Sea Urchin Fertilization Lab

Hawai'i Institute of Marine Biology
Education Program
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Part I: Pre-activities for the classroom

Science background

Production of gametes (eggs and sperm) is a fundamental characteristic of sexually reproducing organisms. Evolutionarily, sea urchins are on the same lineage that led to mammals, and the size and shape of sea urchin eggs and sperm are similar to our own. Because they are easily studied, urchin eggs and sperm provide valuable information on fertilization and development that applies to many organisms, from jelly fish to humans. In this sense, urchin gametes provide excellent model embryos for a unique understanding of development and sexual reproduction.

Spawning is a term to describe the release of eggs and sperm into the water column. Under a very special set of circumstances, spawning is followed by the uniting of two gametes in a process called *fertilization*. A successfully fertilized egg is called a *zygote*, and is the first step in the creation of a new individual. As you might expect, the beginnings of such an important event is quite complex and involves a suite of processes that all must take place sequentially in order to be successful. By studying the eggs and sperm of sea urchins, scientists have begun to understand fertilization at both the cellular and molecular levels.

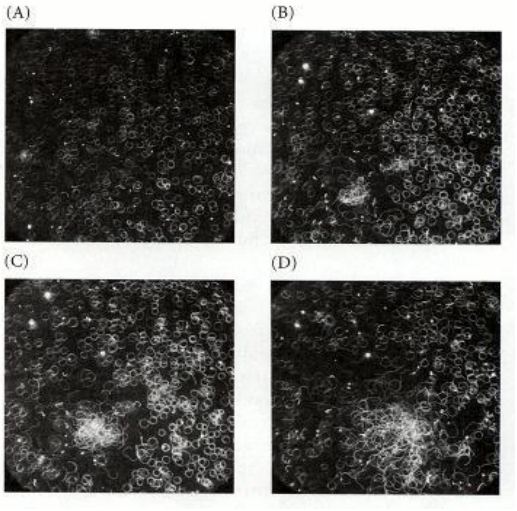

Figure 1: One second photomicrogaph exposures showing the response of sea urchin *A. punctulata* sperm to A) control, B-D) 20, 40, 90 second exposure to one nanoliter of a 10-nM solution of resact, a molecule involved in chemotaxis (see text). Note clumping of sperm cells at point of entry of resact. From Ward et al. 1985.

Sperm Egg Interactions: Sea urchins release their gametes into the environment which may be as small as a tide pool or as large as an ocean. Immediately following release from the adult, two challenges confront the potential fertilization: 1) the unlikely chance of sperm and eggs meeting in the water in such a dilute concentration, and 2) the sperm being prevented from fertilizing eggs of another species that may also be in the water column at the same time. The high local abundance of adults and the shear quantity of sperm and eggs that are produced by individual sea urchins is one strategy to increase the odds of gametes making contact with each other in the water column.

Many species have further evolved a mechanism where the egg emits a chemical that attracts the sperm towards the egg in a process called "chemotaxis" (Figure 1). What is even more astonishing is the sperm is only attracted to the eggs of its own species; in other words the chemical attraction is *species-specific*. At least one

chemotactic molecule, a 14-amino acid peptide called *resact*, has been isolated from the sea urchin *Arbacia punctulata*. Eggs produced by *A. punctulata* are surrounded by a jelly coat that contains resact, while sperm produced by the same species have receptors on their surface (plasma membrane) that bind resact. In recent studies, the binding of resact to these receptors has been shown to activate the machinery that controls movement in the sperm's tail, propelling the sperm through the egg's jelly coat (Ward et al, 1985).

Sperm Motility: The scientific term for the tail of the sperm is *flagellum*, which is the mechanism by which sperm move. Sperm motility is an important factor in successful fertilization, since sperm must travel through the water column to reach the egg, and then penetrate the egg to unite the male and female gametes. The flagella beat in a rapid undulating motion, propelling the sperm in a forward direction. Under a microscope this movement is easily observed, and in healthy sperm looks like an almost random and rapid movement of small particles.

