Introduction:

This experiment will be addressing the effect of temperature on tadpole maturation, more specifically; *Xenopus* metamorphosis. The process of metamorphosis is significant, providing the foundation for lung development, deformation of the gills, changes in the digestive tract, changes in the skin, limb development, and tail reabsorption. (McLeod, pg. #2) Without this integral process, the indirect developing organism has no chance at survival. Any disturbance in the natural event of metamorphosis, whether the process is delayed or stopped, an organism’s chance to survive greatly diminishes. If the process is stopped, vital organs will not have the chance to develop, impeding future maturation. If the process is delayed or slowed, the tadpole’s development may last into the winter months where, depending on the tadpole’s location may result in the death of the organism. *Xenopus* was chosen for this experimental procedure due to the size of the tadpoles, the clear anatomical landmarks present on the organism’s body which allow easy observations of specific anatomical structures, and ease in both caring and handling the eggs during the procedure.

In this study, my hypothesis was that an increase in temperature will cause *Xenopus* larvae to undergo metamorphosis at a faster rate than they would in their natural environment. Previous experiments published by Thoman Morgan show that development in *Xenopus* embryos are dependent upon temperature. (Morgan, pg. #168) Morgan showed that lower temperatures tend to retard development while higher than average temperatures (>25°C and <30°C) usually speed up development in *Xenopus*. (Morgan, pg. #168) Further research published by Ian A. McLaren suggests an equation that directly relates variations in temperature to the rate of metamorphosis of Xenopus; \[ V = a(T - \dot{a})^b \]. Here \( V \), the metabolic rate is directly related to \( T \), the temperature, \( a \), is a constant proportional to egg diameter, \( \dot{a} \) varies inversely with latitude and altitude, while \( b \) is a constant for all populations. (McLaren, pg.#1071) McLaren also published a graph whose curve illustrates the relation between temperature and development time. (McLaren, pg. #1074) Although these experiments allude to the relationship between temperature and the rate of development, it must be stressed that too high a temperature, around 30°C, begins to retard the progress of *Xenopus* development while temperatures slightly higher have been known to result in death of the organism. (Morgan, pg. #168)

In this experiment, two experimental setups were maintained at various temperatures along with a control. The
xenopus eggs were exposed to these various temperatures while the rates of metamorphosis in each individual xenopus were recorded. Observations of the xenopus embryos, while in their early larval stage, were recorded over the next four days.

Materials:

1 Test Tube Rack
1 250mL Beaker
7 mini Petri dishes (4cm in diameter)
1 fish tank (10 gallons)
2 25 Watt, 8 gallon fish tank heaters
Tap water
3 Corning Test Tubes
1 Dissecting microscope with camera/video capabilities
1 Computer with BTV Pro program
1 Printer
4 Glass Petri Dishes (10 cm in diameter)
2 Finger Bowls (11.4 cm in diameter)
3 thermometers (1 room thermometer, two fish tank thermometers)
Tape (9-1” pieces)
1 Permanent marker
1 Plastic pipette with cut off tip
4 Large finger Bowls (20 cm in diameter)
1 Salt Water Tank set at 15°C
9 tadpoles at early tadpole stage
1 15cm ruler

Procedure:

Three setups were used in this experiment; one at room temperature, one at 15°C, and the third was set to 29°C. One trial, the second trial was successfully completed, while the first proved to be an essential learning opportunity. The second trial lasted for four consecutive days. Each setup was constructed in its own individual manner so that it maintained its respective temperature throughout the entirety of the procedure.
In the Control setup, three mini Petri dishes were filled with tap water so that the meniscus lay just below the top of the dish. A tadpole was then placed into each one of the labeled Petri dishes and placed on the lab bench in a designated, protected, area which maintained a constant 25°C. In order to ensure the temperature at which the embryos are maintained, a thermometer was placed beside the specimens on the bench. See figure 1.1 for a photograph of the actual Control setup.

