AP Biology Semester Research Project: 2016-2017

I. Title

The Effect of Water Temperature on the Development and Survival of *Danio rerio* Embryos

II. Abstract

Air pollution and deforestation are factors that affect temperatures of stream systems and watersheds (Beschta, Bilby, Brown, Holtby, & Hofstra, 1987). These changes in water temperature can affect the behavior of mature fish and can also affect the development of fish embryo (Eldridge,1963). The purpose of the experiment was to test how differences of temperature affect embryonic development and growth. *Danio rerio* (Zebrafish) embryo were placed in wells that were exposed to different temperatures (ranging from 24-29 degrees celsius). Embryo were suspended in water baths maintained at certain temperatures and the amount of embryo death and amount of embryo hatched were recorded daily. The results of the data suggest that the development at 24 degrees celsius was delayed due to the hatching rate being significantly lower than the other temperatures. Though the data showed this significant trend, the trials would have to be repeated on a larger scale to confirm the legitimacy of the trend and the effect of temperature on the development of the embryos. If the trend was confirmed, the data could be used by Environmental Protection Agency (EPA) and advocates against pollution.

III. Introduction

Pollution is a prevalent problem on earth today, and as air pollution is known to directly affect humans in a variety of ways, it also can affect humans in indirect ways that most people don't realize. Pollution not only affects the atmosphere surrounding earth, but it also affects the temperatures of water and aquatic ecosystems. Factors such as deforestation and air pollution consistently affect watersheds and their temperatures around the Pacific North (Beschta, Bilby, Brown, Holtby, & Hofstra, 1987). Excessive air pollution can reduce solar radiation by 15 to 20% (Development and Communications Office, 1992), and both lack and excess of solar radiation can cause temperature change in watersheds. In addition to air pollution's effects, logging/deforestation can remove forest vegetation along streams/channels that provide a buffering effect for water temperature (Beschta, Bilby, Brown, Holtby, & Hofstra, 1987). But why does changing water temperatures matter? Besides the fact that inconsistent water temperature changes multiple aspects of the water treatment process and affects the oxygen concentration in water, temperature change can greatly affect aquatic ecosystems and life (Eldridge, 1963). When water temperature rises, mature fish have "increases in metabolic rates and oxygen requirements, in sensitivity to toxic materials, in reduction in swimming speed, and in increased avoidance reactions" (Eldridge, 1963). Mature fish are affected by changes in water temperature that could become exaggerated by pollution and logging. These reactions to the changes in water temperature could have a potentially negative outcome on aquatic ecosystems.

In addition to the fish being affected by water temperature change, fish embryo can also be affected. The purpose of this experiment is to test to see if variation in temperature affects the development of the *Danio rerio* (Zebrafish) embryo. The *Danio rerio* was chosen because of its common location and its abundant availability for testing. Zebrafish, part of the minnow family, are naturally found in slow-moving bodies of water (Reed & Jennings, n.d). They live an average lifespan of around 3.5 years and have been selected for this experiment not only for their availability but also for their easy maintenance. They receive no further parental care after the embryo are fertilized and Zebrafish need no seasonal change to naturally breed and can breed year round. (Reed & Jennings, N.d). Zebrafish embryo need to be kept at a temperature around 28.5 degrees celsius. It is hypothesised that if the temperature is altered from natural temperature, then the development of the embryos will be altered or halted because the temperature change may cause denaturing in the process of development. In the experiment, wells containing embryo will be exposed to 4 different temperatures, including the control group. The temperatures will range from 24 - 29 degrees celsius; At each temperature, 4 wells will be tested and results recorded. The results of the experiment recorded that at temperature 4.5 degrees lower (the 24 degrees celsius embryos) showed a delay in development and hatched 48 hours later that the other wells (embryos usually hatch around 60-72 hours post fertilization (Schirone & Gross, 1968). Environmental agencies like the EPA may be interested in the data to know some effects temperature difference may have on aquatic ecosystems. It may also concern environmentalists who need examples and studies to argue about decreasing pollution. The

research is necessary to explain/prove the detrimental effects water temperature change has on aquatic life and connect it to the excessive amount of pollution (due to sun radiation and logging) that may cause the temperature change.

IV. Materials & Methods

Materials

Materials used in the experiment were 4 well plates (12 well), approximately 160 Danio rerio embryo, 3 fish tanks, 3 water heaters, 3 thermometers, an incubator, microscope, stock instant ocean, pipettes, tap water, plastic bins, labeling supplies (marker and tape), and waste beakers.