Acrosomal Reaction: Inside the tip of the sperm cell is a highly specialized membrane bound storage compartment called the *acrosome*, which contains a variety of enzymes that can break-down, or *digest*, proteins and sugars. When a sperm cell makes contact with the outer coating of the egg, the contents of the acrosome burst out in a process known as the *acrosomal reaction* (Figure 2 A-D). In sea urchins, the acrosomal reaction is thought to be initiated when a sperm cell touches the outer jelly coat of the egg and sets off the fusion of the acrosomal membrane with the sperm cell membrane (Figure 2 A-C). These enzymes that are now outside of the sperm cell break down the jelly coat surrounding the egg and form a narrow pathway that allows the sperm to approach the egg while constantly being propelled by its flagella. In fact, the substances in the egg jelly that initiates the acrosomal reaction are highly specific to each species of sea urchin, and represents a second species-specific mechanism that ensures the correct sperm fertilizes the correct egg (Summers and Hylander 1975).

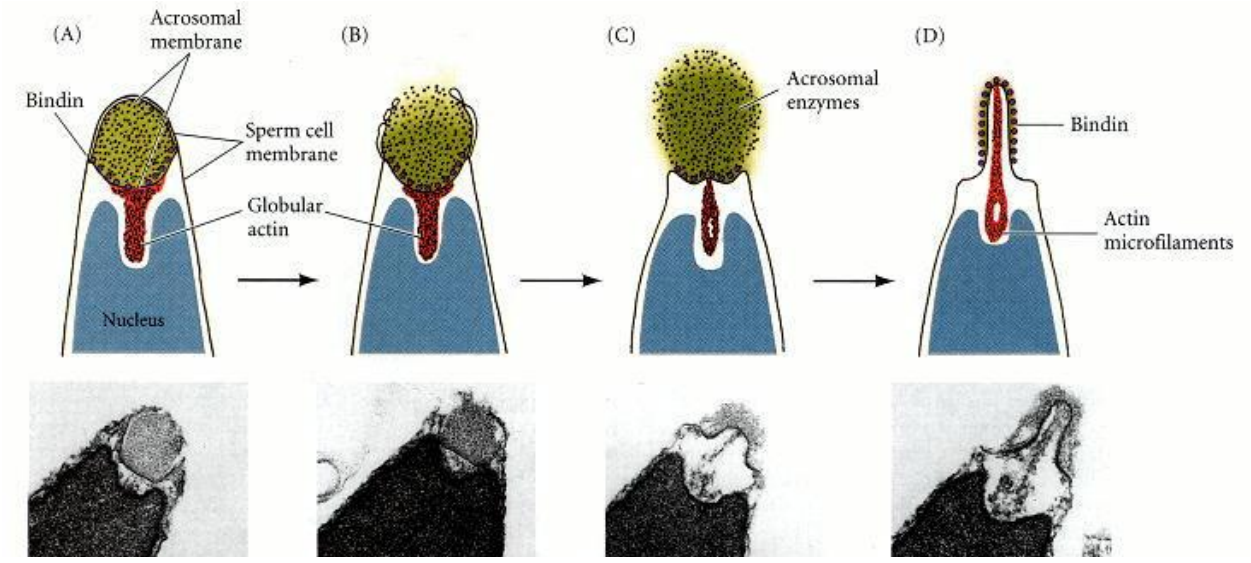

Figure 2. Summary of the acrosomal reaction in sea urchin sperm. A) The tip of a sperm cell showing the main components that will take part in the acrosomal reaction. B) The portion of the acrosomal membrane lying directly beneath the sperm cell membrane fuse together with each other. C) The result is the release of the acrosomal enzymes that digest the egg jelly coat. D) Production of microfilaments extends the acrosomal process outward exposing the bindin and ensuring species recognition of egg and sperm. Actual photographs of the acrosomal reaction in sea urchin sperm are shown below the diagrams. After Summers and Hylander 1974; photographs courtesy of G. L. Decker and W. J. Lennarz.

Sperm and Egg Fusion: A third mechanism that ensures a species-specific fusion of the sperm with the egg involves a protein called *bindin*. Bindin has been found to be closely associated with the acrosomal elements (Figure 2D) that are present in the head of the sperm, and only becomes exposed at the end of the acrosomal reaction. Scientists have isolated bindin from the acrosome and found it to be capable of binding to eggs of only the same species of sea urchin. Just like how a lock and key works, receptors (e.g., the 'lock') on the egg cell surface only recognize the bindin protein (e.g., the 'key') originating from sperm of the same species. Recognition of the bindin protein allows for the initiation of the fusion of both egg and sperm membranes, resulting in the next phase of the fertilization process.

