as a result, warming in the ocean. Ocean temperatures are expected to increase 2–4°C by the end of this century (Masson-Delmotte et al. 2018).

THE CHEMISTRY OF OCEAN ACIDIFICATION

When CO_2 is absorbed by the ocean it mixes with the seawater (H_2O), forming carbonic acid (H_2CO_3). Carbonic acid will then dissociate and lose one hydrogen ion which forms bicarbonate (HCO_3^-). Dissociation will take place a second time causing the bicarbonate to lose another hydrogen ion creating carbonate (CO_3^{2-}). As this process occurs, the concentration of hydrogen ions continues to increase resulting in the seawater becoming more acidic. See the step-by-step chemical formula breakdown below and supplemental materials.

Step 1: $CO_2 + H_2O \rightarrow H_2CO_3$
Step 2: $H_2CO_3 \rightarrow H^+ + HCO_3^-$
Step 3: $HCO_3^- \rightarrow H^+ + CO_3^{2-}$

COASTAL ACIDIFICATION

Shallow coastal habitats, like estuaries and bays, are especially susceptible to environmental changes such as acidification and changes in water temperature. Nearshore waters, which are shallower and have smaller volumes of water relative to the open ocean, are more susceptible to variability in pH and temperature (Hall et al. 2020; Yates et al. 2007). In addition, increases in coastal development, urbanization, and freshwater inputs in coastal areas also make these habitats more vulnerable, causing an accelerated pH change rate compared to the open ocean (Bauer et al. 2013). In some cases, these environmental extremes can result in marine organisms reaching or exceeding their environmental tolerance thresholds which can alter their natural behavior and cause stress, injury, or mortality.

EFFECTS OF TEMPERATURE AND REDUCED PH ON INVERTEBRATE LARVAE

Future ocean conditions pose a significant threat to marine organisms, especially during their earlier life stages which are often more sensitive to environmental extremes like changes in pH and temperature (Albright et al. 2010; Chan et al. 2011; Kurihara 2008). Earlier-life stages of marine invertebrates experience more sensitivity and less tolerance to environmental changes than adults usually because they are still developing the physiological mechanisms needed to compensate for environmental variability (Gravinese et al. 2020; Whiteley 2011). For example, changes in environmental conditions like warming seawater temperatures and acidified waters have been shown to shorten the duration between developmental stages and result in reduced survivorship of crustacean larvae (Costlow et al. 1960; Gravinese 2018; Gravinese et al. 2018; Ong & Costlow 1970).

CRUSTACEAN LARVA ARE DISPERSED USING VERTICAL SWIMMING BEHAVIORS

Many benthic crustaceans often begin their lives as a free-swimming larva and are dependent on ocean currents for dispersal. Several coastal crustaceans can synchronize their larval release with these currents to help direct the larvae toward offshore waters where environmental conditions are more stable and conducive to completing development (Cowen & Sponaugle 2009; Epifanio & Cohen 2016; MacTavish et al. 2016; Sulkin 1984). The timing of larval release with outgoing currents helps the planktonic larvae to be transported offshore, away from the hatching site and helps to avoid higher predation pressure in coastal nearshore habitats (Arana & Sulkin 1993; Cowen & Sponaugle 2009; Gravinese et al. 2019; Krimsky et al. 2009).

Once larvae are offshore, they maintain their vertical position by swimming up and down in the water column in response to certain abiotic stimuli (light, pressure changes or depth, gravity, etc.; Naylor 2006). As larvae develop, their response and sensitivity to these stimuli may alter their swimming speed and directional behavior which can change their overall vertical position in the water column. In addition, larvae may exhibit either kinesis (non-directional movement) or taxis (directional movement) behaviors in response to stimuli. Since larvae rely on currents for transport, changes in their vertical position can influence the distance and direction the larvae are transported which may impact their distribution (Arana & Sulkin 1993; Epifanio & Cohen 2016; Sulkin 1984). In addition, environmental stressors like acidification and warming may impact the larvae's natural response to these external stimuli further altering swimming behavior. One external factor stone crab larvae experience in the water column is gravity. The natural larval response to gravity is known as geotaxis which is the active movement toward or away from a gravitational force. A positive response is a response directed towards the center of the Earth and a negative response is a response away from the center of the Earth (i.e. swimming to the surface; Arana & Sulkin 1993). Similarly, larval movements directed toward light would be termed a positive phototaxis, while movement away from light would be a negative phototaxis. Sulkin (1984) proposed a negative feedback model for depth distribution of larval crustaceans. The model proposed that a larvae's response to external environmental cues (light, pressure, gravity, etc.) can modify larval behavior to counteract negative buoyancy (sinking) by changing the individual's directional orientation (swim up or down) and/or locomotor activity (change in swimming speed; Sulkin 1984; Figure 1).

