EFFECT OF SALINITY ON EMBRYONIC AXOLOTL DEVELOPMENT

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Effect Of Salinity On Embryonic Axolotl Development

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Abstract

The axolotl, *Ambystoma mexicanum,* is an aquatic salamander native only to Laguna Alchichica in Puebla, Mexico and to the system of water channels and lakes nearby Mexico City. These water systems, under the influence of urbanization, are experiencing adverse effects due to decreasing water levels and increasing salinity. Many species are already unable to survive in these water systems due to this. I questioned the salinity at which axolotl embryos will experience decreased survivorship or increased morphological deformities. I used a freshwater solution as well as increasing saline solutions (with the highest being similar to the current salinity conditions in Laguna Alchichica), in order to determine at what point axolotl embryos cease to develop into viable young. I collected survival rates, developmental stages, number of deformities, and hatching rates as well as measurements of body length, head length, intraocular distance, and gill length of the hatchlings. The embryos reared in the 4 ppt NaCl/L solution showed the greatest amount of abnormalities, including many displaying a very distended, fluid filled abdominal cavity. Generally, embryos reared in the 1 ppt NaCl/L solution grew to be significantly larger than the others, based on the analysis of various two-tailed T-tests. The embryos reared in the saline concentration that was similar to that found in the lakes in central Mexico failed to develop. These results support that there is a threshold salinity at which axolotl embryos may cease to exist in the wild (although wild axolotls may have already developed a higher tolerance than the embryos I tested). These animals are already on the Critically Endangered section of the IUCN Red List so it is important to understand the potentially toxic effects of elevated salinity on embryonic axolotl development in order to be able to slow or reverse the decline of the axolotl.

Keywords: Axolotl, Salinity, Central Mexico

1. Introduction

The Mexican axolotl, *Ambystoma mexicanum,* is an aquatic salamander, native only to the system of water channels and lakes near Mexico City.[9] They are typically 6” to 12” long, heavy-bodied salamanders with broad heads and long, laterally flattened tails.[9] Axolotls exhibit neoteny, or paedogenesis, which is the retention of juvenile or larval characteristics after achieving sexual maturity.[7] They are distinguished from terrestrial salamanders by their fully aquatic lifestyle, external, feathery gills, dorsal fin, and small eyes that lack moveable eyelids.[9] The axolotl is normally a muddy gray color, but albinos, with bright gills, are not uncommon.[7] In captivity, they mainly consume small fish, zooplankton, and insects.[17]

Figure 1. The Mexican axolotl, *Ambystoma mexicanum*

Axolotls face multiple risks in their natural habitat that threaten their survival as a species. The axolotl is considered 'Critically Endangered' on the IUCN Red List (the final classification before extinction) due to the recent rapid decrease in their population density.[17] Threats to axolotls include the slow but steady drying out of their native canal systems and lakes. Secondly, the population is threatened by being overharvested for use in the pet trade, through competition with other species, and the spread of diseases.[17] Thirdly, the effects of urbanization, increased salinity, pollution, and the introduction of exotic predatory fish with similar diets can negatively impact their ecosystem and niche. Paedomorphic (and neotenic) species are especially susceptible to these types of threats as they are unable to transition to land due to the retention of their external gills. Many times, an entire population of neotenic salamanders will inhabit a single lake. When all members of a population are endemic; introduced fish, pollution, and drainage can endanger the entire population of the susceptible species, making it much harder for populations to rebound.[9]

Axolotls are easily raised in captivity and have well documented embryonic stages, making them ideal for experimentation. Embryos are most sensitive to stress during major developmental events, such as gastrulation, neurulation, and morphogenesis (gut formation, and the development of brain and limbs), making these stages ideal for experimental purposes. Axolotls are popular in developmental biology studies because they possess a well-known model system, having been studied and described for over 100 years, and although they are endangered in the wild, they are commonly reared in captivity for both scientific research and the pet trade.[9] Most developmental studies performed on axolotls are conducted because humans are interested in their ability to regenerate tissues, organs, and limbs with minimal scarring.[12, 13] Scientists hope to distinguish what trait makes this possible, and then look into how this may be leveraged for human use in the future.[12] If the processes of wound repair in animal models can be described and understood (including hemostasis, inflammation, tissue remodeling, etc.), then new therapies may be attempted in mammalian models, and then these could eventually be applied to humans.[12]

Environmental changes have been shown to negatively affect all age classes in amphibians, from eggs to adults. This can be done through the suppression of their immune system and changing of reproductive behavior, which has led to decreased production of gametes and fertilization failure. A variety of environmental toxins including trace metals, pesticides, industrial chemicals and their byproducts may be influencing various amphibian population declines.[5] Retardation of growth may have various negative effects on survival rates of different amphibians. For example, rapid growth is a trait that is commonly selected for in amphibian species that undergo size-specific predation because rapidly growing and large larvae spend less time in a smaller, more vulnerable stage.[5] Different types of toxicants can directly impair the ability of amphibians to grow, escape predation, and increase their susceptibility to disease.[5]