Blocks to polyspermy: *Polyspermy* is a term used to describe more than one sperm penetrating the egg membrane, a case that would result in a non-viable zygote. Mechanisms that prevent this from occurring are called *blocks to polyspermy*. A rapid, or *fast block to polypsermy* occurs when the fusion of sperm and egg initiates a sudden (e.g., one to three seconds) change in the sodium ions in the egg that spreads over the cell membrane in an electrical wave of activity. This electrical event provides short-term prevention against additional sperm entering the egg. In addition to this 'fast block', the sperm and egg fusion also initiates a process called the *cortical reaction*, which is a series of reactions that ultimately modifies a protein coat on the outside of the plasma membrane (the *vitelline layer*), causing it to be released from the membrane. The vitelline layer separates and lifts away from the egg surface, resulting in the 'elevation of the fertilization envelope' (Figure 3). This process is known as the *slow block to polyspermy*.

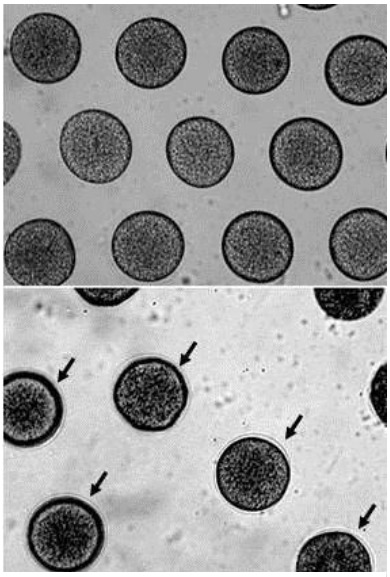

Figure 3. Photographs of unfertilized (top) and fertilized (bottom) sea urchin eggs. Appearance of the fertilization envelope (arrows) is being used as an indicator that fertilization has taken place. Note the lack of fertilization membranes in unfertilized eggs (top photo).

While the 'fast block' occurs in a blink of an eye and is difficult to observe, the raising of the fertilization envelope is visible through a compound microscope. As the fertilization envelope elevates, non-fertilizing sperm are lifted away from the egg plasma membrane, as they are not able to pass through the fertilization envelope.

Meiosis, Mitosis and Cleavage: *Meiosis* is the process that produces the gametes (sperm and eggs) necessary for sexual reproduction. During meiosis, maternal and paternal chromosomes within a 'parent' cell exchange genetic material in a process called *recombination*. The parent cell then divides twice to produce a total of four daughter cells (the gametes). In the end, the daughter cells contain only half the chromosomes (i.e. are 'haploid'), and because of recombination, are **not** exact copies of the original parent cell. After fertilization (when the gametes from two parents fuse), a full set of chromosomes (diploid) is restored.

Fusion of the sperm and egg membranes is followed by the nuclear contents of the sperm being pulled into the egg, giving rise to a *zygote*. The zygote is a diploid cell, and its formation signifies the unification of a complete set of homologous chromosomes; half from the sperm and

half from the egg. Formation of the zygote is then followed by a series of rapid cell divisions without cell growth known as *cleavage* (Figure 4). Essentially the cytoplasm of each cell is divided into smaller and smaller cells called *blastomeres*. During these continual cell divisions, the total intracellular volume of the embryo itself remains unchanged, but the number of cells within the embryo increases. This process of cell replication where the cell separates the chromosomes in its nucleus into two identical sets, in two separate nuclei, is called *mitosis*. Different from meiosis, mitosis produces two daughter cells that **are** exact copies of the parent cell (see Table 1). The function of mitosis is to form somatic (body) cells, which are produced during development, growth, cell replacement, regeneration and asexual reproduction.

	Mitosis	***Meiosis***
Function	somatic cells	gametes
Cell division	1x	2x
Daughter cell #	2	4
Ploidy	Diploid	Haploid

Table 1. Basic differences between the processes of mitosis and meiosis.

