The Effects Different Seawater Salinities Have On Fertilization Success and Fertilization Envelope Lift Off

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I. Introduction

In this experiment I studied fertilization of sea urchin eggs in sea water of differing salinities. Fertilization is the process in which gametes react with one another to form the union that initiates development of an embryo (Wilt & Hake, 2004). Fertilization is an important developmental process to study because without fertilization an embryo can not begin development, and proceed on its path to adulthood. This experiment is significant because sea urchins live and breed in an environment that is affected by weather and continually changing. The experiment explores the question of if and how the environment where fertilization occurs has an affect on the fertilization success of sea urchins.

Sea urchins have been favored organisms for developmental biologists since the mid 19th century, because their development is easily studied. They are excellent organisms to use in developmental studies because their gametes are readily spawned, and can be collected in large numbers (Kozlowski & Tyler, 2003). Sea urchins are of the class called Echinoidea, and are commonly found in the intertidal zone, on rocky shores and in tide pools (Castro & Huber, 2000). The salinity of sea water does not fluctuate much in the open ocean, but does fluctuate widely in the intertidal zone. The definition of salinity is the total amount of dissolved salts measured in parts per thousand (Sumich, 1999). Salinity is altered by processes that add or remove water from the sea, such as precipitation and evaporation. Evaporation is the process in which water is removed from the sea, causing an increase in the concentration of salt, and thus an increase in salinity. On the other hand, precipitation decreases the salinity by diluting the sea salts. Also, cells are affected by osmotic pressure. Osmotic pressure is the pressure that must be applied to stop the osmotic movement of water across a semi-permeable membrane (Raven & Johnson, 115). Water moves to areas with higher solute concentrations. Thus, if more solute resides outside the cell than inside the cell, water will leave the cell, causing it to shrivel. On the other hand, if more solute resides inside the cell than outside, water will move into the cell, causing it to inflate. As a result, it is important for animal cells to maintain isosmotic conditions. Organisms that live in the ocean adjust their internal concentration of solutes to match that of the surrounding seawater. Once they are isosmotic with respect to their environment, there is no net flow of water into or out of their cell (Raven & Johnson, 115). In this experiment I fertilized eggs of the sea urchin species Lytechinus variegates in sea water of different salinities, and observed and calculated the percent of successful fertilizations under the conditions. In my first experiment, fertilization envelope lift off represents a fertilized egg. Thus, the percent of eggs with fertilization envelope lift off represents the percent of fertilized eggs in that sample.

The average salinity of sea water is about thirty-five parts per thousand salts. However, when I began my experiment I took a salinity reading of the seawater in the sea urchin tank, and it read 31 ppt. As a result, the control in my experiment is 31 ppt. I tested fertilization success in three different salinities: a control at 31, a low at 27 ppt, and a

high at 38 ppt. In addition to this experiment, I conducted a trial in which the sperm of Lytechinus variegates was removed, and the eggs were fertilized by the calcium ionophore A23187 in seawater of each of the designated salinities above. I hypothesized that fertilization success rates would be different for sea urchin eggs in salinities different from the control of 31 ppt, and that fertilization lift off would be affected by the different salinities.

II. Materials and Methods

In this experiment I first had to make the two different seawater salinities, a low of 27 ppt, and a high of 38 ppt from a control salinity of 31 ppt. I calculated the low value salinity with a theoretical precipitation yield of one inch, which would dilute the tide pool salinity, at a control of 31 ppt, by ten percent to give 27 ppt. For the high salinity value I based my calculations on a theoretical evaporation yield of one inch, which would increase the salinity of the tide pool at 31 ppt by ten percent, and give a new salinity of 38 ppt. The control salinity seawater was removed from the sea urchin tank. Two 250 mL beakers, a graduated cylinder, and a salinometer were used to make the different salinity seawater. Artificial seawater salt was added to the seawater at 31 ppt to achieve the high salinity of 38 ppt. Distilled water was used to make the seawater at 27 ppt. For trial one of experiment one, water samples of the three distinct seawater salinities: 31 ppt (control), 27 ppt (low) and 38 ppt (high) were needed. Sea urchin eggs of Lytechinus variegates were injected with .53 M isotonic KCL, and shed in NSW. Professor Morris provided the shed sea urchin eggs in NSW. Three sea urchin testes from Lytechinus variegates were used. A Nikon Eclipse E200 Microscope and a Digital Interface Camera were used to view and take images of the eggs. BTV Pro software, on a Macintosh computer, was used to open and save the images that were taken. Further, a watch was used to watch the time between the addition of the sperm and fertilization envelope lift off.

In trial two of experiment one, the distinct seawater salinities were used again: 31 ppt (control), 27 ppt (low), and 38 ppt (high). The sea urchin eggs were shed in NSW as for the previous trial. In this experiment, dry sperm was used and provided by Professor Morris. The same microscope, camera, computer, software, and a watch were used in this trial as described in trial one.

