Sea Urchin Fertilization Lab

Christine Ambrosino
‘Aleʻalani Dudoit
Kyle Landers
RULES

• No eating, drinking, gum chewing in the classroom.
• Lunch area is outside the sliding doors within view.
• Don’t wander around without checking with us.
• Wash your hands before you leave the lab.
• Wear shoes.
• Don’t pour sea water down the drain. Empty sea water into a bucket and dispose outside.
• Do not drag scopes on the desk tops.
• Clean up before you leave the classroom.
Identify the proper sequence of events that characterizes the fertilization process.

A. Spawning, cortical reaction, acrosomal reaction, cleavage.
B. Spawning, cortical reaction, cleavage, acrosomal reaction.
C. Spawning, acrosomal reaction, cortical reaction, cleavage.
D. Spawning, cleavage, acrosomal reaction, cortical reaction.
E. Spawning, acrosomal reaction, cleavage, cortical reaction.
What is the primary purpose of the fertilization envelope?

A. It provides nutrients to the developing embryo.
B. It protects the fertilized egg from being eaten by predators.
C. It contains the DNA from both the egg and sperm.
D. It prevents additional sperm from entering a fertilized egg.
E. It stores the urchin's sperm and eggs until they are ready to spawn.
Which of the following is false:

A. During meiosis there are 2 cell divisions.
B. The daughter cells of mitosis are genetically different from the parent cell.
C. The final number of daughter cells of mitosis is 2 and of meiosis is 4.
D. The final daughter cells of mitosis are diploid and of meiosis are haploid.
E. The function of meiosis is to form gametes.
Why are urchins important to coral reefs?

A. They prevent algae from overgrowing the reef.
B. They help to shade corals from intense sunlight.
C. They improve seawater quality by filter feeding and removing bacteria.
D. They are an important source of food and energy for corals.
E. Their gametes are an important source of food for juvenile fish.
What would be your control experiment if you wanted to test the effect of increased salt concentration on fertilization?

A. Monitor fertilization success in tap water.
B. Monitor fertilization success in regular sea water.
C. Monitor fertilization success in a mixture of sea water and tap water.
D. Monitor fertilization success without water.
E. NONE OF THE ABOVE
Which of the following is an example of a poorly worded hypothesis?

A. If sea urchin gametes are exposed to unusually high salt concentrations, then the fertilization rate will decrease because salt desiccates the eggs.

B. If pesticides are added to seawater with urchin sperm, then sperm motility will be slower because the chemicals interfere with the sperm metabolism.

C. If sea urchin spawn is white, then the sea urchin is a male because males prefer the color white.

D. ALL OF THE ABOVE

E. NONE OF THE ABOVE
Which of the following facts about urchin morphology is FALSE?

A. The anus and mouth are separate openings.
B. Gametes are expelled from 5 separate openings.
C. They have teeth.
D. All species of sea urchins have spines.
E. Eggs and sperm are different colors.
Which of the following is true for a fertilized sea urchin egg?

A. The egg becomes opaque when fertilized.
B. The fertilization envelope is visible through the microscope.
C. The sperm cell is visible inside the egg cell.
D. The fertilized egg is haploid.
E. It has completed the first cycle of mitosis.
PRE-EVALUATION
I understand how to make and test a hypothesis.

- A. Strongly Agree
- B. Agree
- C. Unsure
- D. Disagree
- E. Strongly Disagree
I care about protecting Hawai‘i’s marine environment.

- A. Strongly Agree
- B. Agree
- C. Unsure
- D. Disagree
- E. Strongly Disagree
I am aware of the relevance of marine science to my life.

- A. Strongly Agree
- B. Agree
- C. Unsure
- D. Disagree
- E. Strongly Disagree
I am interested in the subject of marine science.

- A. Strongly Agree
- B. Agree
- C. Unsure
- D. Disagree
- E. Strongly Disagree
I am interested in a career in marine science.

• A. Strongly Agree
• B. Agree
• C. Unsure
• D. Disagree
• E. Strongly Disagree
Sea urchins

Echinometra mathaei
Rock boring sea urchin

Echinometra oblonga
Black rock boring urchin

Colobocentrotus atratus
Helmet sea urchin

Heterocentrotus mammillatus
Pencil sea urchin

Diadema paucispinum
Long spine urchin

Tripneustes gratilla
Collector sea urchin
Sea urchin fertilization

1. spawning
2. acrosomal reaction
3. cortical reaction
4. cleavage
Meiosis VS Mitosis

<table>
<thead>
<tr>
<th></th>
<th>Meiosis</th>
<th>Mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Function</strong></td>
<td>gametes</td>
<td>Somatic cells</td>
</tr>
<tr>
<td><strong>Cell division</strong></td>
<td>2x</td>
<td>1x</td>
</tr>
<tr>
<td><strong>Daughter cells #</strong></td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Daughter cells</strong></td>
<td>Haploid</td>
<td>Diploid</td>
</tr>
</tbody>
</table>

**Meiosis:**
- Synapsis and crossing over occur
- Homologues align independently
- Homologues separate
- Daughter cells form
- Daughter nuclei are not genetically identical to parent cell

**Mitosis:**
- Chromosomes align at the metaphase plate
- Daughter chromosomes separate
- Daughter cells form
- Daughter nuclei are genetically identical to parent cell

**VIDEO**
How does water quality affect fertilization?
Water quality effects on the ecosystem
Research at HIMB

Collaboration with The Nature Conservancy Hawai‘i, the Division of Aquatic Resources
What is a scientific hypothesis?
• A proposed explanation (i.e., an educated guess) for something you can **observe** and is **testable**.

