The Effects of Caffeine on the Development of Zebrafish Embryos

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ABSTRACT: *Zebrafish were used because they grow quickly, they don't take up a lot of space, and their embryonic development is a lot like humans and it is outside the mother and easily visible. Caffeine was chosen because a lot of adults drink it daily. The 12 well plate was divided into 4 sections and each section was dedicated for a different concentration. Each day the embryos were viewed and counted to see how many hatched and how many were alive. All the embryos from the 1.0 mg/mL solution died while most of the control ones survived. The 0.05 mg/mL and 0.25 mg/mL solutions only had a few embryos die. A t-test was conducted which showed that the 0.05 and 0.25 mg/mL solutions had no significant effect while the 1.0 mg/mL had an extremely significant effect. The original hypothesis was supported by the data since all of the embryos in the 1.0 mg/mL solution died during the experiment.*

INTRODUCTION: Zebrafish are used for this type of experiment because they grow fast, don't take up a lot of space, and their embryonic development is a lot like humans and it is outside the mother and easily visible. Caffeine was chosen because a lot of adults drink caffeine daily. This experiment pertains to environmental science. 80% of adults drink caffeine daily (Karen Fernau, 2013) and these 80% intake 400 mg a day (Mayo Clinic, 1998). If an expecting mother drinks a lot of caffeine it can cause birth defects to the baby which can affect the baby throughout its life.

This experiment tested the effects of caffeine on the development of zebrafish embryos. Three different concentrations of caffeine were put in wells with the embryos to see if the different amounts affected their development. For this experiment, put 10 embryos in each well and had 3 wells for each concentration.

If zebrafish embryos are put into higher concentrations of caffeine, then less of them will survive because the caffeine affects their development and growth. In the control group more fish will survive and grow normally, since it is the closes to its normal habitat. As the caffeine was added and gets into a higher concentration, the more fish will die or have some difficulties growing.

MATERIALS: 600 mL beaker, 150 mL beaker, 0.5 mg/mL caffeine, 0.25 mg/mL caffeine, 1.0 mg/mL caffeine, instant ocean embryo media, 1.0mL and 3.0mL pipettes, incubator, well plates, dissecting microscope, compound microscope, zebrafish embryos from UW Milwaukee's Science Education Partnership Award, gloves, and expo marker.

METHODS: Ten embryos were placed in each well of a 12-well plate. One section was left for the control group and filled halfway with instant ocean embryo media. In a twelve well plate, using gloves, 0.5mg/mL, 0.25mg/mL, and 1.0mg/mL of caffeine was added in a section of three wells. A cover was placed the top on the well plates and the well plates were put into an incubator for 24 hours. Grab a beaker and your well plates. The 1.0 mg/mL pipette was used daily to remove and then replace the liquid. A 3.0 mg/mL pipette was used to remove all the dead embryos, which were placed in the disposal beaker. This process was repeated for all

wells. New clean solution was placed into each well making sure it's the same treatment from the day before. A dissecting compound microscope was used to make sure all dead embryos were removed. Placed lid back on and return them into the incubator. Use gloves when doing this experiment so you don't get exposed to dangerous chemicals which can and will harm you.

RESULTS:

The reason for this experiment was to test the effects of caffeine on zebrafish embryos. This experiment helps scientists because human embryos are very similar to zebrafish embryos and the results are very similar. They are similar because they both develop in similar ways because they both have stages of development and the zebrafish embryos are easily studied since they develop outside the mother. If zebrafish embryos are placed into higher concentrations of caffeine, then more will die compared to the ones in the Embryo-Media Solution due to the effects of the caffeine on their developing body.

These results all supported the original hypothesis. They supported it because the hypothesis said that the higher concentration of caffeine would kill more embryos/fish which it did. The results also help because less embryos hatched on Day 2 in the higher concentrations. At the end all of the embryos in the 1.0 mg/mL concentration died which was what was stated in the hypothesis. Most of the embryos/fish survived in the control group which also helps prove the hypothesis to be correct. The statistical t test shows that it is not statistically significant that the lower concentrations, 0.05 mg/mL and 0.25mg/mL of caffeine, was the cause of increased mortality in zebrafish embryos. It does, however, show that it was extremely statistically significant that in the 1.0 mg/mL wells all of the zebrafish embryos died due to the caffeine.