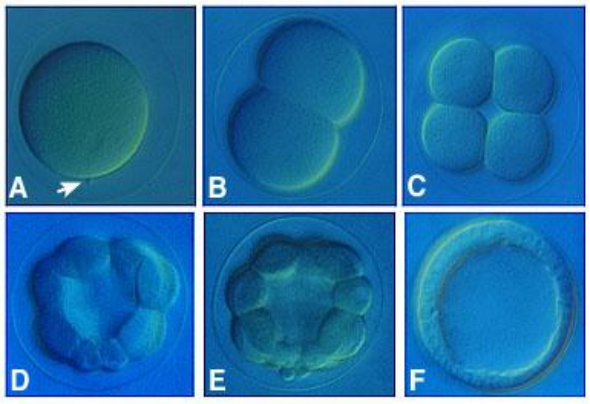

Figure 4. Sea urchin, *L. variegatus* zygotes. A) 1-cell zygote; the fertilization envelope is visible as a large 'halo' around the embryo. The arrow points to the site of sperm penetration. B) 2-cell. C) 4-cell. D) 16-cell. E) 32-cell. F) Hatched blastula.

Source: worms.zoology.wisc.edu/urchins/SUcleavage_stages.html

Environmental Application: Previous studies have found that anthropogenic disturbances (e.g. from runoff into the nearshore environment or other marine pollution) can endanger coastal reef processes such as fertilization of sea urchins and other marine invertebrates (Richmond 1993; 1996). In sea urchins, many of the mechanisms involved in the fertilization process are sensitive to very small changes in surrounding environmental chemicals and therefore their gametes are commonly used as indicator organisms in environmental studies. The Environmental Protection Agency utilizes sea urchin embryo developmental standards to test for the presence of water pollution. They employ a standardized sea urchin sperm/fertilization assay to detect anthropogenic sources of heavy metals and synthetic pesticides that are sometimes present in dangerous levels coastal seawater. The same assay is also used to test for the presence of anthropogenic biological contaminants such as sulphates and nitrogen-containing contaminants. 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, and ultimately us.

Research at the Hawai‘i Institute of Marine Biology

Figure 5. A male sea urchin, *Tripneustes gratilla*, spawning. Note the sperm (whitish cloud) at the base of the sea urchin and spreading away from the individual. Source: www.himb.hawaii.edu/html/beef/projects.php?project=urchin

Dr. Florence Thomas leads the Biophysical Ecology of Environmental Fluctuation Lab at HIMB, and some of her work involves studies of the fertilization process in free spawning invertebrates including sea urchins. Dr. Thomas also investigates how this process is influenced by the local water flow patterns and by physical characteristics and movements of spawned gametes. This research provides insight into how adults space themselves in the environment in order to maximize opportunities for successful fertilization. In addition, research from the Thomas Lab also indicates that gamete characteristics such as their size and shape play an important role in the fertilization process.

Figure 6. The collector urchin, *Tripnuestes gratilla*, is being targeted for artificial propagation in order to generate large numbers that will be released into wild in an attempt to control the spread of invasive algal species. Photograph © Jennifer Smith.

Dr.’s Eric Conklin, Jennifer Smith, and Cynthia Hunter, working with The Nature Conservancy Hawai‘i, the Division of Aquatic Resources and the University of Hawai‘i, are currently engaged in trying to bring two particularly virulent invasive algal species under control: *Gracilaria salicornia* (also known as gorilla ogo), and the gristly yellow-green *Eucheuma denticulatum*. Both species grow into thick, tangled mats that destroy natural habitat by smothering coral and native algal communities. As a project affiliated with HMB, and with funding support through National Oceanic and Atmospheric Administration’s (NOAA) Community Based Restoration Program, Sea Grant College Program and others, these researchers have been studying the effectiveness of the Super Sucker, an ingenious device which is essentially an underwater vacuum cleaner outfitted with a 40-horsepower diesel engine and runs on bio-diesel fuel. The vacuum itself is a Venturi system which means there are no fans or blades that the collected algae pass through. This is important for two reasons: 1) any marine life that is inadvertently collected can be returned alive, and 2) because alien algae can reproduce by fragmentation, the fewer fragments generated during the vacuuming process, the better. But cleaning the reef of alien algae is only half the battle. *Gracilaria* and *Eucheuma* can quickly return and spread at a rate of greater than 300 meters a year. To prevent any new growth,

researchers plan to grow and release native sea urchins that feed on the alien algae and plant native algae in the cleaned areas to re-populate the reef. It is anticipated that it will take a combination of physical removal and biological control with local sea urchins to bring the invasive species under control.