Introduced and invasive species have changed the distribution of native species in many aquatic ecosystems, causing different trophic cascades to occur.[17] Trophic cascades occur when changes (such as extinction of a species) at the top or middle of the food web causes alterations in the ecosystem processes that travel all the way down to the bottom of the food chain.[6] The predicted lack of flexibility in the diet of the axolotl as a top predator indicates that this species may be particularly vulnerable to ecosystem changes.[6] One study examined the trophic interactions and niche overlap between the axolotl and two exotic fish species in central Mexico, the tilapia and the carp.[17] These two fish were introduced into Lake Xochimilco (which is close in proximity to Laguna Alchichica) for aquaculture purposes over twenty years ago and since then have thrived and begun to dominate the ecosystem. As the populations of carp and the tilapia have increased, the axolotl population has dramatically decreased. Carp and tilapia occupy a greater amount of the same niche than the axolotls, meaning that they have adapted to prey on similar species, introducing a power struggle between the exotics and the native species in this area.[17] The introduction of these two species and the subsequent increased competition for food that has followed is likely to have had a negative effect on the already decreasing axolotl population.[17]

If axolotls do become extinct, there will likely be unfavorable impacts on species that normally prey upon them, as well as to the species they prey upon. For example, the disappearance of axolotls could cause increased competition among their predators (birds and large fish). If one species of predator proves to be more adept at handling ecosystem changes, or has a wider range of prey choices, then they may be able to outcompete the others. The potential extinction of axolotls could also trigger a boom in their prey populations, including small fish, zooplankton, and insects, affecting the entire food web.[17]

The skin of amphibians is thin, permeable to water and salt, highly glandular, and needs to be kept moist in order to function.[7] They are not typically found in saline bodies of water because they are unable to osmoregulate.[1] The salinity of many lakes that axolotls are able to survive in is heavily influenced by land drainage and the continuing growth of Mexico City. Laguna Alchichica is of particular interest to researchers because of the variety of organisms that continue to survive there, despite the rising salt concentration.[11] These species include diatoms, fish, and some neotenic salamanders.[11] Even though these species have been able to survive in these abnormally high saline conditions, if the salt content continues to increase this trend may not continue, leading to the ultimate extinction of the axolotl.

Salt lakes are not rare in Mexico, but with the exception of very salty lakes, they have not received much attention.[1] Most saline lakes form in endoheric basins (closed drainage basins that do not allow outflow to other external bodies of water and equilibrate through evaporation) in semi-arid regions between 20° and 40° N latitude. These conditions encompass about 65% of Mexico, which helps explain why there are so many saline lakes there.[1] Salt lakes are categorized according to salinity and from least to greatest, these include: subsaline, hyposaline, mesosaline, and hypersaline.[4] The term 'brackish' is thought to refer to dilute seawater and is not applied to the saline lakes described here. Currently the salinity in Laguna Alchichica where axolotls are native ranges from 8.3-9 grams of dissolved salt (Na-Mg and Cl-HCO_3) per liter of water.[11] This is approximately one quarter the salinity of typical seawater. Increasing salinity of water is a challenge to many species, especially those that are exceptionally vulnerable to the changes that can affect osmoregulation, such as neotenic salamanders.[9]

Anthropogenic salinization of soil and water has been reported on all continents except for Antarctica,[10] and can be caused by a variety of factors, including human induced run-off of deicing salts in temperate regions and various irrigation techniques. Natural salinization is when saltwater intrudes into groundwater, and can be driven by hurricanes, tsunamis, or an increase in storm frequency.[10] Although we may not have control of many of these natural causes, we do have control of the anthropogenic ones.

One study has found evidence that suggests that the development of internal gills in amphibians is necessary for various species to develop an enhanced tolerance to salinity.[16] Axolotls are one of few amphibians that retain external gills, which may suggest that they are unable to endure the same increased levels of salinity as those amphibians with internal gills. In many amphibians, like tadpoles, gills are the principal organs responsible for maintaining ion and water balance. The animals are unable to cope with this high osmotic stress until metamorphosis occurs and their internal gills develop.[16]

Karraker et al. found that survival rates of the Ornate Pigmy Frog, *Microhyla ornate*, were two times higher in fresh, low, and medium salt water concentrations (0.9% and 6.2% seawater) than when compared to those reared in the high salt concentration (19% seawater).[10] This experiment was run on multiple other tadpole species as well, and while not all were as sensitive to increased salinity, many of them showed decreased size and survivorship when exposed to the high concentration of saltwater.[10] The responses of amphibians to increased salinity varies, but reduced survival and delayed development of embryos and larvae as well as larval malformations have been documented.[10]

