The Effects of pH on the Embryonic Development of Zebrafish

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Introduction

The purpose of this lab is to determine the effect of different pH levels on the development of *Danio rerio* (zebrafish). The average pH of a zebrafish embryo is 6.6 to 8.2 (Kwong, Kumai, Perry, 2014). When the pH of the environment is thrown off, the fish could react to the difference of pH; causing deformities. Down to the chemical level, changes in pH cause proteins in the body to react differently; making the protein's function difficult. In this experiment, the zebrafish will be placed in pHs of 4, 7, and 10 and compared to how the zebrafish in Instant Ocean develop. Instant Ocean is an ideal environment containing the essential nutrients for growing zebrafish. Instant Ocean has a pH of 8.2 ("List of frequently asked questions and expert answers from Instant Ocean"). The differences in developments of zebrafish can be used to represent how fetuses develop incorrectly when the uterus is not at an optimal pH. An optimal pH for a human embryo is 7.2 to 7.4 and anything different from this could cause problems similar to the zebrafish ("Embryologists: Why You Should Test Your pH", 2019, para. 4). It is hypothesized that extremely acidic (pH 4) and extremely basic (pH 10) environments would cause the most developmental delay, if not death, in the zebrafish embryos.

Methods and Materials

This lab required three 500 milliliter flasks, three buffer packets (pH 4, pH 7, pH 10), Instant Ocean stock solution, a spot plate with 12 wells, largemouth pipettes, medium mouth pipettes, smallmouth pipettes, 120 alive Danio rerio (zebrafish) embryos, deionized (DI) water, a dissection microscope, a compound microscope, a sharpie, and a lamp.

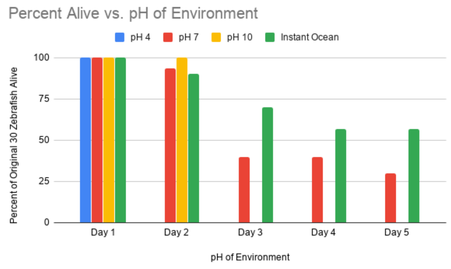
Each well of the spot plate was labeled with the solution and the group number with a sharpie. The buffer solutions were then prepared at each pH, 5 milliliters of Instant Ocean was added, as well as one pipette tip full of methylene blue. The wells were then filled with 10 zebrafish embryos each, and the remaining solution was removed with a small mouth pipette. Next, three wells were filled with 1 milliliter of pH 4, this was repeated with pH 7, 10, and the control (Instant Ocean stock solution). Once filled, each well was placed under the dissection microscope to reaffirm that 10 embryos were in each well. This information was recorded in the data table under "Day 1". The fish were then placed in the incubator which was set at 28.5 degrees Celsius. Photos were also taken as a reference point for development.

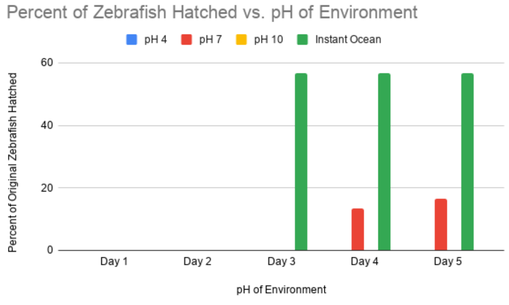
Days 2-5 followed the same procedure daily. First, the fish were removed from the incubator. The fish were then placed under the dissection microscope and the number of alive and dead fish was recorded. The old solution was removed with a small-mouthed pipette and the clean solution was injected into each well. Afterward, certain fish from each group were removed and examined under the compound microscope. These fish were also photographed and observations were recorded. The fish were then placed back into the incubator at 28.5 degrees Celsius.

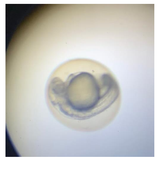
Accurate counts of living and dead fish were crucial in daily observations. This allowed for the analysis to be based upon the percentage alive in each group when comparing.

Abstract

A huge factor for the development of all organisms is the environment(s) they are exposed to. The species *Danio Rerio* is greatly affected by the solution the embryos are placed in. This species is considered a model organism for humans, and this experiment aimed to compare the results to what would take place if the human embryo experienced such changes in the amniotic fluid. Factors like embryonic development and overall deaths were assessed to determine the possible effects of pH (environment) changes to a human fetus inside of the mother. Our results indicated that the zebrafish had the least amount of survival in the pH 10 and 4 environments. The zebrafish experienced a normal rate of survival in the pH of 7, but ultimately had developmental delays in comparison to the control group. These results signify that a change in pH in the amniotic fluid of a mother carrying a child could have developmental effects on the child.

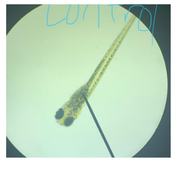




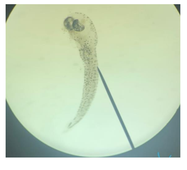


Day Two, Instant Ocean

Day Two, pH 10



Day Five, Instant Ocean



Day Five, pH 7

Results

Zebrafish that were in environments with pHs of 4, 7, and 10 were compared to zebrafish in Instant Ocean. The control group was the zebrafish in Instant Ocean. The independent variable was the environment pH and the dependent variable was the growth and development of the zebrafish.

The top chart shows the percent of the zebrafish that survived throughout the experiment compared to the environment. Percentages were used because they were the most simple way of presenting and comparing the data. The zebrafish in pH 4 were observed to die very quickly. The zebrafish in the pH 10 were also observed to have been developing way slower. The zebrafish in Instant Ocean had the best survival rate.

The bottom chart shows the percent of the total number of zebrafish that hatched throughout the experiment compared to the environment. On the final day of observations, only 16.6% of the zebrafish in pH 7 hatched compared to the 56.67% of zebrafish that hatched in Instant Ocean. The zebrafish in Instant Ocean reached the early larva phase, whereas the zebrafish in pH 7 had only reached the protruding mouth phase.

Discussion

The evidence from this lab suggests that straying from the optimal pH of an embryo can cause developmental delays. Even just having a difference of one pH can have severe effects on development. For example, the Instant Ocean and pH 7 were just one pH level apart, however, the zebrafish in Instant Ocean survived more and developed quicker. The hypothesis was supported by the results of the experiment. On day two, all of the zebrafish in pH 4 died and on day three, all of the zebrafish in pH 10 died. In order to improve the accuracy of this lab, more trials could be performed at various pH levels. The trials could have pH levels closer to the ideal pH level of the zebrafish in order to show the defects that occur when there is only a slight change in the pH.

Works Cited

Embryologists: Why You Should Test Your pH. (2019, July 24). Retrieved from https://fertility.coopersurgical.com/why-you-should-test-your-ph/

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