Classroom Laboratory

Researchers do a considerable amount of preliminary investigation by reading previously published results in order to become informed of the latest information on a particular topic area. There is already a considerable amount of information available and accessible on the internet. Students should conduct their own research[1] on sea urchin fertilization and prepare step by step illustrations of the fertilization process that includes the following elements:

- morphology of a sperm cell
- morphology of an egg
- acrosomal reaction
- bindin protein molecule
- vitelline membrane
- cortical reaction
- cleavage to 8-cell stage

As extra credit, students should illustrate on single sheets of paper several types of sperm cells from different organisms that reproduce sexually (e.g. vertebrates, invertebrates, and plants). Be sure that the size of the sperm cell is indicated as well as the location of the acrosome for each type of sperm cell that is illustrated. Be sure to list any references and sources used for future verification purposes. Are there features of sperm morphology that are similar across different species, different phyla? Why would that be?

The fertilization process will take place at the microscopic level and students should be able to visualize the events in order to fully appreciate what they will be observing and testing while at HIMB. To see if students possess a good understanding of the fertilization process they should be able to answer the following questions:

- The acrosome is located in:
    - the middle of the egg
    - just above the tail of the sperm cell
    - in the head of the sperm cell
    - in the membrane of the egg cell

- Identify the proper sequence of events that characterize the fertilization process
    - acrosomal reaction, spawning, cortical reaction, cleavage
    - cortical reaction, acrosomal reaction, spawning, cleavage
    - spawning, acrosomal reaction, cortical reaction, cleavage
    - spawning, cleavage, acrosomal reaction, cortical reaction

[1] An excellent site to get started with is the *Virtual Urchin*, found at: www.virtualurchin.standford.edu

- The bindin molecule is responsible for:
    - the egg to survive in the water column
    - recognition of sperm and egg being from the same species
    - initiation of the acrosomal reaction
    - initiation of sperm motility

- The fertilization envelope is found:
    - just after cleavage
    - just before cleavage
    - just before the cortical reaction
    - just after the cortical reaction

What to expect during the field trip day

During your visit to HIMB you will observe how sea urchins are spawned in order to obtain their gametes in a controlled fashion. In this manner, you will be able to conduct a series of observations at both the macroscopic level (means using just your eyes) and at the microscopic level (using a compound microscope). Using these basic tools you will be conducting both observations as well as hypothesis driven experiments in order to gain insight into the fertilization process. Students are also encouraged to bring products (e.g., laundry detergent, dish soap, household cleaning products, bleach, vinegar, etc.) from home to investigate impacts that household agents have on gametes and/or the fertilization process.

Please bring a copy of this lab with you to HIMB.

Part II: Field trip day at HIMB

Introduction

A) To investigate fertilization requires that we control the availability of gametes and how they interact with each other. For that reason, the first activity in the laboratory will focus on the controlled acquisition of sperm and eggs from the sea urchins. During this initial phase the instructors will be artificially inducing male and female sea urchins to spawn, and the students may be able to observe the various steps that are undertaken.

B) During the next phase, students will expose the sperm and eggs to various treatments in order to simulate situations that occur naturally (e.g., rainfall) or are man-made (e.g., non-point source pollution). By conducting specific experiments you will be able to test some hypotheses regarding impacts these events have on the fertilization process in sea urchins. This is possible because we can visually see some of the impacts on the sperm (e.g., sperm motility) resulting from the various treatments. For eggs, we will be using the presence or absence of the fertilization membrane as an indicator of treatment effects. While the focal point of the activities at HIMB will be sea urchins, the results you obtain should also provide insights as to the potential impacts that natural or anthropogenic events have on other marine organisms.

C) The last phase of the activity at HIMB will be focused on observing the culminating event of the fertilization process which is the production of a zygote, or the beginning of a unique individual. Confirmation that this has successfully taken place is recognized when the fertilized egg begins to cleave. We can say this because watching cleavage taking place also means that incorporation of genetic material from both the egg and the sperm has successfully occurred.

