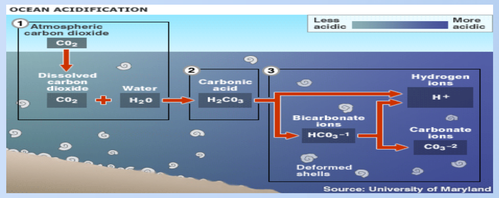
Effects of pH on *Danio rerio* Development

By Alex Navin

Introduction

Since the beginning of the Industrial Revolution, the amount of carbon dioxide (CO_2) produced and released into the atmosphere from manufacturing and burning fossil fuels has increased exponentially. About 25% of all the carbon dioxide released into the atmosphere is absorbed by the ocean. The ocean absorbs carbon dioxide by dissolving it into the water or the carbon dioxide will react with the water and decrease the pH. The ocean has, it's been estimated, absorbed 525 billion tons of carbon dioxide. The decrease in pH is due to the carbonic acid, an intermediate, dissolving into the water and releasing hydrogen ions, thus lowering pH. (Bennett 2016)

Zebrafish development can be broken into many steps. Upon fertilization, the blastodisc is formed. A blastodisc is simply an animal embryo in early development, when it is only a hollow ball of cells. The blastodisc starts as one cell and repeatedly divides and the blastodisc begins to spread around the yolk. The layer formed around the yolk is an epiboly – a spreading of embryo cells that allows for dramatic physical reconstruction. Epiboly formation continues into the Gastrula Period and, once completed, somites, which are body segments with similar internal structure, begin to form. In the Segmentation Period, the embryo elongates, forming the tail and general body shape. Finally in the Hatching Period, the larvae hatch from their egg. Primary organ systems continue to develop and cartilage formation begins. (Hill 2017)



Abstract

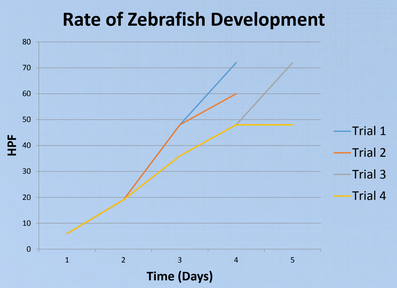
One of the biggest problems that contribute to the broad term of "climate change" is ocean acidification. Ocean acidification is a process in which carbon dioxide emissions from humans are absorbed by the oceans. The result of this process is a drop in the ocean's pH. The purpose of this experiment is to determine the effects of lowering pH levels on organisms that live underwater.

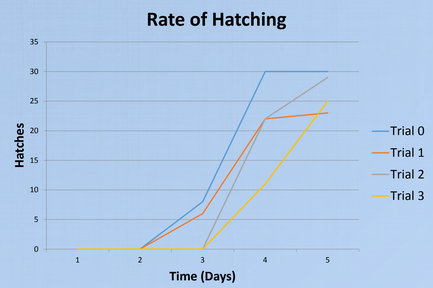
The experiment showed that the lowering pH levels can inhibit the development of zebrafish embryos. The zebrafish that were exposed to relatively normal pH levels exhibited no change in development, but zebrafish exposed to lower pH levels experienced a slower rate of development and. These trends show that the carbon dioxide emissions are having a very profound impact on underwater organisms. (Bennett 2016)

Data Analysis

Upon comparing the data gathered of certain characteristics of all the trials side-by-side, important trends can be identified. The rate at which the zebrafish are developing, measured in HPF, decreases significantly as the pH of the surrounding solutions decreases, or becomes more acidic. Also, the rate at which eggs hatch follow a similar trend – as the pH of the eggs' surrounding solutions decreases, then the eggs tend to take longer to hatch.

It may also be noted that there was also a significant difference in the behavior of the fish depending on the surrounding solution as well. The fish that were exposed to lower pH levels seemed to move less and react less to movement of the well tray. Meanwhile, those that were exposed to relatively normal levels of pH seemed to be more active, move faster, and react more frequently to movement of the well tray. While no data was gathered to support this, it is very possible that the pH was responsible for this observation and another experiment may be developed in order to determine its relation to pH levels.





Materials and Methods

Materials used in this experiment include: 5ml Instant Ocean Solution, 1L dechlorinated water, 120 zebrafish eggs, carbonated water, one pH meter, one burette, four 50mL beakers, one 1L flask, one 50mL graduated cylinder, one well tray containing twelve wells, an excess of pipets, one light microscope, one compound microscope, indented slides, and a lamp.

The Instant Ocean stock solution was created by mixing 1L of dechlorinated water with 5mL of the Instant Ocean mixture. The pH of the stock was measured with the pH meter and then recorded. The solutions for each trial were made by titrating the carbonated water into 20mL of the Instant Ocean Stock. The amount titrated into each solution was measured and recorded. Each well was filled with approximately 3mL of the trial's corresponding solution. The zebrafish eggs were then evenly distributed in every well – ten eggs each. Prior to the eggs being put into incubation, observations were recorded of the eggs from each trial. These observations include written description, pictures, and a count of alive and dead eggs. Once observations were taken, the eggs were put in incubation for 24 hours. From Day 2 until the end of the experiment, the eggs were taken out of incubation and observed 24 hours after they had been placed there. Photographs and a microscope were used to determine the age, in hours post-fertilization (HPF) of the embryos / hatchlings. Once observations were complete, the solutions from each trial were recreated and used to replace the solutions used the previous day. Once replaced, the tray with the eggs and new solutions were put into incubation for the following day.