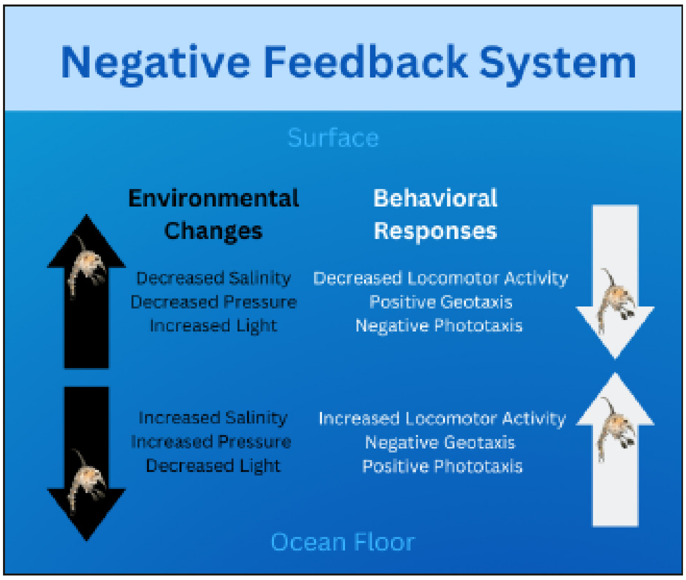

Figure 1 summarizes the larval responses and swimming trends in correlation with environmental changes observed with depth (Gravinese 2018; Sulkin 1984). Geotaxis is the animal's movement toward (positive) or away from (negative) Earth's gravitational force. Phototaxis is the animal's movement toward (positive) or away from (negative) a light source. Larval images were taken by Philip Gravinese. Illustration by Abigail Smith.

THE FLORIDA STONE CRAB: AN IMPORTANT FLORIDA FISHERY AND OUR MODEL ORGANISM

Marine invertebrates make up a significant portion of the United States fisheries contributing to nearly half of the species harvested (Feely, Doney & Cooley 2009). By understanding the impacts of predicted future ocean conditions on invertebrate larvae, we can determine how commercial fisheries will be impacted in the future. The Florida stone crab (*Menippe mercenaria*) is a commercially important species valued at ~30 million dollars annually in the state of Florida. However, the fishery's mean annual catch has declined since the early 2000s (Muller et al. 2011). One possible contributing factor to the fishery decline could be the intolerance of stone crab larvae to the changing environmental conditions. Behavioral experiments can be conducted to determine how environmental changes such as reduction in pH and increases in temperature may affect the larval development and behavior throughout the stone crab's life cycle.

Stone crabs are reproductive during the spring and summer seasons. Adults inhabit coastal waters, where females brood their eggs for about two weeks. Once released, the larvae are transported offshore by surface currents to complete larval development before returning to coastal habitats as juveniles and young adults which restarts the cycle (Bert et al. 2021; Gravinese 2018). Adult stone crabs inhabit coastal waters which have direct exposure to anthropogenic stressors such as increased runoff and eutrophication which can amplify reductions in seawater pH (Wallace et al. 2014). These changes in seawater pH may impact the ability of stone crab larvae to vertically swim, fragment the population, and limit the population's ability to migrate away from adverse environmental conditions (Alaerts et al. 2022; Gravinese et al. 2019).

RESEARCH QUESTION AND HYPOTHESES

The purpose of these guided-inquiry activities is to determine how future ocean pH and temperature projections may impact the swimming behavior of Florida stone crab larvae to an exogenous cue like gravity. By observing swimming behavior across different larval stages, scientists can determine how the life cycle and the success of the species may be impacted under future ocean conditions. In activity one, students will study observational differences in the five larval stages of the Florida stone crab and make connections to the life history of the species. In activity two, students will view geotaxis videos to determine if reduced pH and elevated temperature impact larval swimming ability. Students will evaluate a subset of videos across three larval stages: stage 1, stage 3, and stage 5 larvae. Students will compare the swimming ability in all three larval stages between the control (ambient) and the experimental (reduced pH and increased temperature) treatment groups. These videos are subsets of data collected from an ongoing study being conducted by scientists at Eckerd College and Louisiana State University. While viewing the videos, students will analyze the following: 1) the swimming direction of the larva (up vs. down), 2) the distance (cm) traveled by the larva, and 3) the speed (cm/sec) of the larva in order to determine how swimming behavior may change during exposure to present-day and future ocean conditions.