In experiment two, the same distinct salinities were used as in the previous experiment. Again, sea urchin eggs shed in FNSW, prepared and provided by Professor Morris were used. Six microliters of the calcium ionophore A23187 were prepared and used in this experiment. Stock A23187, at a concentration of 2 mM, was ordered and provided by Professor Morris. A working concentration of 40 uM was prepared by Professor Morris in lab. The same microscope, camera, software, computer, and a watch were used in this experiment. Further, Microsoft Excel was used to create graphs showing the results of both experiments.

Before I began my experiments I first had to makeup the different salinities of seawater. First, I obtained three 250 mL beakers. For my control I took some water from the sea urchin tank and measured its salinity with a salinometer. The salinity was 31ppt, and this was my control. To create seawater with a low salinity I added distilled water in increments of about 5mL to 250 mL of NSW at 31ppt, and checked the salinity of the water with the salinometer. I continued to add the distilled water until the salinity reached 27 ppt. To create seawater with a salinity of 38 ppt I took another 250 mL of the NSW at 31 ppt and added artificial seawater salt to the water until the salinity reached 38 ppt. Again, I used the salinometer to measure the salinity of the water as I added the salt, and stopped when I reached 38 ppt

I used two different methods for the two trials in experiment one so they must be explained separately. In trial one of experiment one, I used shed sea urchin eggs in NSW and three sea urchin testes, two of which were sacrificed for my experiment. First, I took the eggs in NSW and using a pipette transferred the solution to a plastic test tube; I then let the eggs settle to the bottom. Then using a pipette, I transferred 1-2 drops of eggs in NSW on to a glass slide. I viewed the eggs under a Nikon Eclipse E200 Microscope under 10x and Ph1, and took images using BTV Pro and a Digital Interface camera. I then added 1-2 drops of sperm in natural seawater to the eggs on the slide. I then viewed this under the same microscope, and took images with the same camera. I waited for five minutes to pass, imaging in the mean time, and then calculated the percent of eggs with fertilization envelope lift off, or fertilized eggs, in that sample. This was my control. I then filled a test tube with 5mL of seawater at 27 ppt and using a pipette added 1-2 drops of the concentrated eggs from the NSW to the test tube. Again, I allowed the eggs to settle and become concentrated. For the sperm I obtained two small Petri dishes, and added 2 drops of seawater to the first. I then cut a piece of the sea urchin testes carefully and placed it in the seawater at 27 ppt. This step served as a washing. Next, I placed several drops of low salinity into the second Petri dish with a pipette, and with tweezers transferred the sea urchin testes to this dish.

Using tweezers I then squeezed the testes five times, so that sperm was released into the low salinity water. I then took the eggs, now in the low salinity seawater, and added 1-2 drops of the solution to a glass slide using a pipette. Using the Nikon Eclipse E200 Microscope I focused the eggs under 10x and took an image in Ph1 using BTV Pro. Next, using a pipette, I added a drop of sperm from the sperm solution in low salinity directly to the eggs. I took images using BTV Pro under the same standards, 10x and Ph1, and after five minutes calculated the percent of eggs with fertilization envelope lift off in that sample of eggs. I then repeated the same steps above for the fertilization of the sea urchin eggs in the high salinity seawater.

My second trial of this experiment was conducted on a different day, and my method was adjusted due to the resources available to me on that day. In this experiment dry sperm instead of sea urchin testes were used. First, I obtained three glass slides and several pipettes. Using a pipette, I obtained a very small amount of dry sperm and placed it in a test tube of 1mL NSW. I then obtained sea urchin eggs in NSW, and using a pipette, transferred 1-2 drops of the eggs in NSW on to the slide. I then observed the eggs under 10x of the same microscope as in the first experiment, and took an image using BTV Pro. I added one drop of sperm in the NSW directly to the eggs on the slide. Again, I observed the eggs and sperm with the Nikon Eclipse E200 Microscope under 10x, took images in Ph1, and calculated the percent of eggs with fertilization envelope lift off in that sample of eggs after five minutes. For the experiment of fertilization in high salinity seawater, I added 1-2 drops of sea urchin eggs from NSW into 1mL of high salinity seawater in a test tube. Using a pipette I transferred 1-2 drops of sea urchin eggs in high salinity on to a glass slide. Again, I observed the eggs under the microscope, focused, and took an image under 10 x magnification. I then dipped the tip of another pipette into the dry sperm solution, and transferred the dry sperm into 1mL of high salinity seawater. Using a pipette I then added 1-2 drops of the sperm solution in high salinity seawater directly to the eggs on the slide, and calculated the percent of eggs with fertilization envelope lift off after five minutes. To observe fertilization success of sea urchin eggs in low salinity seawater I repeated the steps above, replacing the high salinity seawater with low salinity seawater.

