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Examining the Effects of Altered Water Quality on Sea Urchin Fertilization Success and Embryo Development

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ABSTRACT As a result of shifting marine environmental conditions caused by global climate change and localized water pollution, marine organisms are becoming increasingly exposed to changing water quality conditions. For example, they are exposed to more extreme salinity fluctuations as a result of heavier rainfall, melting polar caps, or extreme droughts. Additionally, polluted coastal runoff into near-shore marine habitats has become more prevalent, carrying contaminants such as fertilizer from farmland or golf courses and other man-made harmful chemicals. At the Marine Science Research Learning Center at the Hawai'i Institute of Marine Biology, we focus on teaching hypothesis-driven and inquiry-based labs to stimulate students' scientific thinking and to increase interest in the fields of marine biology and environmental science. In this lab, we raise awareness of marine pollution and allow students to experiment with the consequences of reduced water quality on sea urchin fertilization success and early embryonic development. The labs consist of three sections: (1) a preparatory literature research and group discussion; (2) hands-on hypothesis-driven science activities; and (3) evaluation, data analysis, and scientific writing. In this article, we discuss background information on water quality changes and sea urchin fertilization, thoroughly describe the three components of the lab, and discuss possible alternatives to the lab's implementation.

KEYWORDS hands-on, high school, inquiry-based learning, place-based education

INTRODUCTION

In sea urchins, many of the mechanisms involved in the fertilization process are sensitive to very small changes in surrounding environmental chemicals. Therefore, sea urchin gametes (sperm and eggs) are commonly used as indicators in environmental research. Studies have found that anthropogenic disturbances (e.g., runoff into the near-shore environment, ocean acidification, global climate change) can endanger biological processes such as fertilization of sea urchins and other marine invertebrates (Richmond 1993, 1996). For example, Kurihara (2008) found that ocean acidification has a negative impact on the fertilization, cleavage, larval settlement, and reproductive stages of several marine calcifiers

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(organisms that produce a calcium carbonate skeleton), including echinoderms, bivalves, corals, and crustaceans. As a result, future changes in ocean acidity will potentially impact the population size and dynamics as well as the community structure of calcifiers (Jokiel and Brown 2004; Kurihara 2008). Water quality changes have also been found to impact the fertilization success of fish species. In a study on pacific herring, *Clupea pallasi*, Griffin et al. (1998) found that sperm motility was inhibited at both elevated and reduced salinities. Also, progression to hatching was delayed in both lower and higher salinities for those embryos that completed development. Likewise, salinity was not found to impact final maturation and spawning in the striped mullet, *Mugil cephalus* (Lee et al. 1992), but it was demonstrated to influence sperm activation and, ultimately, percent fertilization of spawned eggs.

The Environmental Protection Agency utilizes sea urchin embryo developmental standards to test for the presence of water pollution (such as anthropogenic sources of heavy metals, synthetic pesticides, sulphates, and nitrogen, which are sometimes present at dangerous levels in coastal seawater) through employing a standardized sea urchin male gamete assay on its ability to fertilize (Wagner and Nacci 2012). Some public aquariums also use the health of adult sea urchins as an indicator of the water quality in their tanks. Understanding what environmental factors can disrupt or alter normal development in sea urchins provides a sensitive and reproducible indicator for what might harm other life in the sea.

By studying sea urchin gametes, scientists have begun to understand fertilization at both the cellular and molecular levels. As an urchin male gamete enters an urchin female gamete, the outside of the female gamete's plasma membrane lifts away from the surface, resulting in the elevation of the fertilization envelope (Figure 1). Fertilization gives rise to a zygote, and the formation of the zygote is followed by a series of rapid cell divisions without cell growth, known as cleavage. The cytoplasm of each cell is divided into smaller and smaller cells called blastomeres. Like the fertilization envelope, cleavage is visible through a compound microscope and can easily be observed (Figure 2).

In this article, we will describe a hands-on, hypothesis-driven curriculum designed for high school students, focusing on the central driving question "How are sea urchin fertilization success and embryo development affected by water quality?" This inquiry-based lesson promotes interest in marine science and environmental stewardship. The lab consists of three components, which will be thoroughly discussed in the following section. Additionally, we provide possible alterations to this lab in case performance of the lab as described below is not feasible.