Axolotls, as well as many amphibians, are typically restricted to freshwater environments. Some eyewitness accounts, however, state that axolotls have been seen surviving along with other salamanders in some of the hyposaline lakes in central Mexico.[11] Even though there is some discrepancy in the eyewitness accounts of which species of *Ambystoma* have been seen in the Laguna Alchichica, there is no doubt that some of these neotenic salamanders are able to osmoregulate enough to live in this hyposaline environment. Because of the studies completed by Suetsugu-Maki et al.,[13] Zambrano et al.,[17] Carpenter,[6] Karraker,[10] Alcocer and Hammer,[1] Carey and Bryant,[5] and Oliva et al.,[11] as well as concern for the Mexican axolotl, it is important to understand the conditions that are necessary for their continued survival. Comparison of developmental rates of the embryos reared in different treatments is crucial in order to determine how sensitive axolotls are to saline water. I hope to help define how much more saline lakes can become without restricting the growth and survival of these organisms.

These issues led me to wonder if increased salinity was fundamentally affecting axolotl development. I chose to study the development of axolotl embryos reared in a range of saline concentrations including, and surpassing, the salinity in Laguna Alchichica, in the hopes of determining which salinities are detrimental to normal embryonic axolotl development. At the low concentrations of salinity, I expected the embryos to experience retarded growth and speed of embryo development, and an increased numbers of malformations. At the greater saline concentrations, I did not believe the embryos would be able to develop into functional or viable adults. If these solutions proved to be too high to yield sufficient data, I planned to decrease the salinity so that only the highest concentration provides embryos that cannot survive, while the rest of the solutions were low enough for at least some of the embryos to hatch.

I hypothesized that in the hyposaline solution (8.5 ppt NaCl), embryos would experience adverse developmental effects, while in the 15 ppt, 22.5 ppt, and the 30 ppt solutions; embryos would be unable to develop into adult axolotls.

2. Methodology

To compare developmental rates in embryonic axolotls, freshwater was used as a control for presumed normal development. Then a variety of increasing salinities were used as experimental treatments to compare the developmental differences between the groups. Oliva et al. declared that the salinity in the aforementioned Laguna Alchichica ranges from 8.3-9 grams of dissolved salt (Na-Mg and Cl-HCO_3) per liter of water, equivalent to parts per thousand, with an average pH in the basic range, 8.8-10.[11] Consequently, a salinity level of 8.5 ppt NaCl per liter of prepared freshwater was used as the first treatment solution. For the rest of the experimental solutions, salinity levels were increased to 15 ppt, 22.5 ppt, and 30 ppt NaCl per liter of freshwater. I chose these solutions because there is about 15-grams of salinity in normal brackish water and there is about 30 grams of salinity in normal seawater. These ranges are spread out so that they will show the consequences of increasing salinity in axolotl development as well as showing if there is a threshold at which embryos will no longer survive due to salt diffusion.

240 axolotl embryos (in 4 separate batches, 60 per batch) were purchased from The University of Kentucky's Axolotl Colony. Before my first batch of embryos arrived, I prepared all of the salt solutions, beginning with two gallons of artificial freshwater, consisting of 10 mL freshwater stock solution (provided by Stetson's aquatic lab) per gallon of deionized water. It was then calculated that the treatments would consist of; freshwater for the control, 8.5 ppt NaCl to represent the lake salinity, 15 ppt NaCl, 22.5 ppt NaCl, and 30 ppt NaCl. Twenty-five 15mL Petri dishes were labeled, with 5 Petri dishes of 4-6 embryos each per treatment group for the embryos to avoid pseudo replication.

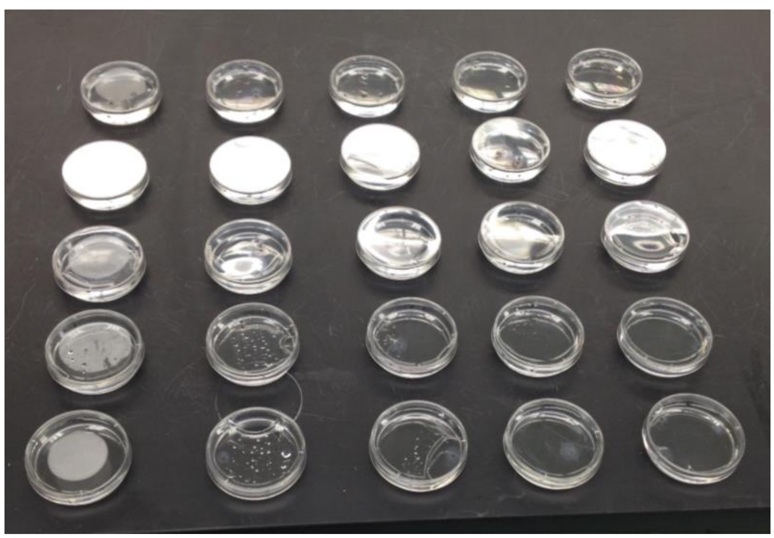


Figure 2: My experiment was set up with all Petri dishes in a column containing the same treatment, and the rows increasing in salinity from left to right.