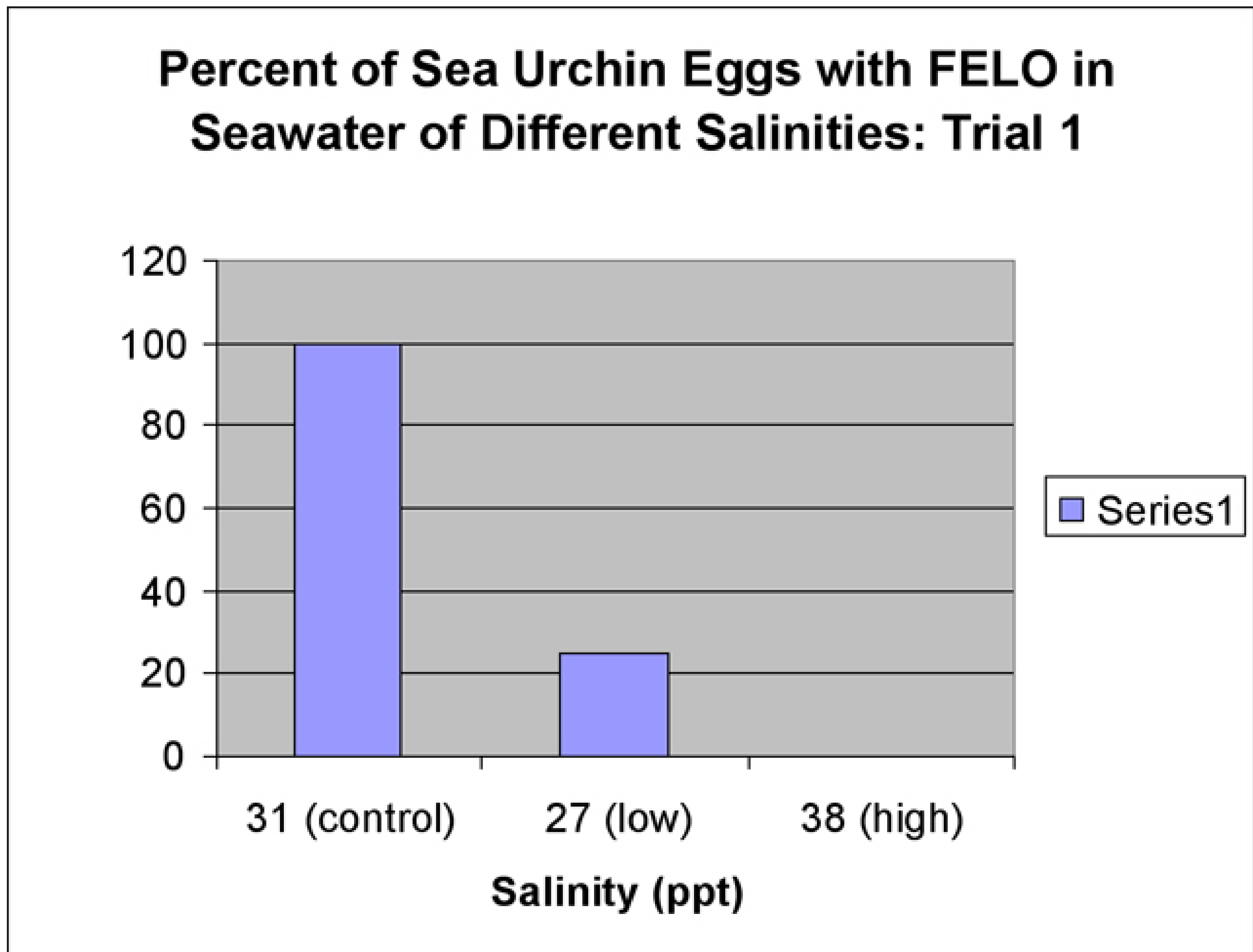
Last, I conducted a trial replacing the sea urchin sperm with the calcium ionophore A23187. First, I placed two drops of sea urchin eggs in NSW on a glass slide, observed the eggs under the microscope and took an image. I then added 2uL of A23187 to 100 uL of NSW in a test tube. Next, I added the 2 uL of A23187 in the 100 uL of NSW to 100 uL, approximately two drops, of eggs in NSW on the glass slide. I then observed the eggs under the microscope, took images, and calculated the percent of eggs with fertilization envelope lift off in that sample of eggs. Again, this was my control. I repeated these steps again for the fertilization success in low salinity and in high. Using a pipette I transferred sea urchin eggs in NSW to one test tube with 1 mL of high salinity seawater, and another test tube with 1mL of low salinity seawater. Then 2uL of the calcium ionophore A23187 was added to a test tube containing 100 uL of high salinity seawater, and another 2 uL of A23187 was added to a test tube containing 100 uL of low salinity seawater. From this point on the steps were the same as outlined above.