The warm temperature setup used a ten gallon tank filled a quarter of the way with tap water. Three more mini Petri dishes were filled as they were in the control setup. Two 25 watt, 8 gallon fish tank heaters were placed on the longer sides of the tank, one on each side. The heaters were suctioned towards one end of the tank as shown in figures 2.1-2.2. The tank thermometer was then placed in the same end of the tank, suctioned to the middle of the shorter side of the tank. Two 11.4 cm diameter finger bowls were then placed in the center of the tank on the same end as the tank heaters. The bowls were placed in the water right side up and then flipped upside-down while under water in order to prevent any air pockets when placed upside-down. These two bowls were placed side by side so that their total length was parallel to the tank heaters. Next, two glass Petri dishes (10cm in diameter) were placed upside down on top of the finger bowls following the same protocol for decreasing the chance of obtaining air pockets as the finger bowls upon their installment. Water was added to each of the three mini Petri dishes in the same manner as the control setup followed by the addition of a single tadpole. These Petri dishes with specimens were then placed on top of the glass Petri dishes in the tank. Next, the heaters were set to their maximum temperature, which was found earlier to maintain the water temperature at a constant 29°C. Finally, tap water was added to the tank using the 250mL beaker, so that the water level outside the mini Petri dishes matched the meniscus of the water inside the mini Petri dishes as close as possible.
The Cold temperature experiment was setup as follows. Three Corning test tubes were filled with water up to their labels. After a tadpole was added to each test tube, the test tubes were placed in a test tube rack. The cold salt water bath (15°C) was setup next. One large (20cm) finger bowl was placed in the salt water bath using the same method to expel air pockets that was used in the previous setups. The other large finger bowl was then placed on top of the other after again expelling possible air pockets that may form. Next, two glass Petri dishes (10cm) were placed side by side so that they fit on top of the second finger bowl, again, after expelling the air pockets. On top of these two glass Petri dishes, the test tube rack was placed. If the Corning test tubes were pushed firmly into the test tube rack they would not float. It was important to make sure that the water level inside the test tubes is equal to or lower than the water level on the outside of the test tubes. This ensured that the water temperature of the cold salt water bath was controlling the temperature of the water inside the test tubes instead of the air temperature of the room (25°C). It was also necessary to make sure that the lip of the Corning test tubes was high enough above the salt water that none of the salt water would enter via splashing. Refer to figures 3.1-3.3 for photographs of the exact setup.
Maintenance for the setups was extremely important. Each day, the water in the mini Petri dishes of both the warm and control setups needed to be refilled with tap water back to their original levels in order to promote optimal heat exchange between their perspective environments as well as prevent the tadpoles from drying out. Tap water was also needed in order to maintain the water level in the warm water tank at the same height as the menisci in the mini Petri dishes.

In order to quantify the progression of metamorphosis in the tadpoles, the specimens were photographed each day using a dissecting microscope and the BTV Pro program. Each specimen was measured individually using several magnifications. For each specimen, a photo of the entire body, head and tail, was captured at .8 magnification. The tail from the tip to at least the beginning of the head region was captured next using a magnification of 1. Finally, just the head was captured using a magnification of 2. The photos were organized using several folders. Each photo was labeled with the date, the specimen ID, and the magnification.

The final data that were analyzed was collected using the .8 magnification images from each *xenopus* specimen. The anatomical landmarks that were used during the measurements were: tip of the head to base of the head (where the outer transparent layer ends on the tail region) in order to get the length of the head and the sides of the head where the tadpole was the widest (transparent boarder to transparent boarder) in order to obtain the head width. See figure 4.1 for a better understanding of what was measured.
These landmarks were measured because they were thought to have shown the most change for a tadpole at its current stage of metamorphosis, the best way to quantify its growth. Head length and width were also one of the few characteristics of the tadpoles that were measurable due to the tadpole’s highly pigmented skin.