When the embryos arrived, they were placed into a glass finger bowl and examined under a dissecting microscope to make sure they were all developing properly. Any abnormally developing embryos were discarded. Each of the viable embryos arrived at around stage 16/17, except for a few in trial 2 that had arrived already neurulated (middle to late neurula).[2]

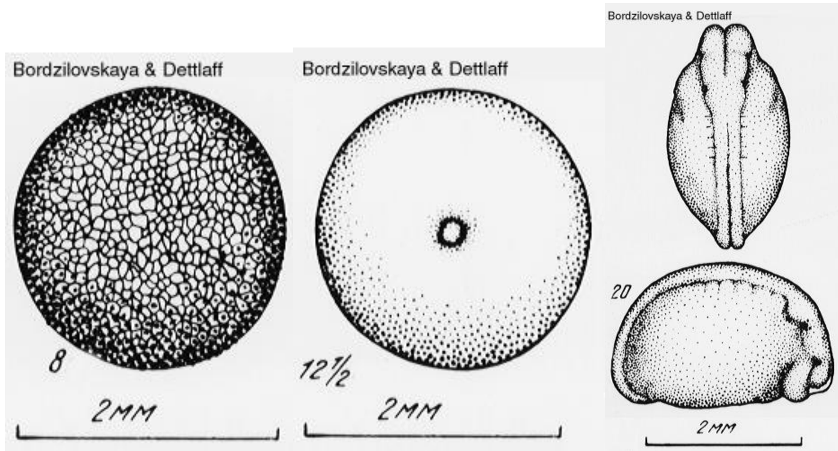


Figure 3: Early blastula, late gastrula, and late neurula axolotl embryo stages, respectively.[2]

The embryos were checked every day in order to record the different developmental stages. Normal developmental stages were used (in accordance with the Bordzilovskaya and Dettlaff staging series) in order to compare developmental rates in control and experimental animals.[2] I also made a partial water change to the solutions every other day, in order to keep the solutions fresh. This water change was accomplished through the removal of half the water in the Petri dish, and the addition of more water from the stock solutions. Life stage differences that occurred were photographed as each of the embryos developed.

Anything that appeared strange or abnormal was recorded and if at any point during the experiment embryos stopped maturing or began to molder, the developmental stage that the embryos had reached was recorded. On day 13, some of the embryos in the freshwater solution embryos hatched from their jelly coat, while all embryos in the saline solutions failed to complete development. All of these occurrences were recorded and the embryos were humanely euthanized with an overdose of MS222. I then put the embryos into labeled vials of Formalin for about a day to preserve them, and then transferred them to Ethyl Alcohol to store until measurements were taken. Measurements of body length, head length, intraocular distance, and gill length were made using a micrometer, Carl Zeiss Stemi DV4 Series Stereomicroscope.

2.1 Second Trial

Since all of the embryos reared in the saltwater failed to develop, salt concentrations were decreased for the next trial of the experiment. I elected to conduct a serial dilution of salt concentrations, with the highest being 8 ppt NaCl per liter of freshwater (around the lowest saline concentration in the first trial). The salt concentrations for the second trial consisted of; freshwater, 1 ppt NaCl, 2 ppt NaCl, 4 ppt NaCl, and 8 ppt NaCl. The same set up was used, and one abnormal embryo was discarded at the beginning of the trial. Most of the embryos in this trial developed fully so two more trials were completed with these same concentrations. Once all the embryos in the freshwater groups had hatched, I released any remaining (salt reared) embryos from their jelly coats using forceps. I also made a note and took photographs of each of the malformations. Statistical analyses were used to determine if the differences in salinities had any statistically significant effect on the developing embryos.

3. Data

All data analyses were made using the lower salt concentrations from the second trial on, 8 ppt NaCl and below. All of the embryos in the 8 ppt NaCl treatment moldered before they developed, so those were not used in the analyses. According to a two tailed t-test assuming unequal variances, mean body length was significantly longer in the 1 ppt NaCl exposed embryos when compared with the control (p-value = 0.00027). Conversely, embryos reared in the 4 ppt NaCl solution were significantly smaller than those reared in freshwater (p-value = 0.00019). No significance was seen between the 2 ppt NaCl exposed group and the freshwater group (see Figure 4).