The first trial of experiment one took me a whole three hour lab period to complete. However, I was able to complete trial two of experiment one and one trial of experiment two in the following three hour lab period. I analyzed my data by calculating the percent of eggs with fertilization envelope lift off in each sample of eggs in each condition. Because my methods changed from trial one to trial two in experiment one, my results from each trial must be analyzed and presented separately, but I can make an overall conclusion from the results of both trials. In both experiment one and experiment two I was looking for different percentages of eggs with fertilization envelope lift off in the different salinities.

In this overall experiment, I had two controls. For the first experiment my control was fertilization of the sea urchin eggs in NSW. For the second experiment my control was eggs in NSW plus A23187. The control in the first experiment was necessary because without it I would not have known whether the eggs and sperm were good, or whether they could be fertilized at all. If I did not prove that the eggs could be fertilized in NSW, then any results I got from the following trials would not mean anything; that is, I would not be able to conclude anything from them. The control was needed in the second experiment, because I needed to prove that the calcium ionophore A23187 could by itself cause fertilization envelope lift in these sea urchin eggs in NSW. If I did not prove this previous to the following experiments, fertilization in high and low salinity, I again would not be able to properly conclude anything from the results. In order to conclude something about an experiment an experimenter needs to be able to compare the experimental results to a control. Thus, both of these controls were necessary for my experiment.

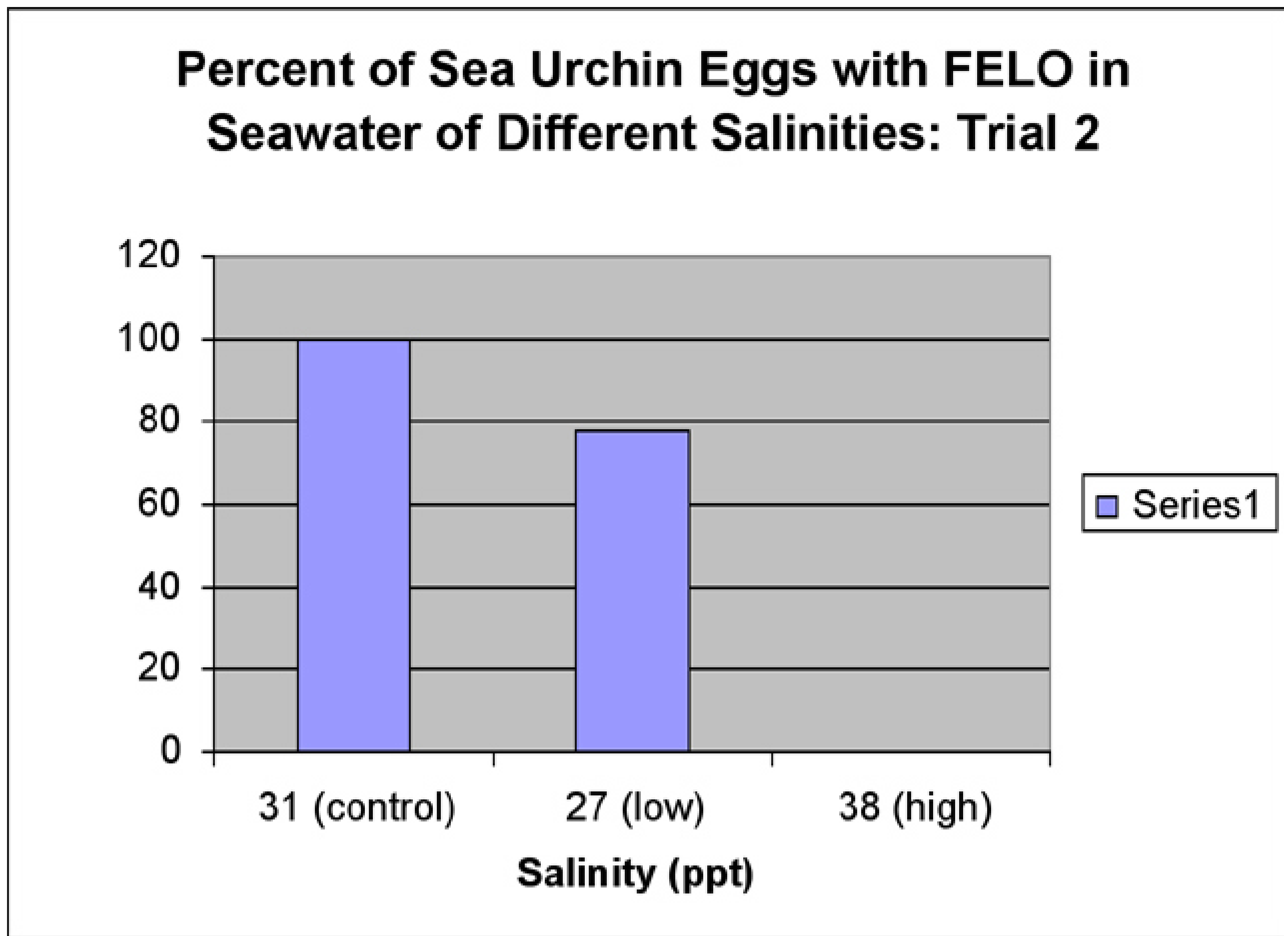
III. Results

Figure 1:



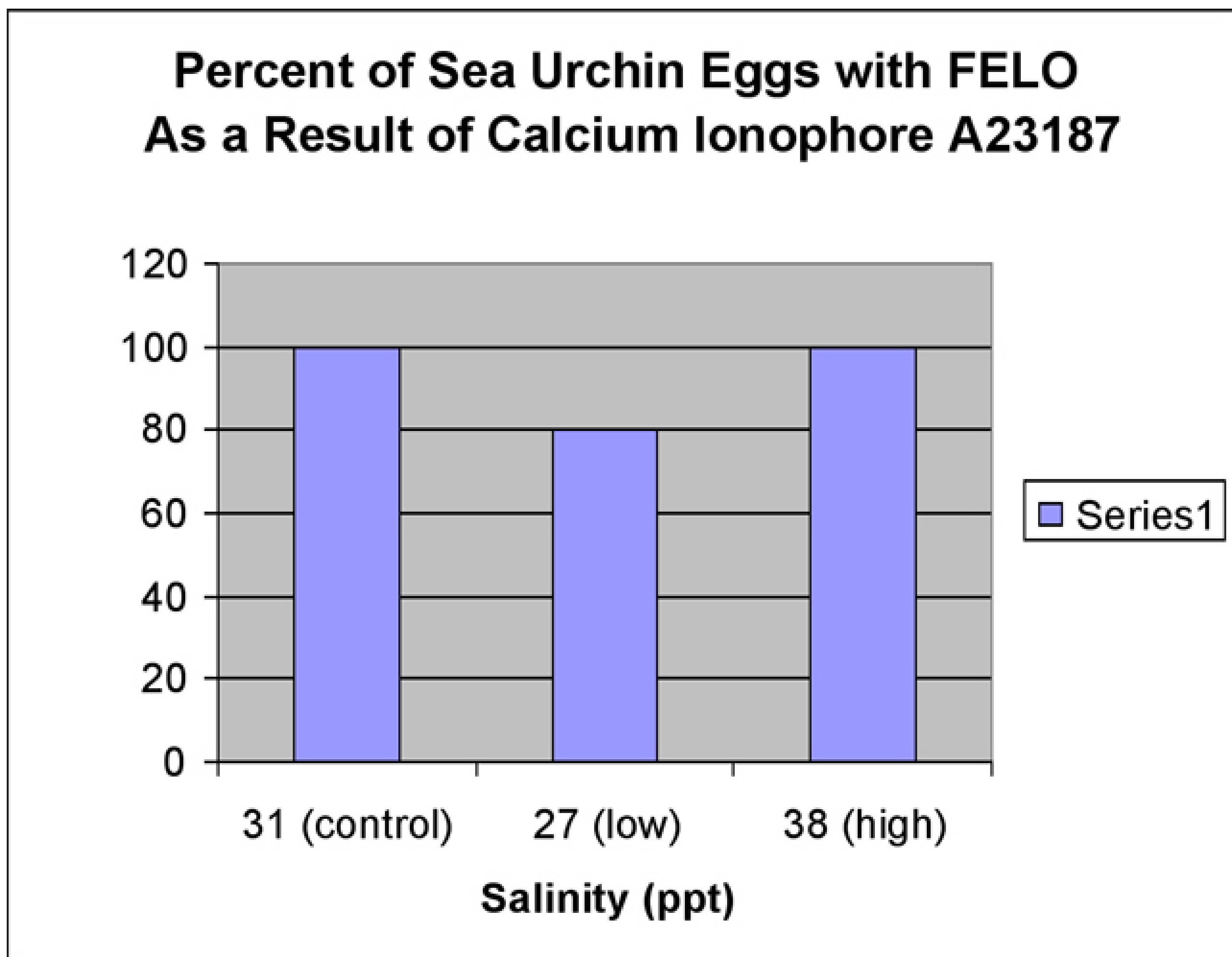
Legend: This graph shows that the percent of sea urchin eggs with FELO was different for each of the egg samples in the seawater with different salinities. The sample size of eggs in the control was ten eggs; the sample size of eggs in the seawater of low salinity was thirty six eggs; and the sample size of eggs in the seawater of high salinity was three eggs.

