Increasing the Acidity of Water May Stunt the Development of Frog Embryos and Egg Jelly Coats

Ian Strachan
Independent Research Project
Bio254 - Developmental Biology
November 28, 2007

Introduction

Amphibian embryos, specifically frog, have been used by biologists since experiments on early development began. This still holds true today as frog, and all amphibian embryos, are excellent test subjects because of their considerable size and availability as they are spawned in large quantities that can be easily collected. Where a chicken may lay one or several eggs a frog might lay hundreds. However it is predominately the size of the amphibian embryo that attracts researchers to use them in experiments. They can be viewed or dissected with relative ease compared to some other speciesÕ embryos, like mice, which are smaller. Biologists such as Hans Spemman and Hilde Mangold were two prominent members of the scientific community that made extensive use of amphibian embryos and their subsequent discoveries popularized the field of developmental biology (Wilt, 2004).

Amphibians are often described as indicator species, which means they are the most sensitive species in the area and are therefore the most affected by changes in environmental conditions, whether they are natural or not. Pollution is a massive factor in the environment today and the disappearance of indicator species may be a warning that the environment is not able to handle such changes. AmphibiansÕ survivability decreases considerably once factors like pollution begin to come more prominent. That is not to say that the adult organism itself is dying because of exposure to chemicals but that eggs might be if they are being subjected to toxins in the area, specifically the water that they were laid and fertilized in. Acid rain is an excellent example of an environmental factor that can have a devastating effect on amphibians. Increased amount of acid rain means a higher acidity (lower pH) in pools and bodies of water, which are places the majority of amphibian eggs develop (www.epa.gov). Once the water has a higher acidity the trapped embryos being to suffer directly, the vitelline envelope can stop expanding which means that the embryo wonÕt ever hatch. Other effects include deformations of the head, tail and organs, and even termination of the embryo (Tyler 14-16).

This research experiment has been designed to observe the impact, if any can be observed, on the increase of acidity (decrease in pH) exposure to frog embryos and the eggÕs jelly layers. The aim is to be able to show with solid data that increased acidity (decreased pH) will have a negative effect on both embryonic growth and embryonic

mortality rate. In this study we appropriated embryos into three groups, 5 embryos per group, there was a low acidity, a high acidity and a control group. Data regarding growth and measurements was observed and recorded.

III. Materials and Methods

a)*Materials*: The acidity for this experiment was manipulated using vinegar (with a pH of 3), obtained from Emerson dining hall in a sterilized container and then stored in the Science Center on a lab bench during the duration of the experiment. The water used was bottled spring water obtained from the Biology 111 Laboratory. The frog embryos were purchased and delivered from Carolina Bio Labs. There was only one trial of this particular experiment and it lasted two days. The embryos involved in the experiment were held into three plastic Petri dishes once extracted. Extraction was done using a modified plastic pipette and a scalpel. These dishes were 8cm in diameter and had loose plastic covers. Oxygen was provided using a Tetratec AP80 air filter, plastic tubing and three glass 50 microliter capillary tubes. Adding the vinegar into the Petri dishes was done with a 1000 nanometer pipetter. Data collection and analysis was accomplished using the dissecting scope and BTV prop color camera in the ICUC of Wheaton College. Images were printed out using an ICUC printer and measurements taken using a metric 30cm ruler.

b)*Method*: The level of acidity was determined through a series of trials using 60mL of water in several Petri dishes. Then vinegar was added in various increments, using the 1000 nanometer pipetter, among each dish with the amounts recorded. Then the pH of each Petri dish, previously filled with 60ml of spring water before the addition of vinegar, was found using pH strips. This allowed for a specific pH to be altered for each dish of embryos. The pHs that were selected were 5.5 (no vinegar), 5.0 (.5ml of vinegar) and 4.5 (2ml of vinegar). Once the dishes were prepared and labeled appropriately the frog embryos were added. This was accomplished by carefully cutting a section of 5 embryos from the entire string with a sharp scalpel. Then once separated they were suctioned up using a plastic pipette with a cut tip to widen the opening allowing the embryos to pass into the pipette. Then gently squeeze them out into the appropriate Petri dish. Oxygen was provided to the developing embryos, by use of a Tetratec AP80 air filter. In order to control the amount of air that entered the Petri dishes containing the embryos a specific apparatus was designed. The plastic tube which attaches to the filter was modified. Three tiny holes were placed in the lining of the tube using a scalpel, and capillary tubes were placed in each of the holes. The end of the plastic tube was then closed off and was taped to a lab bench to prevent it from moving. The three Petri dishes were then placed so that the glass tubes could access the water. The covers were then placed on the tops of the Petri dishes and the air filter was turned on. The air filter remained on throughout the entire experiment.