Safety

Practice proper care with water heater. Handle embryos with proper care and avoid unnecessary agitation.

Procedure

Day 1

3 40-liter fish tanks were filled about halfway with tap water. A water heater was added to each tank. Each tank was set to a certain temperature, 29, 27, or 24 (degrees celsius), and labeled with what temperature they were set at. Thermometers were placed in each tank and the tanks sat for 6 hours (to allow for heating). After 6 hours, tanks were checked for temperature consistency. 4 well plates were labeled A, B, C, and D. In the middle row of each well plate, approximately 10 alive Zebrafish embryos were added to each of the 4 plates. The number of embryo were counted and recorded. The well plate labeled A was placed in a plastic bin and put in the 29 degree celsius water bath, B was placed in the incubator (kept at 28.5 degrees celsius), C was placed in a plastic bin and kept in the 24 degrees celsius water bath, and D was placed in a plastic bin and kept in the 27 degree celsius water bath. The embryo were left in their respective water baths and incubators for 23 hours.

Day 2

The tank temperatures were checked and recorded. Each well plate was removed from the water bath/incubator. The embryos were observed under a microscope. Dead embryos were removed from each well and the total recorded. The instant ocean in each well was removed and replaced. The plates were placed back into their respective water baths and incubator and allowed to sit for 23 hours.

Day 3

The tank temperatures were checked and recorded. Each plate was removed from the incubator/bath. Each well was observed under a microscope for deaths, deformities, and

hatchings. The totals were recorded and photographs were taken. The instant ocean in each well was removed and replaced. The plates were placed back into their respective water baths and incubator and allowed to sit for 23 hours.

Day 4

The tank temperatures were checked and recorded. Each plate was removed from the incubator/bath. Each well was observed under a microscope for deformities, and hatchings. The totals were recorded along with observations, and photographs were taken. The instant ocean in each well was removed and replaced. The plates were placed back into their respective water baths and incubator and allowed to sit for 23 hours.

Day 5

The tank temperatures were checked and recorded. Each plate was removed from the incubator/bath. Each well was observed under a microscope for hatchlings. The totals were recorded and photographs were taken. The instant ocean and Zebrafish and embryos were removed from wells and placed a collective petri dish. Materials were cleaned and put away.

Variables

In this experiment the independent variable was the temperature at which the embryos are exposed to and the dependent variable was the survival rate/affected embryos. The control group was zebrafish embryos at standard/natural temperature and the experimental group was the zebrafish embryos at varying temperatures. Constants were the amount of time in incubator/specific temperature, well type. The sample size is 4 groups of 10 embryos for all 3 different temperatures.

V. Results

The results revealed a trend only with a delayed hatching in the embryos incubated at 24 degrees celsius. Percentage of embryo dead and hatched were compared for days 3 and 5. When compared to the control, the differences in the amount of embryos dead on day 3 or 5 for all experimental values was not statistically significant. For the experimental values 29 and 27 degrees celsius, the hatching percentages again showed no statistical significance when compared to the control. The 24 degree celsius wells did show a delay in hatching compared to all other wells, while the other wells had a 100% hatching rate by day 5, and the wells of the 24 degree celsius well plates hatching percentage ranged from 49.2% to 80%

Well Plate A (29 degrees celsius)

Well	Number of embryos	Number of embryo	Number of embryos	Number of embryos	Number of embryo	Number of embryos	Number of embryos	Number of embryo	Number of embryos

	alive (day 1)	death (day 1)	hatched (day 1)	alive (day 3)	deaths (day 3)	hatched (day 3)	alive (day 5)	deaths (day 5)	hatched (day 5)
1	7	0	0	7	0	0	7	0	7
2	8	0	0	8	0	2	8	0	8
3	7	0	0	6	1	1	6	1	6
4	10	0	0	9	1	3	9	1	9

Well Plate B (28.5 degrees celsius)

Well	Number of embryos alive (day 1)	Number of embryo death (day 1)	Number of embryos hatched (day 1)	Number of embryos alive (day 3)	Number of embryo deaths (day 3)	Number of embryos hatched (day 3)	Number of embryos alive (day 5)	Number of embryo deaths (day 5)	Number of embryos hatched (day 5)
1	8	0	0	6	2	1	6	2	6
2	10	0	0	10	0	1	8	2	8
3	10	0	0	10	0	0	10	0	10
4	10	0	0	8	2	0	8	2	8