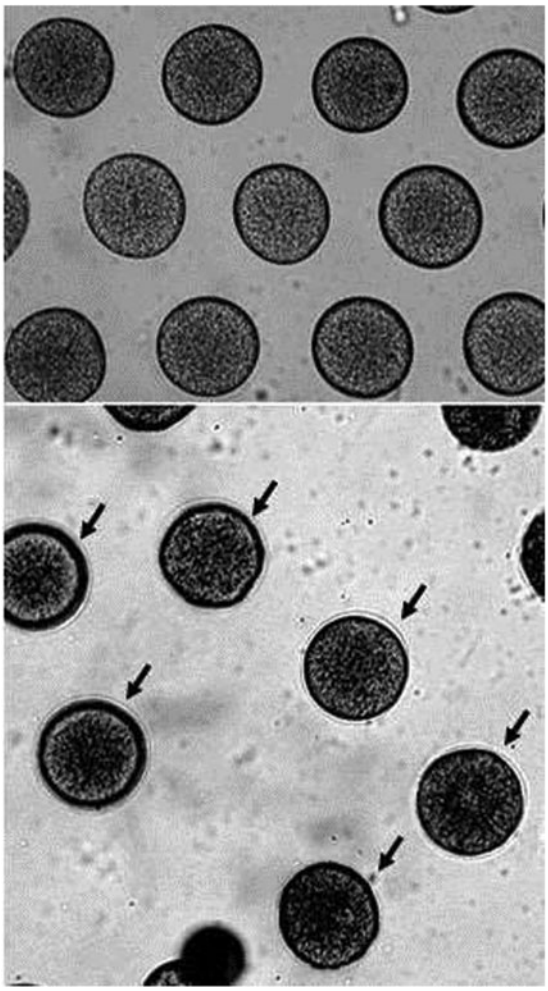

FIGURE 1 **Photographs of unfertilized (top) and fertilized (bottom) sea urchin female gametes. Note the lack of fertilization membranes in unfertilized female gametes.**

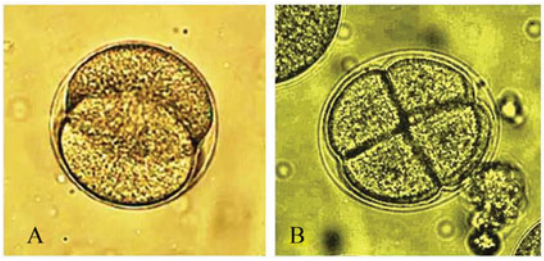

FIGURE 2 **Sea urchin, *Tripneustes gratilla* embryos: (A) 2-cell stage after first cleavage, (B) 4-cell stage after second cleavage (color figure available online).**

MATERIALS LIST FOR GAMETE COLLECTION

1. Approximately 10 *Tripneustes gratilla* urchins
2. One bulb pipette or eyedropper to collect male urchin gametes
3. Several 250-ml glass beakers to collect female urchin gametes
4. Filtered seawater to collect female urchin gametes
5. 100-ml glass beakers: one to prepare the diluted female gamete suspension, one to prepare the diluted male gamete suspension, and one for each group of four students to distribute 40 ml diluted female gamete suspension
6. Test tubes (approximately 8 ml): one to collect male urchin gametes, and one for each group of four students to distribute 5 ml diluted male gamete suspension

Note: If filtered seawater is unavailable, there are three alternatives: (1) Regular seawater may be used. Whether or not microscopic organisms in unfiltered seawater may affect the experiments has not been tested. The effects, if any, are likely minor. (2) Artificial seawater mix may be used. Instant Ocean is a widely used brand and may be purchased at a pet shop or online. (3) Regular seawater may be sterilized using a microwave (Keller, Bellows, and Guillard 1988).

