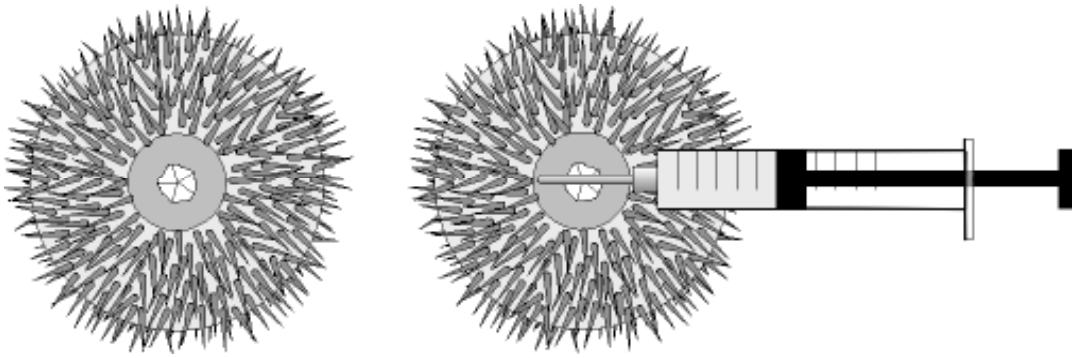
Sea Urchin Fertilization and Water Quality Analysis

Introduction

In **Part 1**, you will observe fertilization under normal conditions and fertilization in the presence of a negative control. In **Part 2**, you will set up an experiment to test the effects of household toxins on fertilization.

The single biggest producer of toxic waste in the USA is the common household, producing more waste than industry, universities, schools, etc. Most of this waste ends up in landfill where it finds its way into our water supplies. Until recently, 3/4 of all used motor oil ended up in landfill. Motor oil is toxic from its organic components, but more importantly from the quantities of highly toxic cadmium and vanadium metals that have worn off of engine components. Detergents, bleach, paints, even salad oil can be hazardous if in high enough concentration and in the wrong place. Most of these toxins end up in our streams, ground water, and water supplies and eventually into the oceans. Once in the oceans the toxins work their way up the food chain in ever increasing concentrations until the ultimate consumer eats them (us!)

Environmental factors appear to play a large role in sea urchin fertilization. Pollution from a wide variety of sources can interfere with normal sperm function, as can temperature, lighting, oxygen levels, pH, and agitation. The Environmental Protection Agency (EPA), in fact, uses sea urchin development as a measure of environmental pollution in a locality.



Procedure

Part 1: Observing Fertilization Under Normal Conditions

1. Sea urchins must be injected with 1 ml of .55 M KCl. Your instructor will do this.
2. Wait to see what sex they are. Sea urchin gametes come out five **gonopores** at the **aboral surface** (opposite the oral surface where the mouth is).
    a. **White gametes** indicate a male producing sperm – gametes should be shed by turning the male upside down in a petri dish placed on ice
    b. **Yellow gametes** indicate a female producing eggs – eggs should be shed into sea water (~pH 8) by inverting them over a beaker full of sea water

3. EGGS SHOULD BE KEPT AT ROOM TEMPERATURE AND THE SPERM SHOULD BE KEPT ON ICE
    a. Sperm are much more delicate than the eggs, so they must be kept undiluted on ice (the seminal fluid has substances in it to keep the sperm inactive and the ice also helps)
    b. Use room temperature sea water in all egg treatments and for fertilization
    c. Sperm must be diluted prior to their addition to the eggs, or *polyspermy* and abnormal cleavage may occur.
4. Use depression slides to view sea urchin fertilization. These slides must be *washed, dried and put away* when you are finished.
5. **View eggs alone** by preparing a slide with a drop of eggs.
    a. Be sure to adjust the microscope light so the eggs on the slide are easily visible.
    b. Place the cover slip on the slide

Instructions for Diluting Sperm

6. Each table should have a test tube of diluted sperm on ice
7. Add one drop of concentrated sperm to 3 ml of seawater in a *labeled* test tube (or beaker) and stir to disperse evenly.
    a. This diluted sperm suspension will only last ~20 minutes, so do it right before you need it, and **keep it on ice**.
8. Put ice in a beaker and put the test tube in the beaker of ice

9. **View sperm alone** by preparing a slide with a drop of diluted sperm.
    a. Be sure to adjust the microscope light so the sperm on the slide are easily visible.
    b. Place the cover slip on the slide
10. **View fertilization under normal conditions**
    a. Prepare a slide with a drop of eggs only.
    b. Be sure to adjust the microscope light so the eggs on the slide are easily visible.
    c. Add one drop diluted sperm and place the cover slip on the slide.
11. Start timing and **record percentage of fertilized eggs** after 1 minute as described below.
    a. Look at 25 eggs and record the number of them that are fertilized. Multiply by 4 to get the percentage of fertilized eggs.
    b. The fertilization membrane is formed immediately after fertilization to prevent polyspermy.
12. Repeat this procedure to record the percentage of fertilized eggs after **1 minute**, **5 minutes** and **10 minutes**
13. If you don't get fertilization try again with another batch of sperm.
14. **Negative control:** While your fertilization under normal conditions experiment is ongoing, you should set up an experiment to observe fertilization with a negative control. We'll use **acetic acid** as the negative control.