			Days of Treatment (# hatched fish/# living)				
Treatment	Well #	# Starting Fish	1	2	3	Final # Live Fish	# Fish Swim
Control	A1	10	0/10	6/8	8/8	8	8
.05 mg/mL	A2	10	0/10	5/10	10/10	10	10
0.25 mg/mL	A3	10	0/10	5/6	6/6	6	6
1.0 mg/mL	A4	10	0/10	0/2	0/0	0	0
Control	B1	10	0/10	4/10	8/10	10	8
.05 mg/mL	B2	10	0/10	10/10	10/10	10	10
0.25 mg/mL	B3	10	0/10	8/9	8/9	9	8
1.0 mg/mL	B4	10	0/10	0/9	0/0	0	0
Control	C1	10	0/10	4/10	9/10	10	9
.05 mg/mL	C2	10	0/10	7/9	9/9	9	9
0.25 mg/mL	C3	11	0/11	11/11	11/11	11	11
1.0 mg/mL	C4	10	0/10	0/10	0/0	0	0

Table 1. This is a data table that shows all of the results for the three days of testing.

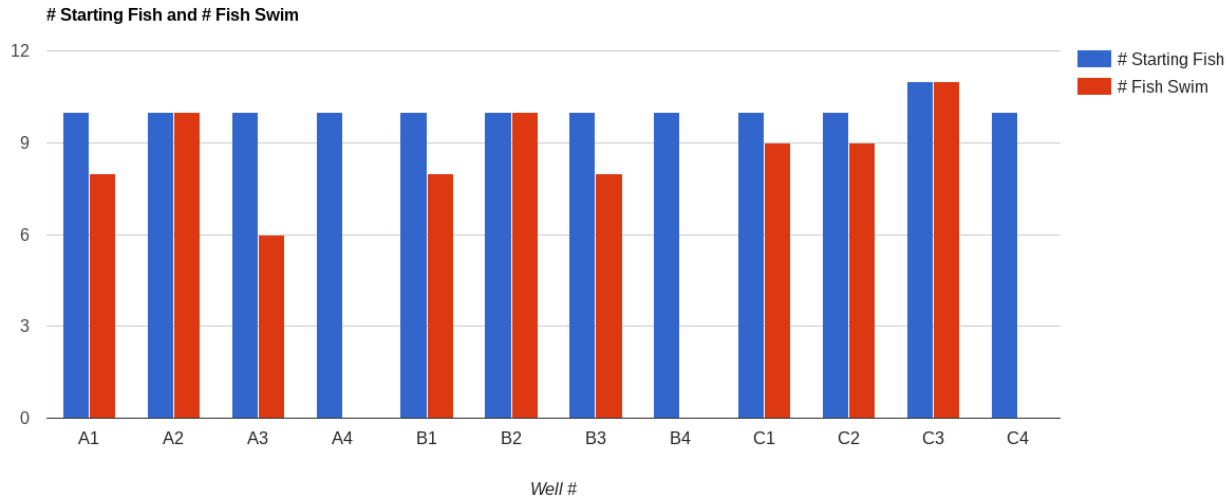


Figure 1. This chart shows the number of starting fish compared to the number after three days of being in the caffeine solution and the Embryo-Media Solution.

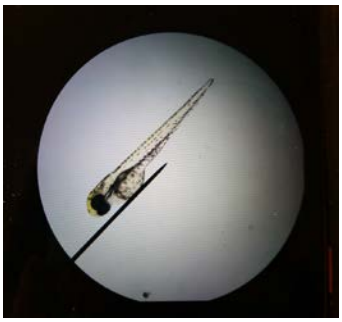
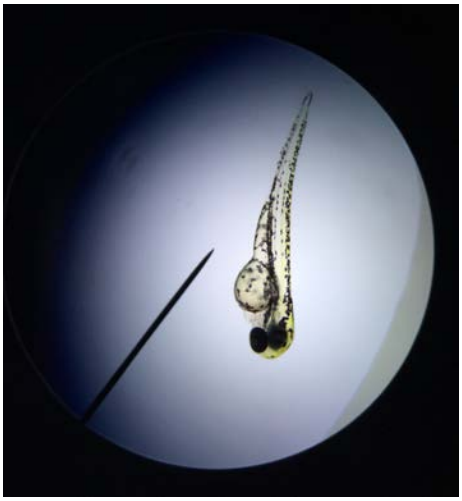


Figure 2. This is a zebrafish on Day 2 from the control wells.