MATERIALS LIST FOR STUDENT EXPERIMENTS

1. One bulb pipettes or eyedroppers per experiment
2. Filtered seawater
3. One test tube (approximately 8 ml) per experiment and a test-tube rack
4. Various solutions to be added to the filtered salt water for manipulating water quality: fresh water, high salt concentrations, prepared liquid plant fertilizer (e.g., Miracle Gro)
5. One 10-ml glass pipette with pipetting ball per group
6. One stopwatch or timer per group
7. At least one compound microscope per group
8. Depression slides and cover slips

PROCEDURE

The lab consists of three components: (1) preparatory literature research and group discussion; (2) hands-on hypothesis-driven lab activities; and (3) evaluation, data analysis, and scientific writing. Although the focal point of the activities at the Hawai'i Institute of Marine Biology (HIMB) are sea urchins, the results also provide insights as to the potential impact that natural or anthropogenic events have on other marine organisms. Below we describe the three components of the lesson plan; a more detailed version may be also downloaded online on the HIMB Outreach Web site (HIMB Education Program 2013).

Component 1: Literature Research and Group Discussion

Researchers do a considerable amount of preliminary investigation by reading previously published research in order to become informed of the latest information on a particular topic. There is already a substantial amount of literature available and accessible on the Internet on the effects of water quality on sea urchin fertilization. Students should conduct their own literature research on this topic and prepare step-by-step illustrations of the fertilization process that includes the following elements: (1) morphology of a male gamete cell, (2) morphology of a female gamete cell, (3) chemotaxis (Ward et al. 1985), (4) acrosomal reaction (Summers and Hylander 1975), (5) bindin protein molecule, (6) vitelline membrane, (7) cortical reaction, (8) first cleavage to eight-cell stage, and (9) mitosis and meiosis. An excellent Web site to get started with is the Virtual Urchin (Stanford University 2013). As an extra-credit extension exercise, students could illustrate on single sheets of paper several types of male gamete cells from different organisms that reproduce sexually, allowing for a deeper understanding of the fertilization process in general. The size of the male gamete cell should be indicated as well as the location of the acrosome for each type of male gamete cell that is illustrated. Be sure to have students list any references and sources used for future verification purposes. Last, the instructor should lead a discussion with the class as a whole about the students' findings from the reading investigation, as well as from the extra-credit assignment.

Component 2: Hands-On Hypothesis-Driven Science Activities

For the following exercise, we use *Tripneustes gratilla* urchins, commonly known as "collector urchins." However, other species of urchins may be used for this lab as well. In case field collections are not possible, *Eucidaris tribuloides* or *Lytechinus variegatus* urchins may be ordered online (Carolina Biological Supply Co., Burlington, NC; http://www.carolina.com). This kit supports the potassium chloride injection method of inducing spawning; however, we highly recommend trying the submersion methods described here to possibly improve urchin survival (based on findings with *T. gratilla*). In order to maintain the urchins in an aquarium, a constant flow of fresh ocean water is preferred, but a saltwater aquarium with proper filtration can also be used. Furthermore, instructors should be sure to research the specific feeding requirements of their urchin species.

Quantifying water quality's effect on fertilization requires that we control the density of gametes in our assays. For that reason, the first activity in the laboratory will focus on the controlled acquisition of urchin gametes. We induce urchin spawning artificially by submerging urchins in seawater after being out of water for 1 hr. This method was developed specific to *T. gratilla* and may reduce postspawning mortalities (unpublished data), but it may be effective with other species as well. Students should observe the various steps that are undertaken. To ensure that there is at least one male and one female urchin, while also accounting for the likelihood that not all urchins will be successfully induced to spawn, it is best to have at least 10 urchins on hand. After resubmerging the urchins into the water, they should be closely monitored for approximately 5 min for gametes being extruded from the gonopores on top of the urchin. A milky white substance identifies a male, and a pink/orange-colored substance identifies a female. Protocols to collect male versus female gametes vary. If the urchin is a male, it should be taken out of the water and placed mouth side down (Figure 3). Male gametes should be collected dry (i.e., without seawater) with a bulb pipette or eyedropper and kept dry in a test tube until ready to perform the experiments. If the urchin is a female, it should be taken out of the water and placed mouth side up onto a beaker filled to the brim with filtered seawater (Figure 4). The female gametes will be shed into the seawater and sink to the bottom of the beaker. It can take 10 to 30 min for a female urchin to finish shedding her gametes. After spawning ceases, urchins should be returned to the water.

FIGURE 3 Placement of spawning male sea urchin to retrieve male gametes (color figure available online).