![Figure 4.1](image)

This data was then converted using a proportion into centimeters. The proportion was calculated using a photograph of a 15cm ruler under .8 magnification of the dissecting microscope. The ratio came out to be 1/14. The average head width and length for each setup for each day was then calculated and compiled into two graphs. There was an average for each set of data for each day except for the warm temperature setup on the fourth day due to the lack of living *xenopus* embryos to quantify.

When examining the specimens under the dissecting microscope, the individual Petri dish may be placed directly on the stage. In the case of the cold temperature set ups, the specimens must first be transferred to a mini Petri dish before they are viewed using the dissecting microscope. It is best to have an auxiliary mini Petri dish and not use one of the already labeled in order to limit the amount that each specimen is handled.

The experimental control in this situation was the room temperature setup. It was necessary to have a room temperature setup in order to compare the rates of metamorphosis in the experimental tadpoles. Due to the fact that the maximum temperature for normal development of the *xenopus* embryos is around 25°C, which is about room temperature, the control allowed us to compare the normal rate of metamorphosis with the rates from the experimental setups. (Morgan, pg.#168)

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**Results:**

From observing the *xenopus* larvae each day, there did not seem to be any significant changes in the rate of metamorphosis nor any significant correlation between temperature and rate of metamorphosis. In general, the cold
temperature specimens outlived the warm temperature specimens and the room temperature specimens outlived both experimental specimens. Day 2 marked the first death, C1. Final photos and data collection were obtained before the specimen was discarded. After this event, my partner and I agreed that the warm temperature setup may be too hot and decided to turn the temperature down by 2°C. On day three, the two remaining warm temperature tadpoles died. One of the cold temperature test tubes (D4) was filled with water, possibly salt-water from the tank. The tadpole died. On day four, the two remaining cold temperature tadpoles were determined to be dead, leaving only the control setup with viable *xenopus*.

The averaged final data does not seem to point in any concrete direction or trend. However, there seems to be some correlation between the cold and control setups whose data remain within .01 of a centimeter with only one exception; the average head width on day 4. It is also interesting that figure 5.1 shows the average head width for the warm experimental setup, dropping to .26cm a day earlier than both the control and cold setups. The only other set of data that seems to have any kind of correlation lies in figure 5.2, where the control and cold setups seem to follow a slight ascending trend from day 2 through to day 4.

![Figure 5.1](image-url)
Discussion:

After one successful experimental trial, results show that temperature has little or no effect on the rate of metamorphosis in *xenopus* larvae. The only clear results, the expediency at which the specimens in the warm temperature setup (29°C /27°C) died as well as their smaller than average head width and length when compared to the cold (15°C) and room temperature (25°C) setups, suggests that a temperature of 27°C-29°C is harmful to frog metamorphosis. Figure 5.1 shows that the cold setup specimens tended to have a larger head width than the specimens at room temperature whereas the room temperature specimens tended to have a longer head length than the cold specimens. This data supports the notion that in colder temperatures (15°C), the head width of the *xenopus* larvae will be larger than that of larvae developing in temperatures around 25°C. It also implies that the average head length will be longer in the 25°C *xenopus* larvae than larvae in colder temperatures (around 15°C). In general, there were no great trends that directly correlated temperature and rate of metamorphosis. Several times a head width or length would decrease significantly one day only to raise significantly the next (cold specimens-in figure 5.1, day 2-4 and in 5.2, day 1-3). This data emphasizes the irregularities in image capturing and measuring techniques used to analyze the data which made trends difficult to determine. Thus, the data in fact refutes the earlier hypothesis which correlated temperature to the rate of metamorphosis, emphasizing that a temperature slightly above that of room temperature would increase said rate.