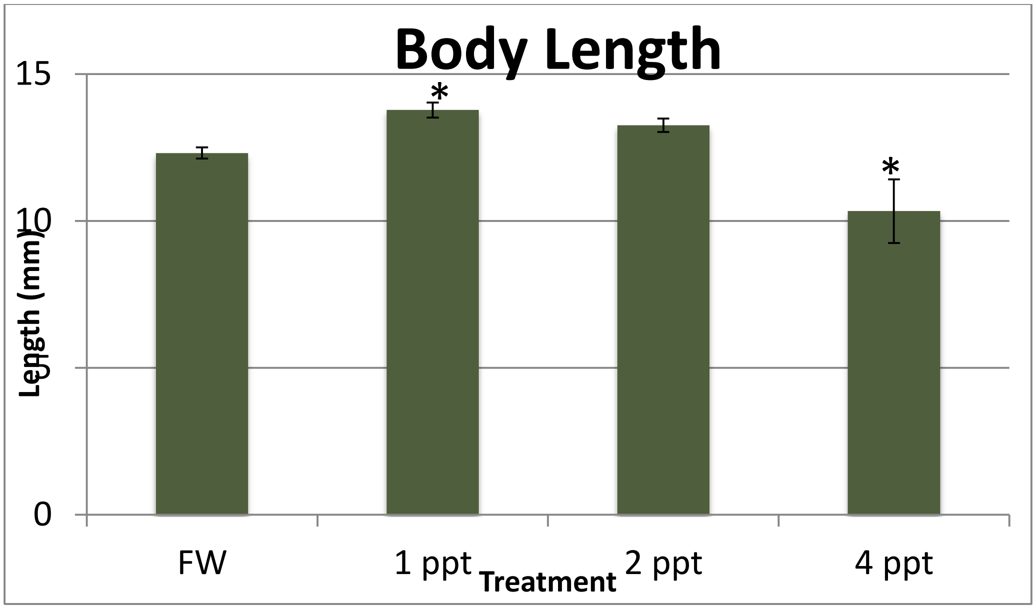


Figure 4: Figure shows salinity compared to the mean body length seen in the embryos (+/- 1 SEM, * indicates statistical significance when compared with freshwater control).

Similar results were seen when the salinity treatments and control compared the mean head length seen in hatchlings. Mean head length was significantly longer in the 1 ppt NaCl exposed hatchlings when compared to the freshwater control, using a two tailed t-test assuming unequal variances (p-value = 0.0163) (see Figure 5) and mean head length of the 4 ppt NaCl exposed embryos was significantly shorter than that seen in the freshwater group (p-value =0.0016).

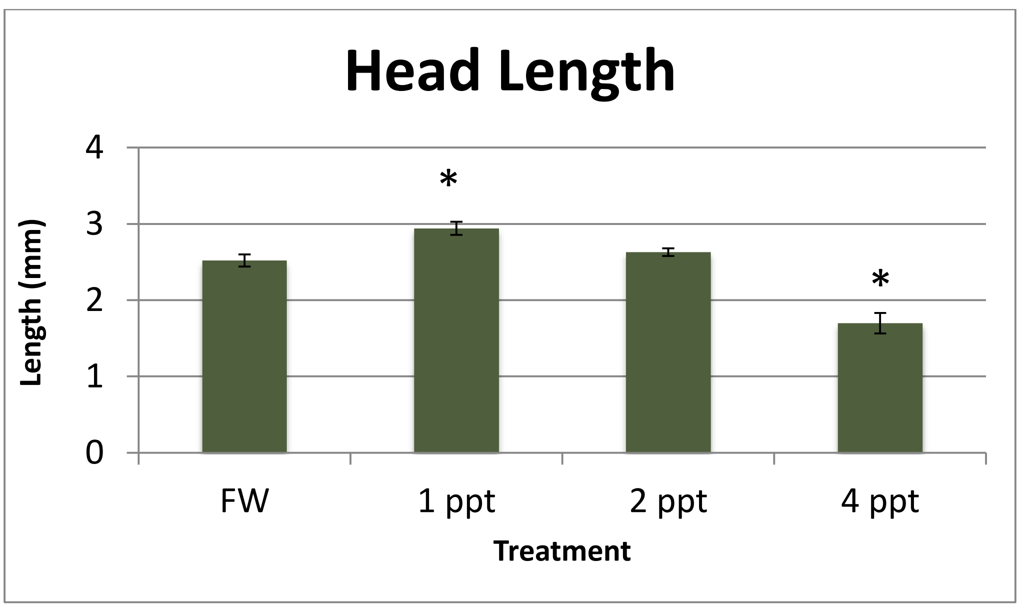


Figure 5: Figure shows salinity compared to the mean head length seen in the embryos (+/- 1 SEM, * indicates statistical significance when compared with freshwater control).

The same trend was observed when comparing intraocular distance for the 1 ppt NaCl exposed and freshwater embryos (p-value = 0.0003) (see Figure 6). The mean intraocular distance was also significantly smaller in the 4 ppt NaCl group when compared to the freshwater (p-value = 0.021). No significance was seen between the 2 ppt NaCl exposed group and the freshwater group for head length or intraocular distance.