ACTIVITY ONE: IDENTIFYING STONE CRAB LARVAL STAGES AND LARVAL MORPHOLOGY

Florida stone crab larvae are released into the water column (Stone Crab Hatching Video) where they undergo larval development in offshore waters. The Florida stone crab has five main developmental larval stages with significant morphological differences in stages 1, 3, and 5. After larvae are released, it takes approximately 20 days for the larvae to complete their development before growing into post larvae (megalopae stage) where they migrate back toward coastal settlement habitats (see supplemental life cycle worksheet). In this activity, students will observe three of the five larval stages and will be challenged to compare and contrast the morphological characteristics and differences between stages 1, 3, and 5.

ACTIVITY ONE MATERIALS

- Computer with internet access

ACTIVITY ONE DIRECTIONS FOR STUDENTS

1. View the larval morphology video to observe the five larval stages. Stone Crab Larval Morphology Video
2. Take note of general observations made from the video.
3. Compare and contrast stages 1, 3, and 5. How are they similar? How are they different?
4. Based on your observations, answer the discussion questions.

ACTIVITY ONE DISCUSSION QUESTIONS (SEE FIGURE 2)

1. How might changes in morphology (particularly size) impact the swimming behavior of stone crab larvae?

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DOI: 10.5334/cjme.117

2. How do the abdominal segments change from stage 1 to stage 5?
3. Explain the relationship between larva size and age.
4. What are some benefits of having a larval stage in the marine environment?

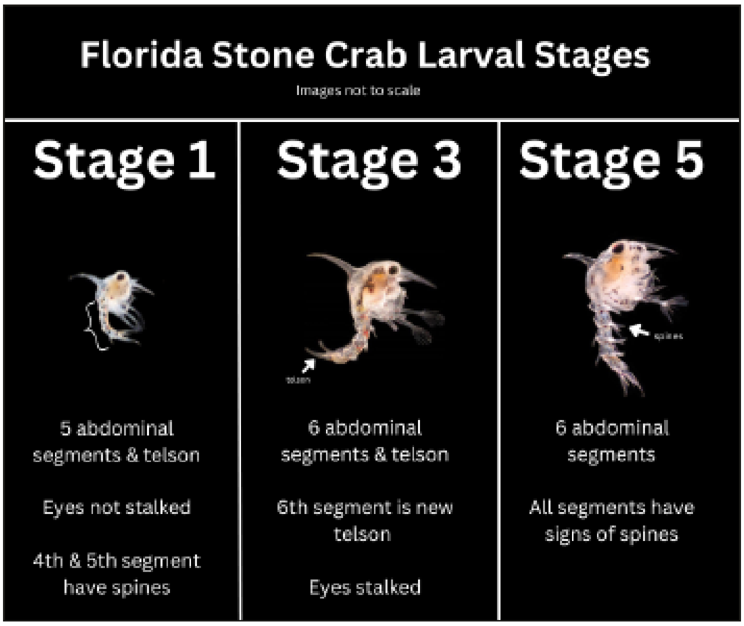

Figure 2 Three larval stages of the Florida Stone crab and observational differences between the stages. The telson is the forked terminal segment attached to the abdomen. Images not to scale. Larval images were taken by Philip Gravinese. Illustration by Abigail Smith.

ACTIVITY TWO: OBSERVING LARVAL STONE CRAB SWIMMING BEHAVIOR AND GEOTACTIC RESPONSE

Previous studies have shown that many crab species in the early larval-stage (immediately after hatching) experience negative geotaxis in response to gravity resulting in an upward migration in the water column. However, environmental conditions such as reductions in pH can confuse the larvae to have an impaired ability to orient to specific exogenous stimuli.

Activity Two will challenge students to observe larval geotactic response within two treatment groups, a control group (pH = 7.9, temperature = 28°C) and a projected end-of-century group (pH = 7.5, temperature = 32°C; Masson-Delmotte et al., 2018).