Figures 3. This is a zebrafish from Day 2 from one well that contained 0.05 mg/mL of caffeine.

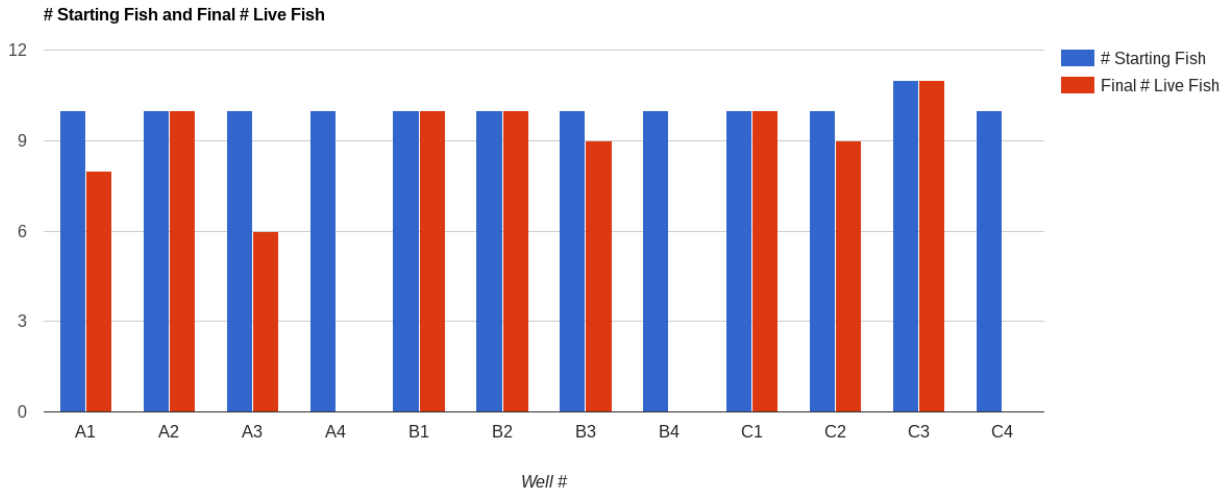


Figure 4. This chart shows the number of starting fish compared to the number which were swimming/hatched after three days of sitting in the solutions.

Figure 5. This picture is from Day 3 from the wells that contained 0.05 mg/mL of caffeine and one of the zebrafish is barely alive.

Figure 6. This picture is from Day 3 from the control wells and it is a healthy zebrafish.

DISCUSSION: In this experiment the original hypothesis was supported by the data. The charts above shows that as the caffeine concentration increased the more zebrafish died. In the control group there was an average of seven zebrafish that survived, and as the caffeine concentration increased the average number of surviving zebrafish decreased. Fewer fish were able to survive in high concentrations of caffeine, that could mean the zebrafish had a weak immune system, heart, or organs.

More studies need to be done to see if humans can have serious side effects due to intake of common daily drinks and foods such as coffee or oranges. These future studies can show if pregnant women should avoid intaking these items to help prevent the chances of their baby having birth defects or having a disease that can be harmful. Women who are pregnant and drink a lot of caffeine have been found to be at a higher risk for miscarriages, they are less likely to become pregnant, and the caffeine can have harsh effects on the baby like tremors, less likely to sleep, and an increased heart rate and breathing rate (MothertoBaby, 2011). While the adults body can handle caffeine with no problems the babies metabolism is still weak and developing. This can affect the movement of the baby during the later stages of pregnancy. Drinking too much caffeine can cause cause a premature labor, low-birth weight of the offspring, and preterm delivery (American Pregnancy Association, 2016). The results of this experiment are relevant because of all the ways humans can possible be affected by join following their daily routine by eating and drinking common foods which can have very dangerous side effects to someone not even born yet.

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