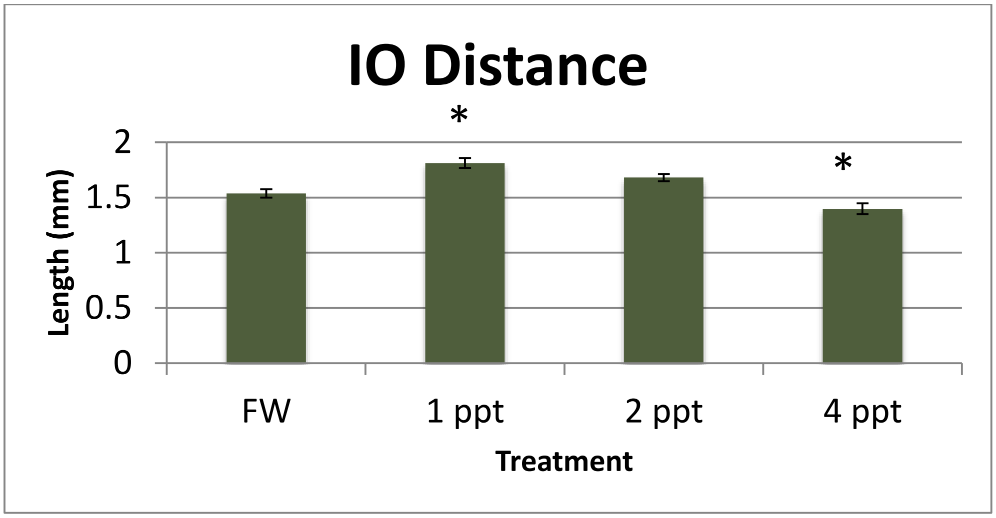


Figure 6: Figure shows salinity compared to the mean intraocular distance seen in the embryos (+/- 1 SEM, * indicates statistical significance when compared with freshwater control).

Gill length exhibited a slightly different trend when the salinity treatments were compared to the freshwater. Mean gill length was significantly longer in the 1 ppt NaCl exposed hatchlings when compared to the freshwater control, using a two tailed t-test assuming unequal variances (p-value = 3.491E-06) (see Figure 7). The gill length of the embryos in the 2 ppt NaCl exposed solution was also significantly larger than the freshwater (p-value = 0.00025). Gill length in embryos reared in the 4 ppt NaCl solution showed no statistical significance.

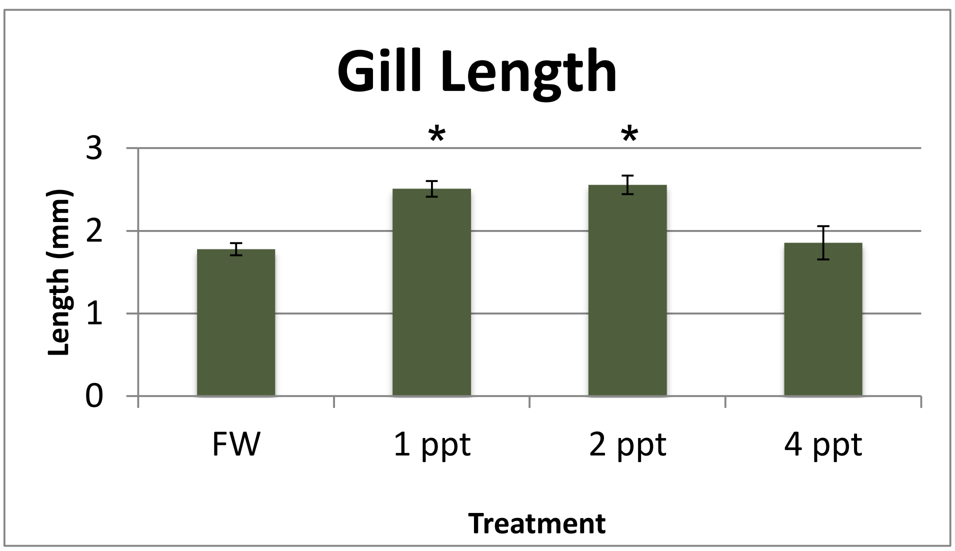


Figure 7: Figure shows salinity compared to the mean gill length seen in the embryos (+/- 1 SEM, * indicates statistical significance when compared with freshwater control).

Embryos developed and began to hatch fastest in the 1 ppt NaCl solution followed by the freshwater solution and then the 2 ppt NaCl solution. Embryos in the 1 ppt solution took an average of 13.75 days to hatch while the hatching rate of the embryos in the freshwater solution had an average of 15.76 days (see Figure 8). This showed statistical significance when compared using a two tailed t-test assuming unequal variances (p = 1.23E-07). Most embryos grown in 4 ppt NaCl did not hatch on their own, and were manually dejellied at 18 - 21 days (depending on the trial). SEM could not be measured because results may have been influenced because I had to assist hatch some of the embryos (13 embryos assist hatched in the 2 ppt NaCl and 29 in the 4 ppt NaCl).