How do you make a hypothesis?
• Make an “If... then...because...” sentence.
• If I change **VARIABLE X**, then I expect **RESULT Y**, because **RELATIONSHIP Z**.

**EXAMPLE**: If you increase the water temperature in an aquarium, then the fish will start spawning because water temperature is a cue for reproduction.
HYPOTHESIS-TESTING

“If I change VARIABLE X, then I expect RESULT Y, because RELATIONSHIP Z.”

EXAMPLE: If you increase the water temperature in an aquarium, then the fish will start spawning because water temperature is a cue for reproduction.

How do you test a hypothesis?
• Control experiment: The “baseline” for comparison.
• Test experiment: Only change one independent variable.

The Variables

a) VARIABLE X = Independent Variable – the variable you manipulate (e.g., water temperature)

b) RESULT Y = Dependent Variable – what happens to this variable depends on what you do to the Independent Variable

c) Controlled Variable – factors that are the same in every test
“If I change VARIABLE X, then I expect RESULT Y, because RELATIONSHIP Z.”

EXAMPLE: If you increase the water temperature in an aquarium, then the fish will start spawning because water temperature is a cue for reproduction.

What is wrong with this “hypothesis”?

“If fish spawn, then the water temperature in an aquarium will increase because of global warming.”
CLASS ACTIVITIES

1. Urchin spawning
2. Develop hypothesis: If we expose sea urchin gametes to water manipulated with (salt? freshwater? MiracleGro?), then fertilization will be and development will be (slower? faster?).
3. Explain your hypothesis reasoning to an instructor.
4. Start experiments.
5. Clean up.
6. Discuss results.
Identifying fertilized eggs
Preparing Egg Solution

1. Your instructor will prepare one 100mL beaker with an egg solution for each group. Add **5mL of egg solution** to each of your test tubes.

2. Add **2mL of test solution** to each of your test tubes. For your control, use filtered sea water. You will now have 7mL in each test tube.
Preparing Sperm Solution

3. In 100mL beaker, add 4-5mm of dry sperm in tip of bulb pipette to 20mL of filtered seawater.

4. Use clean bulb pipette to add 1 drop of sperm solution to each test tube. **Note the time** (T=0).
4. Each student should keep track of ONE TEST TUBE.

- Remove a couple drops from the bottom of the test tube and place this on a slide. **Don’t use up all your eggs!** Return any extra liquid in your pipette back to the test tube.

- Examine the first 20 eggs you come across and quantify the number of fertilized vs. unfertilized eggs.

- **Every 15 minutes**, examine a new batch of 20 urchin eggs.

- As soon as cleavage is observed, begin collecting data on the total number of cleaved eggs.

**Note:** It takes about **40 minutes** for first cleavage to occur.

<table>
<thead>
<tr>
<th>Time (T)</th>
<th>Total fertilized (fertilization envelope OR cleaved)</th>
<th>Total cleaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>T=0</td>
<td>e.g., 16/20</td>
<td>e.g., 0/20</td>
</tr>
<tr>
<td>T=15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T=30</td>
<td></td>
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<tr>
<td>Etc.</td>
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Results discussion

• Was urchin egg fertilization affected by water quality differences?

• How did you detect these changes?

• What do your results tell you about how other organisms may be affected?
CLEAN UP

• Wash all lab materials in the sinks
  – Clean pipettes by sucking up fresh water twice and rinse the outside.
• Use sink sponges to wipe down tables, then use blue/yellow rags and cleaner spray
• Sweep floors
• Place chairs on top of table

• Dry microscope slides and cover slips
• Dry tubes UPSIDE DOWN
i-Clicker Questions
Identify the proper sequence of events that characterizes the fertilization process

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POST - EVALUATION
How would you rate your visit to HIMB overall?

• A. Excellent
• B. Good
• C. Average
• D. Fair
• E. Poor
The science activities were fun and interesting

• A. Strongly Agree
• B. Agree
• C. Unsure
• D. Disagree
• E. Strongly Disagree
I feel I learned something new and important

- A. Strongly Agree
- B. Agree
- C. Unsure
- D. Disagree
- E. Strongly Disagree
Because of my participation in this activity…

I have a better understanding of how to make and test a hypothesis.

• A. Strongly Agree
• B. Agree
• C. Unsure
• D. Disagree
• E. Strongly Disagree
Because of my participation in this activity…

I now care more about protecting Hawai‘i’s marine environment.

- A. Strongly Agree
- B. Agree
- C. Unsure
- D. Disagree
- E. Strongly Disagree
Because of my participation in this activity…

I am now more aware of the relevance of marine science to my life.

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Because of my participation in this activity…

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