Once the embryos were in place in the dishes they were imaged using the dissecting scope and the attached BTV Pro

camera. After all images had been taken of the embryos an image of a ruler was taken at the same magnification and focal distance for measuring purposes. This image of the ruler was then printed and measured by hand to give an image to object size ratio. This was found to be 18:1 as 1 mm on a ruler was 18mm on a sheet of paper when the image was printed. Knowing this ratio allowed for all images to be printed and measured by hand (and ruler) then converted to the objects real size. Embryo and Egg Jelly Layer diameters were measured as can be seen in Fig 1, the Embryo diameter measurement is in red and the Egg Jelly Layer diameter is in yellow. These distances were the numbers that were quantified into graphs as described using the above image to object ratio. These measurements were done for all images/frog embryos and the average diameter distance, (embryo and egg jelly layer) for every group, (low, high and control) was taken and collated into graph form as can be observed in Figures 3 and 4 in the Results section. Each data point is an average of all five embryos in a group.

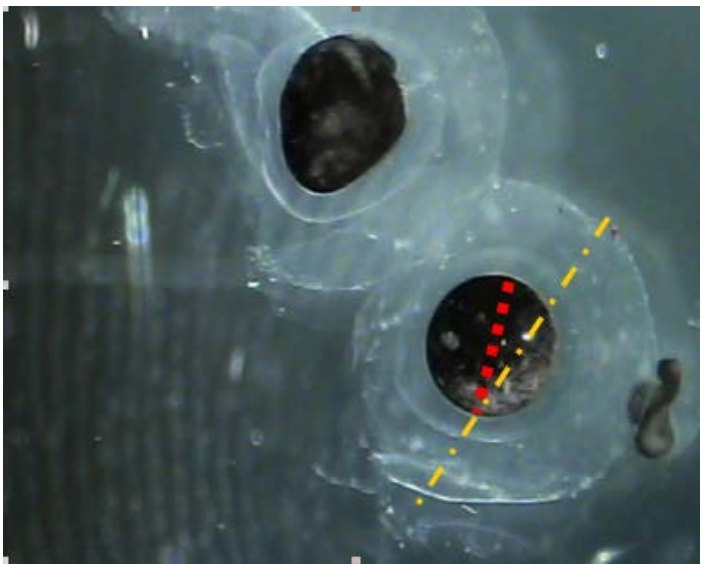

Fig 1. Frog Embryo and Egg Jelly Layer with Measurement areas.

IV. Results

I observed that there appeared to be a trend that the higher the acidity the lower the growth would be, in both the Frog Embryo and Egg Jelly Layer diameters. In fact the high acidity group diameters got smaller in many cases. This can be seen in both Figures 3 and 4. Figure 2 is an example of a typical image from our experiment. Another observation I made is that the high acidity groupÕs embryos promptly died upon commencement of the experiment and therefore showed no growth increases.

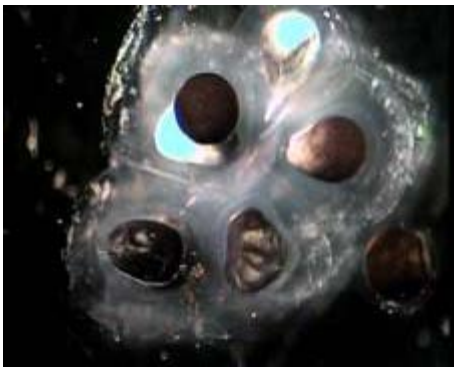

Fig 2. Typical Image of Frog Embryos with Jelly Layer

As there were only two days of data collected it makes accurate predictions very hard as there is a considerable amount of risk in making an error, especially making predictions. However as there were two points of data, a line can be drawn connecting them which gives an indication of a possible trend. These trends can be examined and then compared to our hypothesis that acidity will have a negative effect on the Embryo and Jelly Layer growth to see if it is supported or refuted.

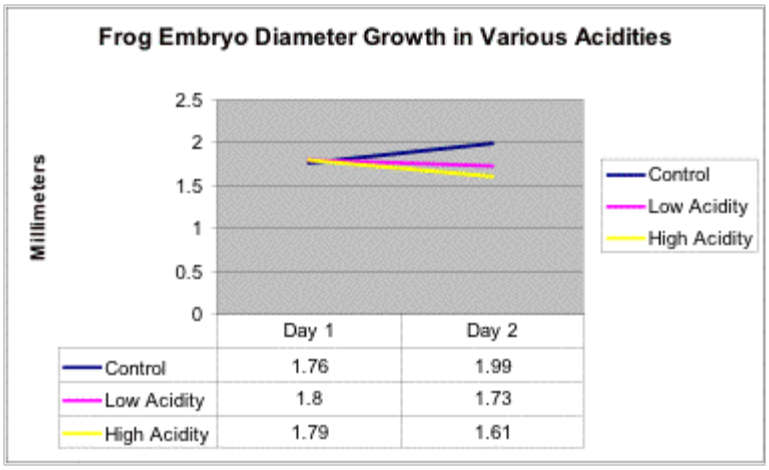

Fig 3. Graph of Frog Embryo Growth in the three different groups.

Figure 3 shows that the EmbryoÕs diameter Control Group increased from 1.76mm to 1.99mm. The Low Acidity group decreased from 1.8mm to 1.73mm and the High Acidity group decreased from 1.79mm to 1.61mm which means that both experimental groups displayed no growth and in fact decreased in size.