Standardized gamete suspensions should be prepared to distribute to students working in groups of four. It is important to use standardized gamete concentrations to successfully fertilize the female gametes during the experiments. These protocols follow directions initially developed at Stanford University (2013). For preparation of the diluted male gamete suspension, a few microliters of male gametes should be added to 100 ml of filtered seawater. Each group should then

FIGURE 4 Placement of spawning female sea urchin to retrieve female gametes (color figure available online).

be provided with a 5-ml aliquot of this diluted male gamete suspension. For preparation of diluted female gamete suspension, 4 ml of female gametes should be taken from the bottom of the beaker and placed into 100 ml of seawater. Each group should be provided with 40 ml diluted female gamete suspension.

The students can expose the gametes to various treatments in order to simulate processes that occur naturally (e.g., rainfall, drought) or are instigated by humans (e.g., polluted coastal runoff). At HIMB, we simulate altered environmental conditions by providing (1) solutions of salt to allow students to artificially increase salinity, (2) fresh water to decrease salinity, and (3) fertilizer to approximate increased nitrogen loads common in coastal runoff from agricultural land and golf courses. Before setting up the experiments, each group has to formulate and write down a hypothesis to test the impact of altered water conditions on fertilization success and embryo development. Students should be lectured on how to formulate a hypothesis and the importance of a control experiment. The hypothesis of each individual group should be checked before setting up any experiments. In this activity, the control experiment is a fertilization experiment in normal unaltered seawater. An example of a hypothesis and experimental methods is available online (HIMB Education Program 2013).

We recommend the following as a guide for setting up fertilization experiments: each experiment includes 5 ml diluted female gamete suspension, 1–2 ml test solution (i.e., regular seawater for the control experiment and manipulated water for the test experiment), and one drop of diluted male gamete suspension (Figure 5). For the test experiment, students should decide which water quality variable they wish to manipulate in their test solution (e.g., plant-fertilizer concentration, salinity). A timer should be started as soon as the male gamete suspension is added to the experiments. To monitor fertilization success, each student should determine the percentage of female gametes with a fertilization envelope in the control and test experiments at T = 0 min. The fertilization envelope develops immediately after a male gamete cell enters a female gamete cell. To monitor embryo development, each student should determine the percentage of each embryonic phase (e.g., two-cell stage, four-cell stage) in the control versus test experiments at several time intervals (e.g., T = 0 min, T = 15 min, T = 30 min). To view the embryos under the microscope, students should perform the following steps: (1) mix the suspension into a homogenous

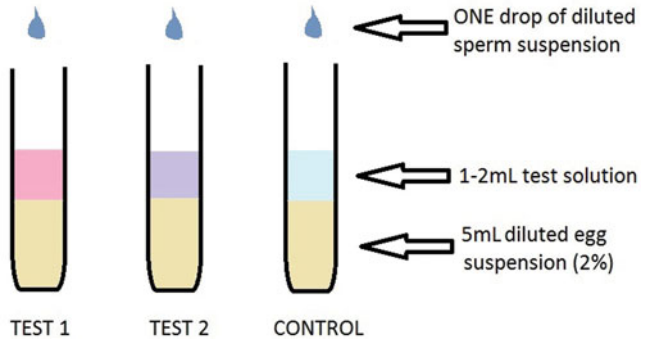

FIGURE 5 **Test-tube volumes for experiments (color figure available online).**

solution using the bulb pipette and take three drops with the bulb pipette, (2) place the drops on a depression slide and cover it with a cover slip, and (3) view under the compound microscope and monitor fertilization success and embryo development in a minimum of 20 female gametes. Group averages should be calculated of the percent fertilization and percentage of embryos found at each cell stage for each time interval and filled out in the tables contained in the Appendix. In *T. gratilla*, first cleavage takes place approximately 40 min after fertilization occurs. Optionally, students may continue to observe development of the embryos from their experimental treatments for the next several days. In this case, students may collect data on a daily basis regarding the further developmental stages, such as the early blastula, hatched blastula, early gastrula, gastrula, prism, early pluteus, and pluteus. Each student should observe 10 embryos from each experimental group and note the developmental stages. All student findings within the same group should be combined, and percentages of each developmental stage for each experimental group should be calculated. Additionally, survival data may be collected. In this case, each student should observe 10 embryos from each experimental group and note survival. Live embryos are characterized by movement, and in later developmental stages a "heartbeat" may be observed. Eventually, all embryos will die due to lack of nutrition in the water or buildup of waste products.