Instructions for Mixing Sperm with Chemicals

a. Mix 1 ml of diluted chemical (acetic acid, etc.) with 1 ml of your diluted sperm solution in a *labeled* test tube.
b. Set up a slide of only eggs.
c. Place slide on microscope stage.
d. Add 1 drop of sperm diluted in chemical solution to the microscope slide
e. Place a cover slip on the slide

15. Start timing and record percentage of fertilized eggs after **1 minute**, **5 minutes** and **10 minutes**.

Part 2: What Conditions Affect Fertilization Rates?

Conditions to Test:

- **Temperature** – lower and higher than the ideal for each species.
- **Salinity** - higher and lower than sea water (1/2 to 2x)
- **Oils and fuels** – salad oil to diesel oils. Is the oil itself toxic or does it need to cover the container to cut off oxygen?
- **Detergents** – again start with a "typical" use level for a household activity and use serial dilutions to a non-toxic level (most of our wastes are diluted when going down the drain).
- **pH** – use test paper to vary pH from 4-10. Small amounts of dilute hydrochloric acid and sodium hydroxide can be used to vary the pH. Does the pH of the solution change over time? [It will take only a drop of 1N HCl or NaOH to affect pH.]
- **Spermicide** – the most common component in spermicides is **nonoxynol-9**. Test what effect nonoxynol-9 has on sea urchin fertilization.
- **Carbon dioxide** – make your artificial seawater with sodium free seltzer water (keep stirring to a minimum as this removes the carbon dioxide from the water). Using varying proportions of the regular seawater and the seltzer seawater. What is the pH of the resulting solutions? How does this relate to the "green house" effect of higher carbon dioxide levels worldwide?
- **Carbon dioxide and acid** – Use a drinking straw to blow CO_2 into your solution. The CO_2 will react with the water to produce carbonic acid.
- **Other pollutants** such as bleach, copper sulfate (used as a fungicide), plant fertilizer and insecticides. NOTE: SMALL QUANTITIES SHOULD BE USED!

1. Spend a few minutes designing your experiments and labeling your slides.
   a. Which 3 conditions will you and your group test?
   b. How will you measure the fertilization rate?
   c. How will you record your data?
   d. How often will you record your data?
   e. What will you use as a control?
2. Check with your experimental design with your instructor before beginning.
3. Carry out your experiment and record all your data

4. Use the laptops in the classroom to graph your data. Check your graph with your instructor before you leave.
5. You can email this graph to yourself or print it out in class.
6. *Wash, dry and put away* the depression slides you have used.

Check with your instructor to determine if you will write a complete lab report or answer the attached questions.

Write up complete lab report describing your experiments, results and conclusions. Your lab report should include:

1. **A background section** of that describes why you are doing this research.
    a. What was the purpose of the experiment?
    b. What is your hypothesis?
    c. What results do you expect to see?
2. **A methods section** that describes what methods you used.
    a. How was the experiment set up?
    b. What condition(s) did you test?
    c. How did you test these conditions?
3. **A data section**. Data should be in the form of tables and graphs.
    a. Graphs *must* be made on the computer.
    b. You should make a graph on the computer that shows normal fertilization, fertilization in the presence of a negative control, and fertilization in the presence of the chemicals you tested.
4. **An analysis and conclusion section**. What does your data mean?
    a. What were you able to learn from this experiment?
    b. Does your data match your hypothesis?
    c. If not, why might that be?
5. Your lab report should be at least 2 pages and no more than 5 pages. You should attach your graph and data tables.

Name _____

Sea Urchin Fertilization

Results/ Data Analysis
Normal Fertilization Conditions

	1 minute	5 minutes	10 minutes
# Fertilized eggs out of 25			
Percent fertilized eggs			

Negative Control (acetic acid)

	1 minute	5 minutes	10 minutes
# Fertilized eggs out of 25			
Percent fertilized eggs			

Condition #1 (_____)

	1 minute	5 minutes	10 minutes
# Fertilized eggs out of 25			
Percent fertilized eggs			

Condition #2 (_____)

	1 minute	5 minutes	10 minutes
# Fertilized eggs out of 25			
Percent fertilized eggs			

Condition #3 (_____)

	1 minute	5 minutes	10 minutes
# Fertilized eggs out of 25			
Percent fertilized eggs			

Questions (Be sure to attach your data tables and graph)

1. Which conditions did you test?

   _____

   _____

   _____

2. What effect did each condition have on fertilization?

3. Why is it important to have a negative control (acetic acid in this case)?

4. Explain how you think your experimental results might be relevant in places like the San Francisco bay. (This should be a paragraph)