Figure 2:



Legend: This graph shows the same relationship as seen in Figure 1, but must be presented separately because a different method was used to collect the results. The sample size of eggs for the control was forty five eggs; the sample size of eggs in the low salinity seawater was twenty seven; and the sample size of eggs in the high salinity seawater was three. Also, an average of two tries was taken for the egg sample in the low salinity seawater, because the eggs were spread out and difficult to count. In the first try in the low salinity seawater, a sample size of twelve eggs was used. In the second try, a sample size of fifteen eggs was used.

Figure 3:



Legend: This graph shows that the percent of eggs with fertilization envelope lift off, due to the calcium ionophore A23187, differs very little in the different seawater salinities. Note, in this experiment sea urchin sperm was not present. In this experiment, the sample size of eggs used in each of the water samples was five.

The two trials in experiment one must be presented and analyzed separately, because a different method was used to obtain the results. However, after analyzing each of the results for the trials separately, I can use the results to make an overall conclusion. For both trial one and two of experiment one, the control had the highest percent of fertilized eggs, followed by the low salinity. Also, in both cases, the eggs in the high salinity seawater did not fertilize. Further, in both trial one and trial two of experiment one, the percent of eggs with FELO, or the percent of fertilized eggs, was different in each of the seawater samples with different salinities (See Figures 1 and 2).

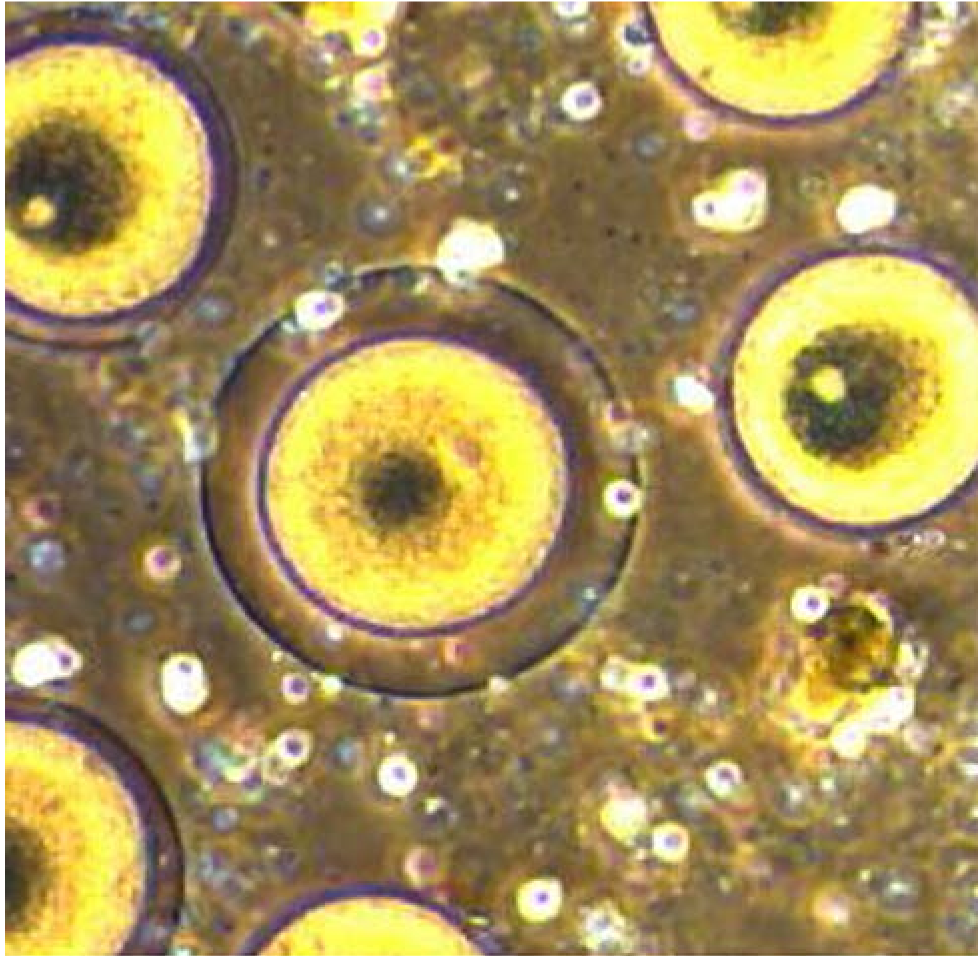
One trial for experiment two was conducted. In this trial, the control had the highest percent of fertilization, followed by the high salinity seawater, and last the low salinity seawater. However, it must be noted that the percent of eggs with FELO, or the percent of fertilized eggs, did not differ much in each condition, even between the control and the low salinity seawater. This data shows that FELO is affected very little by the different seawater salinities (Please see Figure 3).