Component 3: Evaluation, Data Analysis, and Scientific Writing and Communication

Class should start with a general discussion to evaluate the previous components. One discussion point should concern how the results of the literature search

and subsequent class discussion during component one assisted with the completion of the experimental activities during Component 2. The key to the success of this laboratory exercise is in ensuring equal concentrations of gametes for each experiment. Therefore, it should also be discussed if it was hard or easy to obtain gametes using the submersion method and if the number of female gametes that were produced was surprising or expected, and what this might imply for reproduction in nature. Next, students should discuss their data by comparing their test and control experiments and interpreting what the findings might indicate. Each group should share with the class their hypothesis and results. A final discussion point should be about the implications of the research findings to other organisms that are also present in the reef environment.

At HIMB, we also evaluate to what extent the experimental activities impacted student awareness of how humans can affect the environment. In addition to evaluating student awareness, we ask questions relating to their interest in science subjects, environmental stewardship, and science careers, as well as knowledge of content. We do this through the use of pre- and post-activity questions. During this process, students answer questions anonymously to encourage participation and honesty in responding to questions evaluating their understanding of the content. Last, students should produce an in-depth laboratory report including a title, introduction, hypothesis, materials and methods, results, discussion, and conclusion. To practice scientific communication, student groups should present their data in a 5-min oral presentation.

POSSIBLE RESULTS

When urchin gametes are exposed to plant fertilizer or altered salinity levels, decreased sperm motility, deformed eggs, and/or lower levels of percent fertilization may be observed.

POSSIBLE ALTERATIONS

Teaching Urchin Fertilization for Students Younger than the 9–12 Level

When dealing with younger students, it is also possible to remove the hypothesis component from this lab. In our experience, the ability to witness live fertilization and cleavage, and further development of the blastula, is an impressive experience on its own. Additional lecturing on the impressive process of mitosis will have enhanced meaning if students are able experience this firsthand.

No Access to Urchin Gametes

Although this lab is focused on urchins, other organisms (e.g., goldfish) may also be used, as long as it is possible to manually add gametes (and thus control the timing of fertilization) and if female gametes are of an appropriate size for monitoring fertilization and development using a microscope. We recommend contacting nearby universities to obtain information regarding any brood stocks they maintain and the potential for using their spawn.

When All Urchins Are Males

In our experience, it is not unlikely that all of the spawning urchins are males. If this happens, however, it is still possible to conduct the lab activity by focusing instead on the effects of water quality on male gamete motility and using this as an indicator for potential effects on fertilization.

CONCLUSION

This hands-on and inquiry-based biology activity on water quality effect on sea-urchin fertilization and embryo development was developed to provide high school students exposure to the field of marine biology, and to stimulate hypothesis-based thinking. In addition to high school students, we occasionally host younger students as well as recent high school graduates or early undergraduates. Also, we have been contacted by a university on the mainland to use the environmental aspects of this lab for their nonmajor undergraduates to enhance their "regular" urchin fertilization lab. Besides the more typical biology lesson on the fertilization process as commonly taught in many science classrooms, this activity expands to include hypothesis testing, experimentation, and data analysis. The exploration of altered watered quality effects on successful reproduction and development raises student awareness about pollution to marine ecosystems. Moreover, this activity draws from both the *National Science Education Standards* (National Research Council [NRC] 1996) and the newly released *Next Generation Science Standards* (NRC 2013). In other words, the inquiry activity we present

here emphasizes several skills and concepts determined to be important to the early development and training of young scientists. These skills and concepts include: (1) the identification of questions and concepts that guide scientific investigations, as well as the design and conduct of scientific investigations (*National Science Education Standards* [NRC 1996], 9–12 Science as Inquiry Standard A), (2) the role of cell differentiation and arrangement in embryo development and the formation of complex multicellular organisms (*Next Generation Science Standards* [NRC 2013], HS-LS1–4), (3) the role of human impact on the environment (*Next Generation Science Standards* [NRC 2013], HS-LS2–7), and (4) the evaluation of how changing environmental conditions may affect certain species over others and how these effects may ultimately cause species extinction and/or alter species evolution over time (*Next Generation Science Standards* [NRC 2013], HS-LS4–5). We continuously receive positive feedback from teachers as well as the many students who have visited our program thus far, and we recommend the implementation of this lab in other school programs as well.