In order to determine the significance of the gathered data, chi-squared (goodness of fit) test was performed, as shown to the right. The null hypothesis states that there is no difference in zebrafish development when exposed to normal pH or lower-than-normal pH. An alternative hypothesis states that there is a difference in zebrafish development when exposed to normal and lower-than-normal pH. The table to the right shows the observed and expected values – sums of the HPF of all fish during that trial on day 5. The p-value is very close to zero, meaning that there is almost no chance that this experiment could have been performed and support the null hypothesis. (Data is significant)

	Trial 0	Trial 1	Trial 2	Trial 3
Observed Values	288	288	216	144
Expected Values	288	288	288	288

$X^2 = \Sigma(\text{observed} / \text{expected})^2 / \text{expected}$

$X^2 = 2((288-288)^2 / 288) + ((216-288) / 288) + ((144-288) / 288)$

$X^2 = 90$

P-value ~ 0

Results

Independent variables in the experiment include the pH levels in each trial's solution and the substance with which the pH is altered – carbonated water. The dependent variable, and the focus of the experiment, is the development of the zebrafish. The first trial in the experiment, named "Trial 0" acted as a control group since no carbonated water was added to the Instant Ocean solutions, leaving the zebrafish in a natural 8.2 pH solution. The observations throughout the experiment were separated by the day they were taken. Figures 3-6 show the raw data gathered from each days two through five. No data table is shown for the observations on day one since there are no numerical values or photographs documented. It was only observed that the eggs embryos were circular, with a bulbous top (the blastula), and it was determined by the instructor that the HPF of the eggs at that point in time was six hours.

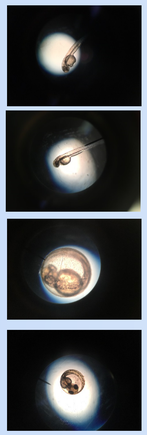
	Trial 0			Trial 1			Trial 2			Trial 3		
	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3
Eggs	10	10	10	10	10	10	10	10	10	10	10	10
Hatchlings	0	0	0	0	0	0	0	0	0	0	0	0
Alive	10	10	10	8	10	10	10	10	10	10	10	9
Dead	0	0	0	0	0	0	0	0	0	0	0	1
HPF	19	19	19	19	19	19	19	19	19	19	19	19

	Trial 0			Trial 1			Trial 2			Trial 3		
	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3
Eggs	7	8	8	7	6	6	11	11	7	10	5	11
Hatchlings	5	2	1	2	3	1	0	0	0	0	1	0
Alive	11	10	9	9	8	7	11	11	7	10	6	11
Dead	1	0	0	0	1	0	0	0	0	0	0	0
HPF	48	48	48	48	48	48	36	30	36	36	36	30

	Trial 0			Trial 1			Trial 2			Trial 3		
	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3
Eggs	0	0	0	0	1	2	4	1	1	10	3	2
Hatchlings	11	11	9	9	8	5	6	10	6	0	3	8
Alive	11	10	9	9	8	7	10	11	7	10	6	10
Dead	0	1	0	0	1	0	1	0	0	0	0	1
HPF	72	72	72	60	60	60	48	48	48	48	48	48

	Trial 0			Trial 1			Trial 2			Trial 3		
	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3	Well 1	Well 2	Well 3
Eggs	0	0	0	0	0	0	0	0	0	0	0	0
Hatchlings	11	10	9	9	7	7	11	11	7	10	6	9
Alive	10	10	9	9	7	7	11	11	7	10	6	9
Dead	1	0	0	0	0	0	0	0	0	0	0	0
HPF	72+	72+	72+	72+	72+	72+	72	72	72	48	48	48

Day 3 Pictures
(Trial 0-3)



Discussion

The zebrafish exhibit a decreased rate of development when exposed to lower pH levels. This prominent observation supports the hypothesis which stated that the lowering of pH of surrounding solutions of zebrafish embryos will hinder their development. The experiment has a few errors, including the fact that the pH of the carbonated water decreased as the experiment continued. This resulted in varying pH levels in each trial, as shown in Figure 9. Also, while changing the solutions in the well tray, some eggs were accidentally removed from their wells and lost. This explains the varying total amount of zebrafish embryos in the collected data in the results.

	Night 0	Night 1	Night 2	Night 3
pH of Trial 0	8.2	8.2	8.2	8.2
pH of Trial 1	7.5	7.0	7.5	6.9
pH of Trial 2	7.0	6.3	6.7	6.3
pH of Trial 3	6.5	6.5	6.3	5.8

Hindered development of zebrafish embryos shows the effects of lowering pH on underwater life. These effects may result in loss of biodiversity, hindered populations, or, in the case of shellfish, hindered lifestyles. The effects of ocean acidification do more than just effect zebrafish; they effect all of underwater life, and thus our own, and will greatly alter life for us humans as well if it continues.