ACTIVITY TWO MATERIALS

- Geotaxis trial videos
- Computer with graphing software (Excel, Google Sheets)
- Stopwatch

ACTIVITY TWO DIRECTIONS FOR STUDENTS

1. View the five geotaxis trial videos for stage 1 for both treatments. *If students are struggling to identify the larvae at this stage, start at stage 5 and work backwards.*
2. Observe behavior in the first 10 seconds of video footage after the chamber is rotated to the vertical position.
3. For each video, take note of the larva's starting point (cm) and ending point (cm) after 10 seconds to determine the total distance traveled by the larva. ***Note:** In some trials, the crab larvae may swim out of frame within the first 10 seconds. For these cases, students will need to record the last point (cm) the larvae was seen before swimming out of frame in addition to recording the actual time (less than 10 seconds).*
4. Calculate the larva's speed (cm/sec) by dividing the total distance traveled by 10 seconds (or by the actual recorded time if less than 10 seconds).
5. Enter these data into the spreadsheet template provided.
6. Repeat steps 2–5 for both treatments for all stages.
7. Using the spreadsheet, calculate the average downward speed for each stage in each treatment. *Remember to make these numbers negative values because they are downward speeds.* To calculate the average in the spreadsheet, use the built-in average

DOI: 10.5334/cjme.117

function by typing the "= AVERAGE(cells)" into a cell. Select the data cells from each group to calculate the average. ***Note:*** *The spreadsheet template should be pre-set with the mathematical formulas. However, if it is not, follow these steps to calculate average, standard deviation, and standard error.*

8. Calculate the standard deviation for each stage in both treatments using the function "= STDEVA(cells)" in the spreadsheet.
9. Next, use the standard deviation value to calculate standard error using the built-in function "= STDEVA(cells)/SQRT(COUNT(cells))".
10. Use the calculated average, standard deviation, and standard error to create a bar graph representing the larvae average downward swimming speed. *Adding error bars is optional based on the educator's preference.*
11. Now, create a second bar graph representing the directional response of the larvae focusing on the percent of larvae that swam upward in each stage and treatment.
12. Calculate upward swimming percentage by dividing the number of larvae that swam up by the total number of larvae in the set. For example, if three larvae swam up, divide three by the total number in the set which is 5. This calculation should provide a decimal percentage that can be used to create a bar graph.
13. Use your created bar graphs to answer the Activity Two discussion questions.

GEOTAXIS VIDEOS

Ambient 28- Stage 1

Ambient 28- Stage 3

Ambient 28- Stage 5

End 32- Stage 1

End 32- Stage 3

End 32- Stage 5

ACTIVITY TWO DISCUSSION QUESTIONS

1. Explain the relationship between the larval stage and the directional movement seen in each treatment. Use the calculations and plots that you created as evidence.
2. Why might it be important to understand how stone crab larvae respond to exogenous cues like gravity?
3. Make hypotheses as to how the stone crab life cycle and overall reproductive success of the stone crabs might be impacted by future predictions in seawater temperature and pH.

INTENDED LEARNING OUTCOMES

Teaching students about climate change science can be a challenging undertaking for educators. Informing students about the threats and challenges faced by marine populations from climate change stressors is essential to reducing the intensity of climate change for future generations and for our future planet (Paşcalău et al. 2021). Educators that tested this lesson in their classroom emphasized that 'reading information in a textbook or modeling climate change without data can be too abstract for many students'. Our lesson provides a hands-on opportunity for students to evaluate the effects of climate change using a model fishery species, the Florida stone crab. This lesson highlights how earlier life-stages of the stone crab's life cycle may be more sensitive to future ocean conditions. This lesson couples a commercial fishery species with climate change research to help frame the importance of learning about the effects of climate change into a more realistic and relatable context. Teaching climate change related topics using real-time research methods and data is crucial in today's classrooms because it keeps the topic relevant, fosters scientific literacy, hones problem-solving skills, and provides a global perspective. By integrating real-world research, climate literacy, and critical thinking, this lesson becomes engaging and relevant to participating students. This engaged learning can motivate climate action among students, promote interdisciplinary learning, raise ethical considerations, and open doors to careers in sustainability. By utilizing a hands-on, real-time approach, students are empowered to become informed and engaged citizens capable of addressing the urgent global challenge of climate change (Ardoin, Bowers & Gaillard 2020).

The objectives of this lesson aim to help students understand and identify the implications of climate change on an economically important fishery species. Participating students are asked a set of questions before and after (~ one week between testing) the completion of the lesson to assess their understanding of ocean acidification concepts and how changes in seawater pH can impact the biology of a commercially important species.

DOI: 10.5334/cjme.117

LESSON EVALUATION

This lesson was piloted with 28 total high school students (juniors and seniors) from the Benjamin School in Palm Beach Gardens, FL. and at Argonauta Academy in Melbourne, FL. The students were given a pretest and posttest, both of which used the same questions. The median pretest score was 37.5%. A Wilcoxon signed rank test was used to analyze the pre-posttest results and showed that posttest scores were significantly higher at 50% (W = 21, p = 0.0003; Figure 3). Overall, these scores represent a median increase of 12.5% in understanding ocean acidification concepts after students completed the lesson which was representative of a 33.3% change/increase in correct student responses. In particular, the lesson evaluation showed significant increases in posttest scores of 28.5% and 42.8% on the students' ability to identify factors that contribute to ocean acidification (28.5% increase; W = 0, p = 0.005) and how climate stressors can impact the biology and swimming behavior of stone crab larvae respectively (42.8% increase; W = 7, p = 0.002).