To assist you in your science based inquiry of the fertilization process, there are a number of guiding questions that you should think about in developing the hypotheses you want to test while at HIMB. The listed questions are to assist you in developing testable hypotheses, which forms the basis for the next set of activities. An example of how a null hypothesis is derived and how to test it is available in the Appendix.

Guiding Questions

- What conditions (natural or man made) might affect fertilization in sea urchins?
- What kinds of things might have a negative effect on sea urchin gametes? On other marine organisms?
- How much (e.g., concentration) of a particular substance will impact sperm motility?
- How much of a particular substance will affect an egg from being fertilized?
- What conditions might affect cleavage from taking place?
- What conditions may affect the rate at which cleavage and embryonic development takes place.

Tools available

Motic digital compound microscope
Cordless digital compound microscopes

Materials available:

- 0.5M potassium chloride (KCl) solution (3.73g of KCl in 100mL of distilled water)
- Filtered sea water
- Petri dishes
- Depression slides
- Glass slides and cover slips
- Small syringes
- Plastic pipettes
- 100 mL glass beakers
- 250 mL glass beakers
- 500 mL glass beakers
- Rulers
- 10 mL test tubes
- Test tube racks
- Urchins to induce spawning of gametes
- Sea salt solutions
- Miracle Grow

A) Production of gametes (instructors will lead this phase): To insure that there is at least one male and one female sea urchin it is best to have approximately 10 sea urchins on hand. These will be available when the class visits HIMB. The instructor may have already performed the following steps prior to your arrival:

Figure 7. Injecting KCl into a sea urchin to induce release of gametes.

Step 1. Vigorously rock the urchin for one minute and see mechanical disturbance induces spawning. If this does not work after one minute, the next step would be to inject about 0.1-0.2 mL of 0.5M KCl solution per inch of sea urchin width into the soft tissue surrounding the mouth of the sea urchin (Figure 7). A total of two injections will usually suffice. It is best to use as small a needle as possible as a larger needle leaves a larger wound.

Step 2. Gently rock the urchin for a few seconds to mix the KCl solution inside the sea urchin. Be careful not to rock them too hard.

Step 3. After the injection, place them mouth side down in a petri dish (Figure 8) and closely watch for gametes being extruded from the top of the urchin. Wait for at least five to ten minutes. A milky white substance identifies a male and a pink/orange colored substance identifies a female.

Male

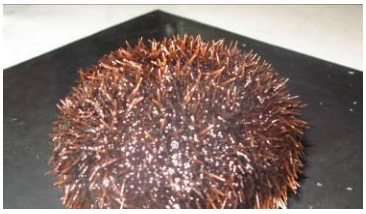

Figure 8. Placement of sea urchin male to retrieve sperm after injection with KCl. Sperm will be exuded through the pores located on the surface of the sea urchin.

- If a male, leave the urchin in the petri dish.
- Collect the sperm 'dry' in a pipet or eyedropper and mix it with 20 mL of filtered seawater. This is called the sperm diluent.
- Place a male urchin back in seawater holding tank only after collecting the sperm.

Female

- If a female, place the urchin mouth side up onto a 500 mL beaker filled to the brim with seawater (Figure 9).
- The eggs will be shed into the seawater and collected at the bottom of the beaker. This can take from 10-30 minutes to finish shedding the eggs.
- After the eggs have stopped flowing, return the female to the holding tank.
- There should be a layer of eggs on the bottom of the beaker. Remove 0.2 mL of eggs from the bottom of the beaker and place them into individual 100 mL glass beakers filled with 50 mL of sea water. Distribute these around the classroom as the source of eggs to be used to conduct your various experiments.

Figure 9. Female sea urchin induced to spawn her eggs into a glass beaker filled with seawater.