During my experiments I also observed that FELO took slightly longer to occur in trial one and two of experiment one with sperm, than it did with the trial of experiment two with the calcium ionophore A23187. Also, I was able to watch the sperm swim towards and react with the egg under the microscope, and watch the progress of the FELO. When A23187 was used in the place of sperm, the FELO occurred more quickly, and there was no progress to watch.

Further, I took images of the eggs in each trial for each experiment before and after the addition of sperm or A23187. However, all eggs that produced FELO looked the same, and all eggs that did not produce FELO looked like normal unfertilized eggs. In all of the trials of this experiment, some of the eggs in each water sample produced FELO, except for the eggs in the high salinity seawater in experiment one (Refer back to Figures 1, 2 and 3). As a result, I have included an image of an egg with fertilization envelope lift off in low salinity seawater, to represent all of the eggs in this experiment that produced FELO. In addition, I have included an image of an egg without FELO from the first

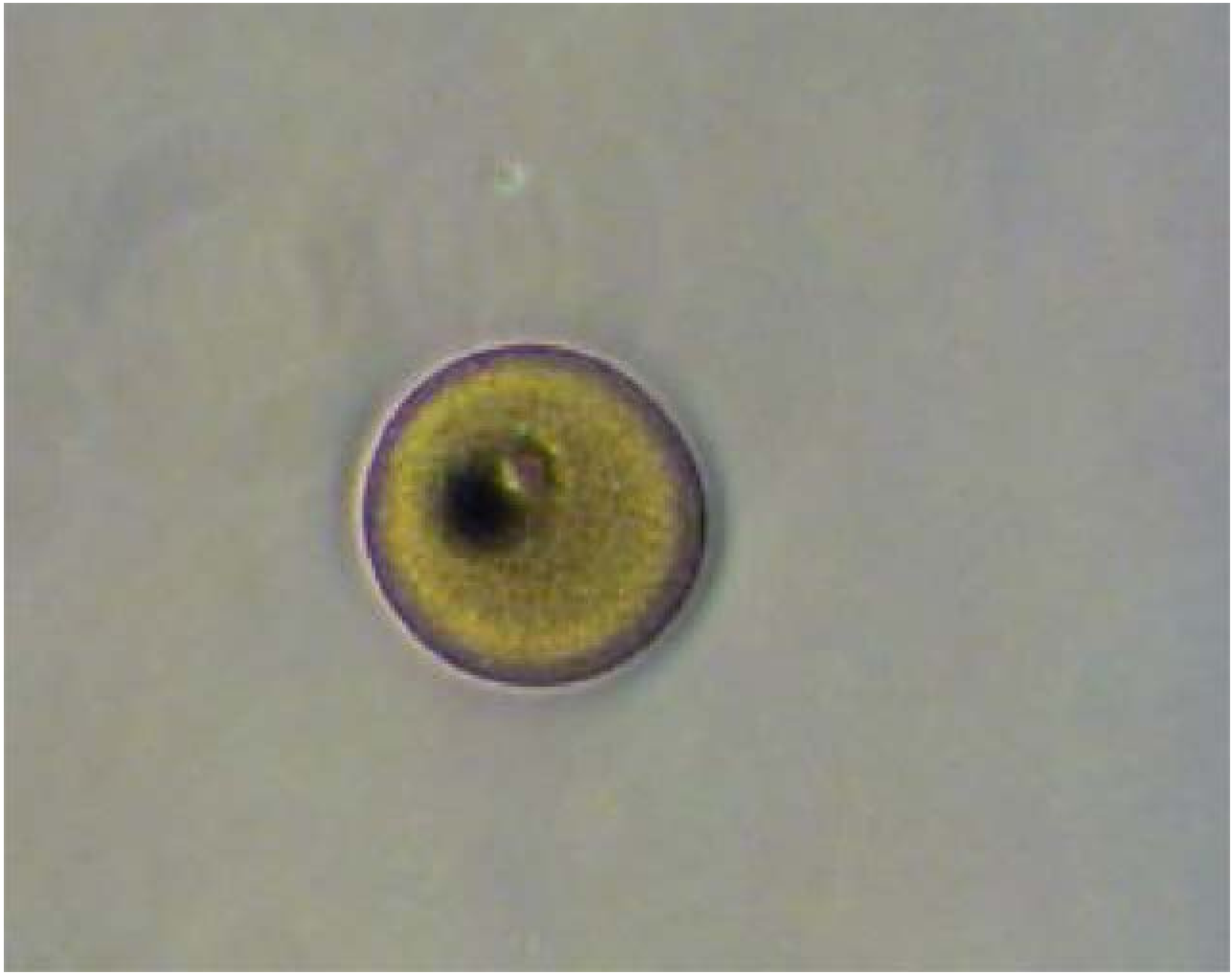
experiment in high salinity seawater (Please see Figures 4 and 5 below).