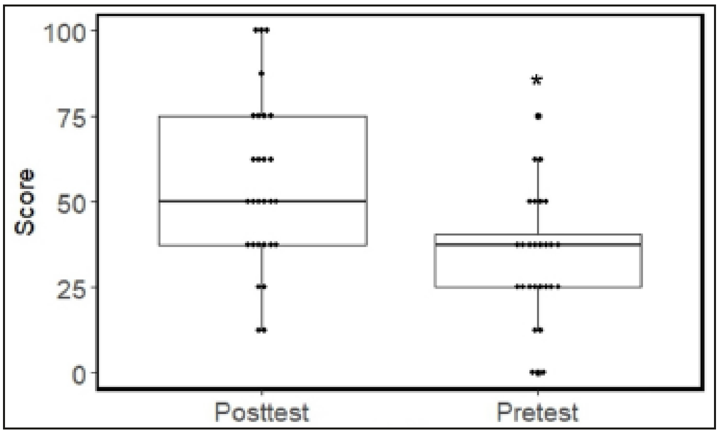

Figure 3 Box plot of pre-test and post-test assessment scores (%) during pilot testing. The solid horizontal black line represents the median while the upper and lower boxes represent the interquartile range. The dots represent individual student scores. The asterisk represents a significant difference at the α = 0.05 level.

RECOMMENDATIONS FOR IMPLEMENTATION AND CONCLUSIONS

Before implementing this lesson in a classroom, ensure that students have familiarity with topics such as the pH scale, land-based run-off into the ocean, and basic crustacean biology (molt, larvae, exoskeleton). To assist with further understanding of the Florida stone crab lifecycle, a worksheet is provided in the supplementary materials to assist students in visualizing this process. In addition, it is recommended that the instructor provide students with training on how to effectively create a plot using an online spreadsheet software as this is critical to the successful completion of the lesson. On average, this entire lesson can be completed in ~three hours which can be broken up into multiple class periods or completed during a longer laboratory setting.

We accept our hypothesis that this lesson increased understanding of both ocean acidification concepts and the potential impacts on the larval biology of the Florida stone crab. While our results show that students performed better in understanding concepts related to ocean acidification and the impacts of reduced pH on stone crab biology, we caution that our sample size (n = 28 students) is low. Further, the students tested were enrolled within a course taught by our partner educators (co-authors).

ADDITIONAL FILES

The additional files for this article can be found as follows:

- **Supplementary Material 1.** Glossary. DOI: https://doi.org/10.5334/cjme.117.s1
- **Supplementary Material 2.** Figure 4. DOI: https://doi.org/10.5334/cjme.117.s2

- **Supplementary Material 3.** Figure 5. DOI: https://doi.org/10.5334/cjme.117.s3
- **Supplementary Material 4.** Figure 6. DOI: https://doi.org/10.5334/cjme.117.s4
- **Supplementary Material 5.** Figure 7. DOI: https://doi.org/10.5334/cjme.117.s5

ACKNOWLEDGEMENTS

We thank the Florida Fish and Wildlife stone crab monitoring program for assistance with animal collection. In addition, we thank Mote Marine Laboratory which provided the laboratory space needed to complete these experiments. We thank the NOAA AOML Lab in Miami for analysis of our seawater carbonate chemistry samples.

FUNDING INFORMATION

This research and lesson were supported by a National Science Foundation grant (Award #2049047) and a NOAA Ocean Acidification Education grant (Award #NA22OAR0170209).

COMPETING INTERESTS

The authors have no competing interests to declare.

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TO CITE THIS ARTICLE:
Smith, A. L., LaBadie, J., Busse, A., Solomon, E., Farrell, C, Holstein, D. M., Xue, Z., & Gravinese, P. M. (2024). Will Climate Change Alter the Swimming Behavior of Larval Stone Crabs?: A Guided-Inquiry Lesson. *Current: The Journal of Marine Education*, 39(2), pp. 3–12. DOI: https://doi.org/10.5334/cjme.117

Submitted: 22 March 2024
Accepted: 25 October 2024
Published: 11 December 2024

Current: The Journal of Marine Education is a peer-reviewed open access journal published by Ubiquity Press.