NOTE: In order for students to view cleavage during their visit to HIMB, the instructors will initiate the fertilization process prior to beginning the other inquiry based activities. Students should follow along with the instructor as the steps below are carried out to demonstrate basic fertilization:

1) Pipet 1 mL of the sperm diluent and place it with the eggs. Be sure to mix gently allowing all of the eggs to be suspended in the water column.
2) Allow the eggs to incubate over the course of 45 minutes to an hour. Begin viewing samples under a compound scope to watch for signs of cell division approximately 30 minutes after introduction of the sperm. Use the Motic Digital Compound Microscope for viewing eggs by the whole class.
3) As an option, arrangements can be made with the instructor to have the fertilized eggs be placed in a plastic bag for transport back to school after leaving HIMB. The eggs will hatch and continue to develop back in school over the course of the next few days.

B) Science based inquiry into the fertilization process (students): Students should work in groups of four, and begin by preparing one 100 mL beaker with 50 mL of sea water and another 100 mL beaker with 20 mL of sea water. These will be used to collect eggs and prepare the sperm diluent. Each group is required to generate a hypothesis to assess anthropogenic and/or

environmental impacts following a similar experimental design. For example, we can examine non-point source pollutants such as those used in agriculture (e.g. fertilizers, pesticides) or natural fluctuations in the environment (e.g. changes in salinity from rainfall). The simplest means of doing this is to compare the effects on fertilization or sperm motility from a particular substance in seawater (e.g. Miracle Grow, laundry detergent, dish washing soap, vinegar, bleach, salt) to normal seawater. Different groups in the class will each be able to formulate their own hypotheses and design an experiment to test it. See the example in the Appendix of a hypothesis and experimental methods.

C) Observing cleavage (students): Lastly, you will have the opportunity to observe one of the most significant events that characterize sexually reproducing organisms which is the creation of a new and discrete individual, or zygote. In this laboratory, first cleavage (Figure 10) is used as an indication of the successful completion of this event. The process is a dynamic one, and the zygote will be undergoing numerous changes. If your classroom at school has compound microscopes, you can take the samples with you and continue to observe development of these embryos and compare them to embryos from your experimental treatments.

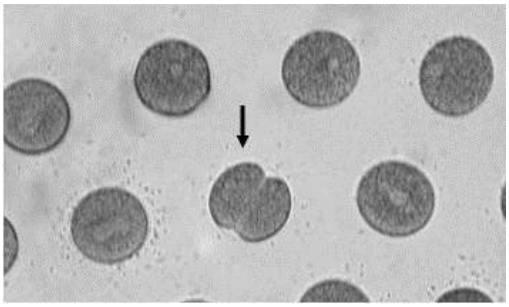

Figure 10. Sea urchin zygotes in various stages leading up to first cleavage. The arrow points out a zygote beginning to cleave.

Part III: Post-activities back in the classroom

Step by step analysis

Begin class with a general discussion of your experience at HIMB. Did you feel that the research you had completed on sea urchin fertilization before you came to HIMB prepared you for the lab exercises and assisted you in the completion of the activities? Did you enjoy your time at HIMB?

A) Controlled spawning is the key to the success of this laboratory exercise. Did the instructor(s) have a hard or easy time obtaining both eggs and sperm using the injection of KCl? Did any of the sea urchins spawn by themselves without any injection? Were you surprised at the number of eggs that were produced? Is there a lot of sperm being produced in a single drop of sperm coming out of the male's body?

B) Were your hypotheses supported by your data? Were there any effects of your treatments on percent fertilization? Do you think sperm motility was affected and/or it had anything to do with fertilization rates? Were any of the potential non-point source pollutants tested and found in your home able to impact sea urchin sperm motility and/or fertilization? If you took your samples with you, did the embryos continue developing? Did they survive and if so, how many? If survival was different in the experimental embryos, why do you think that was the case?

C) What is the relationship of your findings to that of other organisms that are present in the reef environment? Do your findings make you more aware of how humans can impact the environment through non-point source pollutants? What are things that you could do to help prevent such detrimental impacts from occurring?

Lab report

For your laboratory exercise at HIMB, you will be expected to eventually produce an in-depth laboratory report including the following independent sections:

- **Title**: summarize the entire laboratory experiment in several words.
- **Introduction**: in one half to three-quarters of a page describe the process of fertilization and the reasons we use sea urchins as a tool to investigate that process.
- **Hypothesis**: based on your background knowledge of sea urchin spawning and fertilization, describe the hypothesis which you tested.
- **Materials and Methods**: develop and describe in detail the methods you used to test your hypothesis; include all of the materials you used to complete it as well.
- **Results**: compile your data and express them visually and where appropriate, in graphs, tables, or figures.

