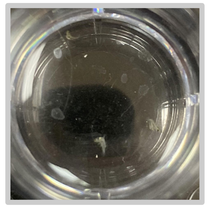

Effect of Caffeine Exposure on Zebrafish (*Danio rerio*) Embryos

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Introduction

Research Question:

The purpose of this experiment is to determine how exposure to different caffeine levels affect Zebrafish Development.

Background:

Zebrafish are minnow family fish that develop as transient embryos. Average time from egg to larvae is 72 hours. Zebrafish are used as model organisms in experimentation because they are known to be very hardy, and they have quick developmental processes. Caffeine is a crystalline structure that harbors significant stimulating biological effects. Caffeine increases heart rate in organisms when exposed. When this increased stress occurs in zebrafish embryos, hatch rate can be affected. Additionally, caffeine can have mirrored effects on human development. Oxford Academic Journal argues that pregnancies with high exposure to caffeine cam limit embryonic development. The University of Wisconsin-Milwaukee recommends experimental caffeine concentrations of 0mg/mL, 0.05 mg/mL, 0.25 mg/mL, and 1.0 mg/mL.

Hypothesis:

The Hypothesis is as stated: As the concentration of caffeine increases, the development of zebrafish will decrease due to the toxic qualities of caffeine. This would mean a higher death rate among higher caffeine concentrations.

Abstract

Experiment Purpose and Importance:

The effects of increased caffeine exposure on zebrafish embryos is an incredibly important study because it will reflect well on to human embryonic development. With the society of today being heavily reliant on coffee, soda, energy drinks, and other beverages containing caffeine, expecting mothers should be made aware of the potential effects of increased exposure of an embryo during pregnancy. If zebrafish embryos have developmental delays because of caffeine exposure, it is likely human embryos would face a similar response to increased exposure. The further study of this concept is critical for ensuring the safety of the next generation prior to birth.

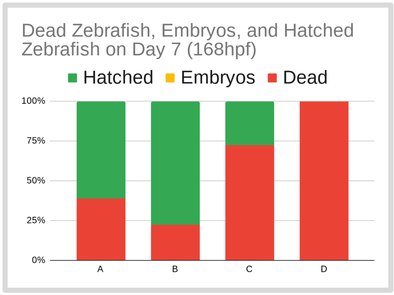
Methods and Finding:

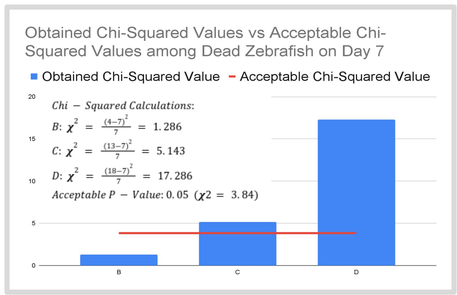
Through the experimental procedure referenced left, 18 zebrafish embryos were exposed to each condition level. The final death, embryo, and hatch counts were recorded and tested using Chi Square Analysis. This analysis yielded the result that the 0.25 and 1.0 mg/mL levels are outside of the accepted null hypothesis. This shows correlation with high caffeine concentrations and lower development of zebrafish embryos.

Results and Human Health:

The correlation between high concentrations of caffeine and lower development of zebrafish could reflect heavily onto humans as well. Further study must be made into the human developmental effects of caffeine exposure to determine risks of caffeine on human embryos. o9

Data Analysis





Graph and Chart Explanations:

Above are two figures: zebrafish status documentation, and a chi square analysis. The status tracks dead, hatched, and embryo zebrafish over time. The chi-squared bar graph displays the value obtained on the final day of the data collection as it related to the statistical likelihood of an event occurring randomly. The above calculations employ the use of the chi-squared formula.

Statistical Tests Used:

A chi-squared test was used in determine if the number of zebrafish dead was a statistical anomaly or if the data collected was true to a cause-and-effect relationship.

Significance of Results:

Through the analysis of data included in the experiment, it can be concluded that the null hypothesis that caffeine has no detrimental effects on zebrafish embryo development is rejected. With solution A as control, the expected value of dead embryos is 7, and in order to be certain that the results are not the result of random chance, a p-value of 0.05 (5%) is used, corresponding to a chi-squared value of 3.84. According to the status documentation at 168 hpf, there is a large increase in deaths in the C and D groupings, showing that there was correlation between high caffeine concentration and increased embryonic deaths.