REFERENCES

Griffin, F. J., M. C. Pillai, C. A. Vines, J. Kääriä, T. Hibbard-Robbins, R. Yanagimachi, and G. N. Cherr. 1998. Effects of salinity on sperm motility, fertilization, and development in the Pacific herring, *Clupea pallasi*. *The Biological Bulletin* 194: 25–35.

HIMB Education Program. 2013. Inquiry fieldtrips. www2.hawaii.edu/~himbed/inquiry-fieldtrips (accessed April 1, 2013).

Jokiel, P. L., and E. K. Brown. 2004. Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawaii. *Global Change Biology* 10: 1627–1641.

Keller, M. D., W. K. Bellows, and R. R. L. Guillard. 1988. Microwave treatment for sterilization of phytoplankton culture media. *Journal of Experimental Marine Biology and Ecology* 117: 279–283.

Kurihara, H. 2008. Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series* 373: 275–284.

Lee, C.-S., C. S. Tamaru, C. D. Kelley, A. Moriwake, and G. T. Miyamoto. 1992. The effect of salinity on the induction and spawning in the striped mullet, *Mugil cephalus*. *Aquaculture* 102: 289–296.

National Research Council. 1996. *National science and education standards*. Washington, DC: National Academy Press. http://www.nap.edu/openbook.php?recordid=4962&page=107 (accessed July 18, 2013).

National Research Council. 2013. *Next generation science standards*. Washington, DC: National Academy Press. http://www.nextgenscience.org/next-generation-science-standards (accessed July 18, 2013).

Richmond, R. H. 1993. Coral reefs: Present problems and future concerns resulting from anthropogenic disturbance. *American Zoologist* 33: 524–536.

Richmond, R. H. 1996. Effects of coastal runoff on coral reproduction. *Biological Conservation* 76: 211–211.

Stanford University. 2013. Virtual urchin. www.virtualurchin.standford.edu (accessed April 1, 2013).

Summers, R. G., and B. L. Hylander. 1975. Species-specificity of acrosome reaction and primary gamete binding in echinoids. *Experimental Cell Research* 96: 63–68.

Wagner, A., and D. Nacci. 2012. Tropical collector urchin, *Tripneustes gratilla*, fertilization test method. US Environmental Protection Agency 600/R-12/022.

Ward, G. E., C. J. Brokaw, D. L. Garbers, and V. D. Vacquier. 1985. Chemotaxis of Arbacia punctulata spermatozoa to resact, a peptide from the egg jelly layer. *Journal of Cell Biology* 101: 2324–2329.

APPENDIX: RESEARCH FINDINGS URCHIN LAB

Date:_____
Group members:_____
Collection area:_____

Data sheet for quantifying percent of fertilized eggs.
Group Results

	T = 0
Student 1:	
Student 2:	
Student 3:	
Student 4:	
Average:	

Data sheet for quantifying percent of zygotes at each developmental stage for several time intervals (the table may be extended with longer time intervals and further cell divisions).
Group Results

	T = 0 min			
	1-cell	2-cell	4-cell	8-cell
Student 1:				
Student 2:				
Student 3:				
Student 4:				
Average:				
	T = 15 min			
	1-cell	2-cell	4-cell	8-cell
Student 1:				
Student 2:				
Student 3:				
Student 4:				
Average:				
	T = 30 min			
	1-cell	2-cell	4-cell	8-cell
Student 1:				
Student 2:				
Student 3:				
Student 4:				
Average:				
	T = 45 min			
	1-cell	2-cell	4-cell	8-cell
Student 1:				
Student 2:				
Student 3:				
Student 4:				
Average:				
	T = 60 min			
	1-cell	2-cell	4-cell	8-cell
Student 1:				
Student 2:				
Student 3:				
Student 4:				
Average:				