- **Discussion**: analyze your data in essay form; discuss the results and emphasize what the results obtained mean to you and your group. You should also propose a new experiment which may help explain the results or test a new hypothesis you had developed during your discussion.

- **Conclusion**: in a paragraph or so, summarize your results and make concise conclusions about them. Also include a sentence or two conveying your general conclusions about your results in the context of how the information obtained by studying sea urchins has value in understanding processes taking place in other organisms, including humans.

References

Science background information condensed and/or compiled from the following sources:

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4) Student Website for Life: The Science of Biology (2004). Seventh Edition, by William K. Purves, David Sadava, Gordon H. Orians, and H. Craig Heller. Retrieved on October 20, 2009 from http://bcs.whfreeman.com/thelifewire/content/chp43/4302001.html

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6) Richmond, R. H. (1993). Coral Reefs: Present Problems and Future Concerns Resulting from Anthropogenic Disturbance. *Amer. Zool.* 33 (6): 524-536.

7) Richmond, R. H. (1996). Effects of coastal runoff on coral reproduction. *Biological Conservation* Volume 76, Number 2, 1996 , pp. 211-211(1)

Relevant Hawai‘i Content and Performance Standards III

SC.BS. 4.3: Differentiate between the processes of mitosis and meiosis

SC.BS. 4.5: Describe the components and functions of a variety of macromolecules active in biological systems

SC.BS.1.1: Describe how a testable hypothesis may need to be revised to guide a scientific investigation

SC.BS.1.2: Design and safely implement an experiment, including the appropriate use of tools and techniques to organize, analyze, and validate data

SC.BS.1.3: Defend and support conclusions, explanations, and arguments based on logic, scientific knowledge, and evidence from data

SC.BS.1.4: Determine the connection(s) among hypotheses, scientific evidence, and conclusions

SC.BS.1.5: Communicate the components of a scientific investigation, using appropriate techniques

Acknowledgements

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Appendix:

The following section describes an EXAMPLE of a possible hypothesis and experiment you could conduct at HIMB. By reading through this example, you will also get a better sense of how to use the tools available in the lab. Your experimental design must be DIFFERENT from this activity, and must directly address your hypothesis. The techniques and tools (i.e., the sea urchin eggs and sperm, glass slides, seawater, microscopes, etc.) described in this experiment are the same ones you will be using, but you must come up with your own question and your own way to answer it.

Hypothesis (EXAMPLE):

Does salinity affect the fertilization success of sea urchin eggs? In this case, the null hypothesis to be tested is that increased salinity will have no impact on the fertilization rate of sea urchin eggs. To test the hypothesis, the following experiment was carried out:

1. I prepared the following solutions in test tubes: a) 10 mL of filtered sea water (FSW) and b) 9 mL of FSW combined with 1 mL of a salt saturated solution (provided by instructors).

2. I then added 1 mL of FSW containing a concentrated amount of eggs to each individual solution, such that each of the solutions now contained approximately the same number of eggs. I recorded all of this information to be used in my lab report.

3. The eggs in each solution of varying salinity were allowed to incubate for 5 minutes. After the 5 minute incubation had elapsed, I slowly decanted and discarded the water from each of the test tubes, being sure to carefully leave the eggs behind. I then immediately refilled each tube with fresh FSW.

4. Next, I prepared a fresh sperm diluent (provided by the instructor) and fertilized the eggs in each test tube using the same amount of sperm per tube.

5. I allowed the eggs to incubate with the sperm for a further 1 minute. Then, using a pipette, I collected the eggs from the bottom of each tube and placed them in individual depression slides (one depression slide per solution tested).

6. I viewed the eggs under a compound microscope using the 10X objective, and counted the number of eggs having fertilization membranes versus those that did not (see Figure 3 for examples of fertilized verses un-fertilized sea urchin eggs). I recorded this data in data table format.

7. To determine the percent fertilization observed in each of the solutions, I divided the number of eggs having fertilization membranes by the total number of eggs (i.e. with and without fertilization membranes) and multiplied by 100.