Figure 4:



Legend: This is an image of a fertilized sea urchin egg, with fertilization envelope lift off, in low salinity seawater. The image was taken under a Nikon Eclipse E200 Microscope at 10x magnification in Ph1 with a Digital Interface camera. BTV Pro was used to capture the image

Figure 5:



Legend: This is an image of a sea urchin egg in high salinity seawater, exposed to sperm, but unfertilized. As a result, no fertilization envelope lift off occurred.

IV. Discussion and Conclusions

The results of my experiment supported my hypothesis that fertilization success rates of sea urchin eggs would be different in different salinities other than the control of 31 ppt (See Figures in result section) The purpose of the experiment with the calcium ionophore A23187 in place of sperm, was conducted to help determine whether fertilization envelope lift off, and thus fertilization success is affected by salinities differing from the control 31 ppt. If the percent of eggs with FELO was different in each of the salinities than I could conclude that fertilization envelope lift off is affected by different salinities. However, the results from experiment two proved that fertilization envelope lift off is relatively unaffected by different seawater salinity. However, it must be noted that in the trial conducted for experiment two, eighty percent of the eggs produced fertilization envelope lift off. This result implies that the lower salinity seawater has some affect on fertilization envelope lift off and ultimately on fertilization success. The conclusion that FELO, and thus fertilization, is relatively unaffected by different salinities, suggests that sea urchin sperm may be affected by different seawater salinities. This indirect result explains why none of the eggs in either trial in experiment one, in the high salinity seawater, had no fertilization envelope lift off. That is, no fertilization occurred. From these results I can conclude that sea urchin sperm itself, and the way the egg interacts and is triggered by the sperm is affected by high salinity seawater. However, because the results of experiment two show that fertilization envelope lift off may be affected by the low salinity, I can not conclude that sperm is affected by both the high, 38 ppt, and the low, 27 ppt, salinity.

A couple of errors may have affected my results. First, because eggs from NSW were being added to relatively small volumes of different salinity water, there was a concern that the salinity may have been altered with the addition of the

NSW and eggs. To minimize this risk, the eggs in the NSW were allowed to settle to the bottom so that a concentrated sample of eggs was collected in the pipette and transferred to the volume of desired salinity water. This helped minimize the amount of NSW transferred over along with the eggs. Second, the concentrations of sperm in each sample, particularly for experiment one, may have been different. A different concentration of sperm has a direct affect on the fertilization success rate, because if the sperm are not abundant, less sperm will reach the eggs, and as a result the percent of eggs with fertilization envelope lift off or fertilized eggs is reduced.

Further, a couple technical aspects that reduce the validity of my results must be discussed. Due to time limit, I was only able to conduct two trials for experiment one. Further, because the two trials were conducted on two different days with different resources, the method in which the trials were carried out differed. As a result, the data cannot be compiled and analyzed together. Instead, the data must be presented and analyzed separately, and then a conclusion made from the results of each. Since the results of the two trials for experiment one showed similar results, I was able to make an overall conclusion based on these. However, error results here because the two methods I used affected the concentration of sperm, and could have had other affects. Dried sperm, which is much more concentrated, was used in trial two of experiment one, and as a result the eggs in this trial were subjected to a much higher concentration of sperm. As mentioned earlier, the concentration of sperm has a direct affect on fertilization envelope lift off, and fertilization.

Also, since I was only able to conduct these two trials for experiment one, and one trial for experiment, two with the calcium ionophore A23187, the power of my results is limited. Having fewer trials for each experiment limits the results, and restricts the conclusions one can make. Ideally, three trials for experiment one and experiment two would have been conducted.

To refine this experiment, I would repeat experiment one and two three times each. Also, I would make sure that either I had enough time to complete the three trials of each, or I would make sure the same resources were available on a different day. I would take these precautions to ensure that my methods would be the same in each trial, so that I could pool my data and take a cumulative percent of the number of fertilized eggs or eggs with fertilization envelope lift off, in each of the designated salinities.

A couple of future experiments could be done to extend on my results. First, it would be interesting to see an experiment done that tests whether sea urchin sperm are affected by seawater salinity at 27 ppt. My results from experiment one show that the percent of fertilized eggs, determined by the percent of eggs with fertilization envelope lift off, in the low salinity seawater are different from that in the control. However, my results from experiment two also show that fertilization envelope lift off is affected by the lower salinity. This future experiment would explore the reason the percent of fertilized eggs in the low salinity seawater in experiment one was lower than the control. The experiment could answer, whether the difference was a result of a combination of the sperm and fertilization envelope lift being affected by the lower salinity, or whether it was a result of the salinity affecting only fertilization envelope lift off in the eggs. Also, further experiments could be done to see whether later stages of embryo development, like mitosis and gastrulation, are affected when the embryo is in different seawater salinities.

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