There were many sources of era in this experiment ranging from maintaining the organisms at a constant temperature to the water used to house the organisms. Maintaining the temperature inside the mini Petri dishes so that it was perfectly constant was not easy. Without coming in every few hours to refill the Petri warm temperature mini Petri dishes, the temperature inside the mini Petri dishes ranged slightly from day to day. As the water evaporated in both the tank and the mini Petri dishes over night into the morning, the temperature of the air surrounding the mini Petri dishes influenced the
Material:

Water inside the Petri dishes more so than the temperature of the surrounding water. This may have caused slight changes in temperature for these specimens. The fluctuating temperatures may also have caused abnormal growth in these specimens. There were also variations in the time of day that at which the specimens were maintained and recorded. For instance, the specimens were recorded and maintained at 1pm on day two and then at 5pm on day 3. This also would lead to variations in water heights due to evaporation and have the same effects upon the specimens as earlier mentioned. The water used throughout this trial was taken from the tap. Towards the end of the trial, it was discovered that dechlorinated water is optimal for tadpole development. (McLeod, pg. #1) The chlorine in our water may have impeded the growth of the specimens. There were also errors in measuring the images taken. Not every image was optimal for measuring the head width and length. In some of the images the tadpoles were laying slightly on their sides or their bodies were construed in some manner. In these cases the measurements were very close but not optimal or data could not be collected at all.

There were many difficulties experienced during this experiment and further refinement of future experiments is greatly stressed. One of the first problems that were encountered was the delivery of the organisms. The company chosen to purchase the eggs from sent the desired organisms earlier than specified. By the time lab began research on the xenopus, the fertilized eggs had already developed into xenopus larvae. The current condition of the specimens along with the uncertainty of future deliveries of xenopus eggs caused my partner and me to drastically change our hypotheses, having to study an entirely different stage of development than we had originally planned. This is why metamorphosis was studied in this experiment instead of neurulation in the gastrula stage of xenopus as originally proposed.

The first trial conducted used an incubator set at 36°C. It must be stressed that specimens at these temperatures must be checked more than once a day for the water evaporates very fast from the mini Petri dishes. None of our specimens survived the first day in this environment. Difficulty in maintaining our original extreme cold specimens which were placed in a refrigerator set to 5°C. These specimens also did not survive past the first day, concluding that 5°C environments are too cold for this species of Xenopus to prosper. This limited our next trial to temperatures around 15°C-30°C. Future experiments may want to explore these ranges, especially on the colder end of the spectrum to see if they can sustain specimens at slightly colder temperatures than used in this experiment in order to better observe the effect of a colder environment on xenopus eggs without killing the specimens. The cold specimens in this lab were also housed in Corning test tubes which are shaped differently than the 4 cm diameter Petri dishes. This would affect the amount of gas that would be able to exchange across the surface of the water in the test tubes, possibly suffocating the tadpoles in the test tubes. A refinement to this procedure would find a way to incorporate either all test tubes or mini Petri dishes into all of the setups of the lab procedure.

Future experiments should take into account the chlorine content of the water, making sure that it is in fact dechlorinated before the specimens are added. There are several ways to do this; treating the water with dechlorination
drops that can be purchased in most pet stores or by letting the water stand in the sun for a few days, vaporizing a majority of chlorine. (McLeod, pg. #2)

Another refinement would be the addition of days of data collected. Although enough data was collected so that it could be tabulated in a linear form, more data could unveil trends that were not clear after just four days of collection. The maximum temperature would also be lowered slightly to 27°C. Fresh tadpoles would also be ideal.

The majority of this experiment was concerned with the development of a completely original method and procedure. It would be ideal for future experiments to focus on the data collection and results now that a procedure is firmly established. Future experiments would take this into consideration extending the period at which the organisms are observed while taking the proposed refinements into consideration. The procedure needs to be repeated with emphasis on data collection as any new procedure would be. The previous research done on this topic suggest that temperature does in fact correlate with the rate of metamorphosis in many species of the *xenopus*, so further research in this topic must be stressed.

Bibliography:


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