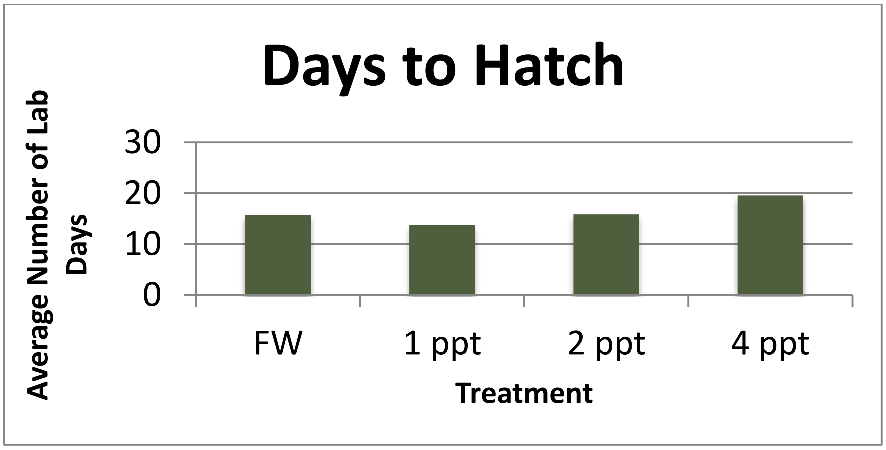
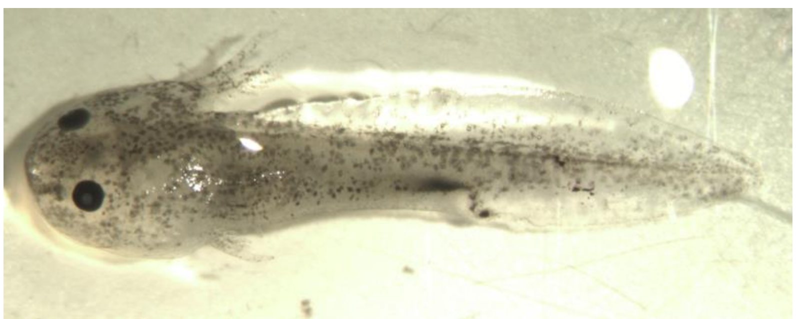


Figure 8: The average number of lab days the embryos took to hatch in each of the treatment groups.

Embryos reared in the 4 ppt solution showed the greatest number of abnormalities. The most common deformity observed was edema of the abdominal cavity (see Figure 9) and was only observed in embryos reared in the 4 ppt NaCl solution. In the other treatments, torsion and a variety of other deformities that were less severe were observed. Some embryos were not included in the initial analysis if measurements couldn't be accurately taken. The edema also seemed to be accompanied by decreased head and gill length in the animals.

(A)



(B)

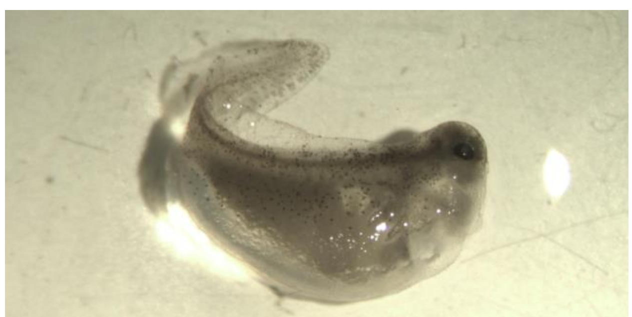


Figure 9: Severe malformations were common in embryos reared in 4 ppt NaCl (B), but did not occur in the other concentrations (A).

To compare the rates of deformities in the different treatment groups, embryos were split in each treatment into three abnormality groups, including normal, mild (kinked/cricked, slight "S" shape), and severe (lack of head formation, edema, and complete torsion). It was found that with the exception of one mildly deformed embryo in the freshwater group, all of the deformities occurred in the 4 ppt treatment. In the 4 ppt treatment group 20.83% were normal, 37.5% had mild deformities, and 41.67% had severe deformities (Figure 10). These results yield statistical significance when a Fisher's Exact test is used to compare the number of observed abnormalities between the treatment groups (p-value = 2.4E-15).

	FW	1 ppt	2 ppt	4 ppt
Normal	18	24	28	5
Mild	1	0	0	9
Severe	0	0	0	10

Figure 10: This figure shows the rates of malformations per treatment group by the classification of normal, mild, and severe.

4. Conclusions

All of the salt concentrations resembling Laguna Alchichica were too high and none of the embryos survived. After the NaCl concentrations were adjusted, the results supported my hypothesis. The embryos in the highest salinity did not develop, those in the lower saline solutions did develop, but experienced severe adverse effects, and those in the lowest saline solution appeared to develop more normally, albeit faster and at a greater size.