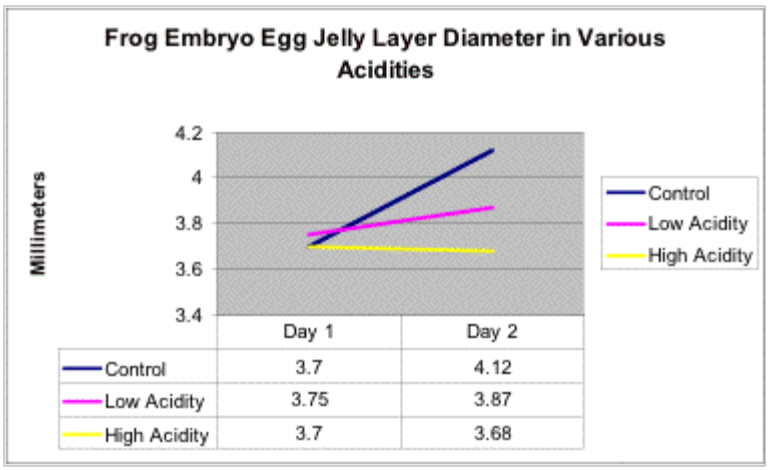

Fig 4. Graph of the Egg Jelly Layer in the three control groups.

Figure 4 shows a large increase in Egg Jelly Layer Diameter for the Control Group, 3.7mm to 4.12mm, a small increase in the Low Acidity group, from 3.75mm to 3.87mm and slight decrease in the High Acidity, 3.7mm and 3.68mm. This displays a trend that the higher the acidity the less the growth of the Egg Jelly Layer.

V. Discussion and Conclusion

The results appear to support my hypothesis in that embryos exposed to a higher water acidity, (lower pH) would suffer a negative effect in their growth and development. The data results show that frog embryos and egg jelly layers both grew in the control group and then that growth decreased in the low acidity group and decreased further in the high acidity group. This data shows a clear progression that the more vinegar that was present, and consequently higher acidity, the smaller the growth increase would be to the point of the observed measurements getting smaller.

That being said there were significant problems with this experiment the foremost being that there is such a little amount of data, only two days worth. This makes viewing trends and analyzing results regarding the embryos very difficult as there is not enough data to show a conclusive pattern. Instead predictions have to be made based of the data that is present, which is not reliable data. Having a limited data pool to draw from makes the entire experiment very narrow as the less data that exists, the less information that can be taken from them, so the conclusions that can be drawn about them are narrowed considerably in that they can focus only on one or two aspects of the study. Also there were issues with our organism, frog embryos, not being delivered at the appropriate times or at all so at first we had to make do with what we had, which were tadpoles. This logistical issue was the main reason for our small data pool.

Another source of error in our experiment was that there was a certain degree of unfamiliarity with the study organism. We had never dealt with them in a laboratory setting so were very unsure as to how to deal with them correctly to ensure survival and good data. Earlier when dealing with tadpoles we were unable to keep them alive for more than a day. The first trial involving tadpoles suffocated due to the Petri dishesÕ lids being on and the second trial died from their water evaporating due to the Petri dishesÕ lids being off. However these errors allowed us to preserve the frog embryos once they finally arrived through the ingenious invention and application of a modified air filtration system and glass capillary tubes.

Lastly the pH testing was far from exact as it was measured using pH strips which use a color comparison chart and is not an exact reading. If this experiment were to be revised perhaps having an accurate reading of each Petri dishes pH would be a good idea.

To make this experiment better I would need to ensure that there was enough time to correctly gather data. The

issues we ran into with our organism not being delivered couldnÕt have been helped but knowing now that there can be issues with shipment, especially with such time sensitive organisms like embryos being used getting them earlier rather than later would be desirable. Also it would have been more desired to actually use acid rain or a solution with the same chemical makeup to give the varying pH as vinegar may have had its own effect on the embryos.

As far as future experiments to extend this studyÕs results I would like to see a longer term study observing the frog embryos up through the metamorphosis of tadpoles and into fully formed frogs. Limb bud and digits like fingers and toes could be observed for mutations or stunted growth as could the eyes which would be interesting.

All in all this experiment showed that frog embryos and egg jelly layers were able to grow in an apparently normal way in the control groups. That same growth was slightly impeded in the low acidity group (with a pH of 5) and perished in the high acidity group (that had a pH of 4.5). These findings are in some part supported on the Environmental Protection Agency Website (www.epa.gov) which states that frog eggs can withstand and hatch in water that has a pH of over 4.0. And while these numbers are not identical they are relatively close which shows that the relationship found in this experiment has been found in the real world as well.

VI. References Cited

1. Wilt, Fred H. Principles of Developmental Biology. W.W. Norton & Company, 2004
2. Tyler, Mary S. Developmental Biology: A Guide for Experimental Study, Third Edition. vade mecum^2 An Interactive Guide to Developmental Biology Version 2.2
3. http://www.epa.gov/acidrain/effects/surface_water.html

4. Amanda Rawson, my lab partner, was an essential part of this experiment and all aspects of the experiment itself was conducted with her or with her consultation and collaboration.