Methods

Procedure:

1. Label well plates and obtain rinsed embryos
2. Fill 6 well plates each with 0.0, 0.05, 0.25, 1.0 mg/mL
3. Place 3 embryos into all 24 well plates, record 30 hpf data counts by well
4. Make observations, record
5. Place Plates into 28.5 ^{0} C incubator
6. (After 24 hr) Remove plates, remove dead embryos using pipette, discard
7. Count embryos, hatched fish, record in proper data table location
8. Remove solution from around embryos, replace with 1 mL solution
9. Make observations, record
10. Place into 28.5 ^{0} C Incubator
11. Repeat 6-10 every 24 hr

Outcomes:

Outcomes were measured via counting each individual well starting with 3 live embryos. The counts were then combined for final analysis via concentration in each condition value.

Statistical Methods/ Data Gathering

Chi Square analysis was used to evaluate the results after the experiment and testing the null hypothesis for data significant.

Resources:

Abdelkader, T. S. (2013, November 1). *Exposure time to caffeine affects heartbeat and cell damageârelated gene expression of zebrafish Danio rerio embryos at early developmental stages.* Analytical Science Journals. Retrieved February 13, 2022, from https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/jat.2787

Qian. (n.d.). *Caffeine consumption during early pregnancy impairs oviductal embryo transport, embryonic development and uterine receptivity*. Oxford Academic Journal. Retrieved February 13, 2022, from https://academic.oup.com/biolreprod/article/99/6/1266/5049471

Zebrafish. (n.d.). Genome.Gov. Retrieved February 13, 2022, from https://www.genome.gov/genetics-glossary/Zebrafish

Results

Experimental Design:

In this experiment, the number of zebrafish embryos, dead zebrafish, and hatched zebrafish was systematically recorded in order to draw conclusions regarding the effects of caffeine on zebrafish embryonic development. In order to ensure proper data was collected, several test groups were used for each treatment. Specifically, 18 zebrafish were used in each treatment group for groups A, B, C, and D. Each of these were split into groups in which 3 of the 18 would occupy a well for a total of 6 wells per treatment group.

Variables in the Experiment:

Due to its alteration, the concentration of caffeine was the independent variable in this experiment. Because of its variability from the caffeine concentrations, the number of dead zebrafish was the dependent variable in the experiment. Additionally, the controls used in the lab include the same wells, the same “instant ocean,” and the same timeframe of observation. The data that was collected shows a cause and effect relationship between in the independent variable (caffeine concentration) and the dependent variable (dead zebrafish).

Results and their Relation to the Purpose:

The analysis of data included in the experiment allows the conclusion to be drawn that that the null hypothesis that caffeine has no detrimental effects on zebrafish embryo development is rejected. In addition to deaths, the overall delay in development may be seen by how green segments of the bar graphs are less present in the solutions with higher concentrations earlier on in the experiment.

Discussion

Data and the Hypothesis:

According to the findings of the lab, the initial hypothesis that caffeine would have detrimental effects in zebrafish embryo development was supported. This is shown by the rejection of the null hypothesis that caffeine has no detrimental effects on zebrafish embryo development in the Chi Square Analysis. Because 5.143 and 17.286 are greater than the acceptable chi-squared value of 3.84, the chances of this sample occurring randomly is minute, and therefore the hypothesis is supported.

Project Limitations and Errors:

In this experiment, the zebrafish selected may have already had problems that would result in their developmental delays and early deaths. In the control, 7 of the 18 initial zebrafish died, thus suggesting that the caffeine was not the cause of their death. If they had some other issue, and more of the fish in other solutions had a similar issue, then the results of the experiment would have been incorrect. In order to minimize the error possible, more zebrafish would need to be used. The more zebrafish used, the more accurate the results of the experiment would come out to be. Additionally, more trials could be performed; more trials would lead to more data to be analyzed, thus increasing the accuracy of results.

Connection to Larger Body of Knowledge:

Zebrafish development may be majorly delayed, or completely stopped due to the presence of caffeine in their environment, as shown by the results of this experiment.According to pre-lab research, caffeine primarily serves as a nervous system stimulant, and this can be connected to the results obtained in the lab. It is possible that due to the overstimulated nervous system, other areas of the zebrafish embryos were not able to develop. Additionally, according to pre-lab research, zebrafish embryos typically hatch after three days; this also occurred in the lab, but less so in the trials with high concentrations of caffeine. Finally, the results from this experiment may be used in order to design future labs that aim to make discoveries regarding the effects of caffeine on other types of development. If caffeine hinders the growth and development of zebrafish, it may also do the same to other organisms, including humans. Additionally, similar experiments may be performed in order to determine if certain areas normally inhabited by zebrafish have been contaminated by substances deadly to zebrafish, and these substances are not limited to caffeine, but rather any that may slow or detrimentally affect development.