4.1 Reduced Concentration Yields Larger Hatchlings

I found it most interesting that embryos reared in the 1 ppt saline solution were significantly larger than those reared in the freshwater by all of the parameters. I could not find any other studies that directly considered beneficial effects of salinity and amphibian development. This finding is unlikely due to random chance, it is supported that the 1 ppt NaCl solution is more optimal in comparison to the freshwater solution.

Brandon et al. completed a study that explored the possibility of the appearance of a new species of neotenic *Ambystoma* in Laguna Alchichica. While completing this experiment, it was found that wild caught salamanders (and offspring) kept in saline water appeared healthier than those reared in freshwater in the laboratory. To explain this, researchers asserted that these species might require salt water close to the 8.3 ppt reported in Laguna Alchichica in order to eat and grow well.[3] If there is an optimal amount of salt (or range from freshwater to a certain point) in which axolotls thrive, this may explain why some species of *Ambystoma* salamanders have been seen in some saline lakes in Mexico, while so many other animals were unable to tolerate the salty conditions. However, it is very clear that this trend is only applicable to the 1 ppt solutions in my own experiment, and that the higher saline concentrations had the opposite, and expected, effect on the developing embryos in the higher saline solutions.

4.2 Energy Expenditure and Osmoregulation

In my experimental trials, most of the embryos in the 1 ppt salt solution (treatment two) hatched before the ones in the freshwater. One study that found a species of tadpole experienced accelerated development if they were exposed to elevated salinity (9 ppt) before they metamorphosed.[15] The lower salinity treatment (3 ppt) in Wu's study allowed the very young tadpoles to build tolerance to maintain normal development if they were exposed to a higher salinity later in their development.[15] This may have been a very high-energy cost to the organism and forced them to metamorphose earlier and at a reduced size. The animals may partake in this altered development in order to avoid stressful situations.[15] The shifts of metamorphic timing seen in this article provided the means for these amphibians to adapt to unpredictable fluctuations in salinity.[15] Another study suggested that multiple amphibian species are able to alter their hatching rates in response to different environmental conditions such as when an egg becomes dehydrated.[14] The study only considered eggs that had been suspended above water, but the same may be true in eggs that are dehydrated because of a factor such as increased salinity. Since axolotls do not typically metamorphose, I considered hatching a similar consequence of energy expenditure for the purposes of comparison in regards to the Wu et al. experiment. It is possible for major developmental changes to occur if an organism experiences environmental stressors such as dehydration.[15]

Wu et al. also found that the treatments of high salinity (9 ppt) always caused delayed metamorphosis, and reduced survival, growth and developmental rates (compared to those in the low salinity of 3 ppt).[15] This is due to the fact that they must expend more energy on osmoregulation than they would in more typical freshwater conditions, similar to what was seen in my high salinity treatments. These embryos very well may have expended a larger amount of their energy toward osmoregulation than those reared in the freshwater and the lowest salinity treatment.

The other result of my experiment that was found to be of particular interest was the appearance of malformations in the embryos and the development of edema in the abdomens in many of the high saline treatment embryos. This may have been due to a failure of the animals to properly osmoregulate in the saline solutions. It was expected that the higher saline treatment groups would experience the most abnormalities and almost all of the embryos had some type of abnormality, only 5 of the embryos that hatched in this treatment group appeared to be "normal". This supports my hypothesis that the higher the salinity the embryos were reared in, the greater chance they had of altered/adverse development. I have been unable to find any previous research that has yielded the same type of results of embryos in regards to a common deformity. It is suspected that this may be a result of the stresses of increased amounts of salinity and osmoregulation in the developing embryos. Consequently, these embryos suffered from vastly decreased head and gill length and were unable to hatch without assistance (nor did they typically appear to still be alive at the end of the trial). These subjects would most likely be unable to survive in the wild, or would experience an amount of decreased survivorship.

4.3 Future Research and Axolotl Survival

I would like to know if it is possible, and how likely it is, that axolotls may be able to develop a tolerance to salinity in the wild. The embryos obtained from the lab would not have the same tolerance as these wild axolotls because it may have been passed down genetically in Laguna Alchichica. This also caused me to wonder at what point the increasing salinity would be cause for much more serious concern for the wild animals.

What would be the best course of action to take in the natural habitats of axolotls in order to ensure their survival? The main reason I set out to study the effects of salinity on embryonic axolotls was to determine their tolerance and hardiness, in order to aid in the overall survival of the species. Because the salinity levels found in the lakes in Mexico proved to be too high for the lab-provided embryonic axolotls that were used in the study, it was concluded that some other variable must play a role. It seems likely that the wild salamanders have a tolerance to elevated salinity. If they have this ability, they may very well be able to continue to survive in the wild, but there is likely to be some limit to their NaCl tolerance. If this limit is not discovered and prevented, axolotls may no longer be able to survive in their only remaining natural habitats. Axolotls, along with every other species, are an important part of ecosystem dynamics and it is important to protect and continue to learn about them so as to avoid any massive ecosystem alteration.

5. Acknowledgements

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