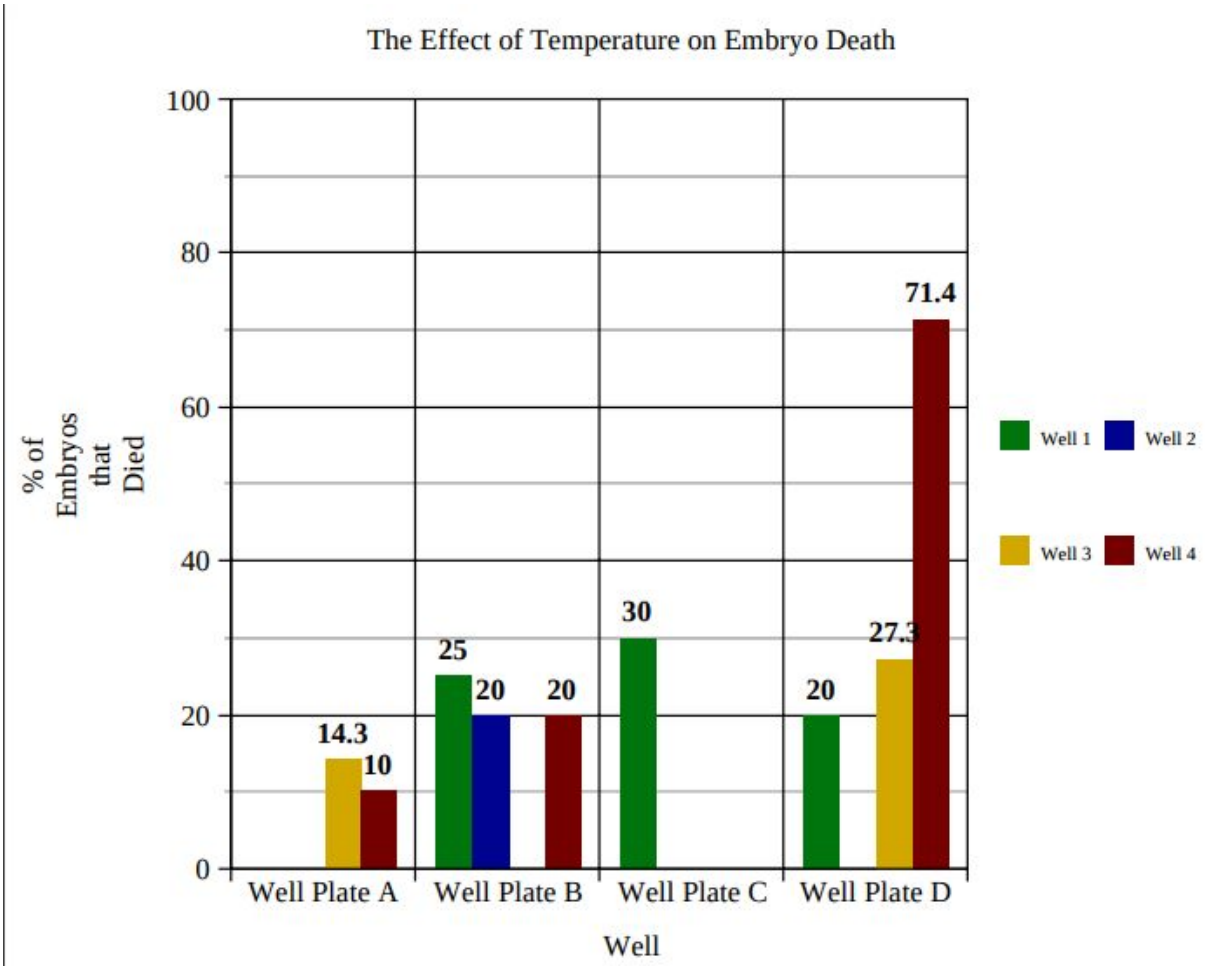
Well Plate C (24 degrees celsius)

Well	Number of embryos alive (day 1)	Number of embryo death (day 1)	Number of embryos hatched (day 1)	Number of embryos alive (day 3)	Number of embryo deaths (day 3)	Number of embryos hatched (day 3)	Number of embryos alive (day 5)	Number of embryo deaths (day 5)	Number of embryos hatched (day 5)
1	10	0	0	7	3	0	7	3	3
2	9	0	0	9	0	0	9	0	5
3	10	0	0	10	0	0	10	0	5
4	10	0	0	10	0	0	10	0	8

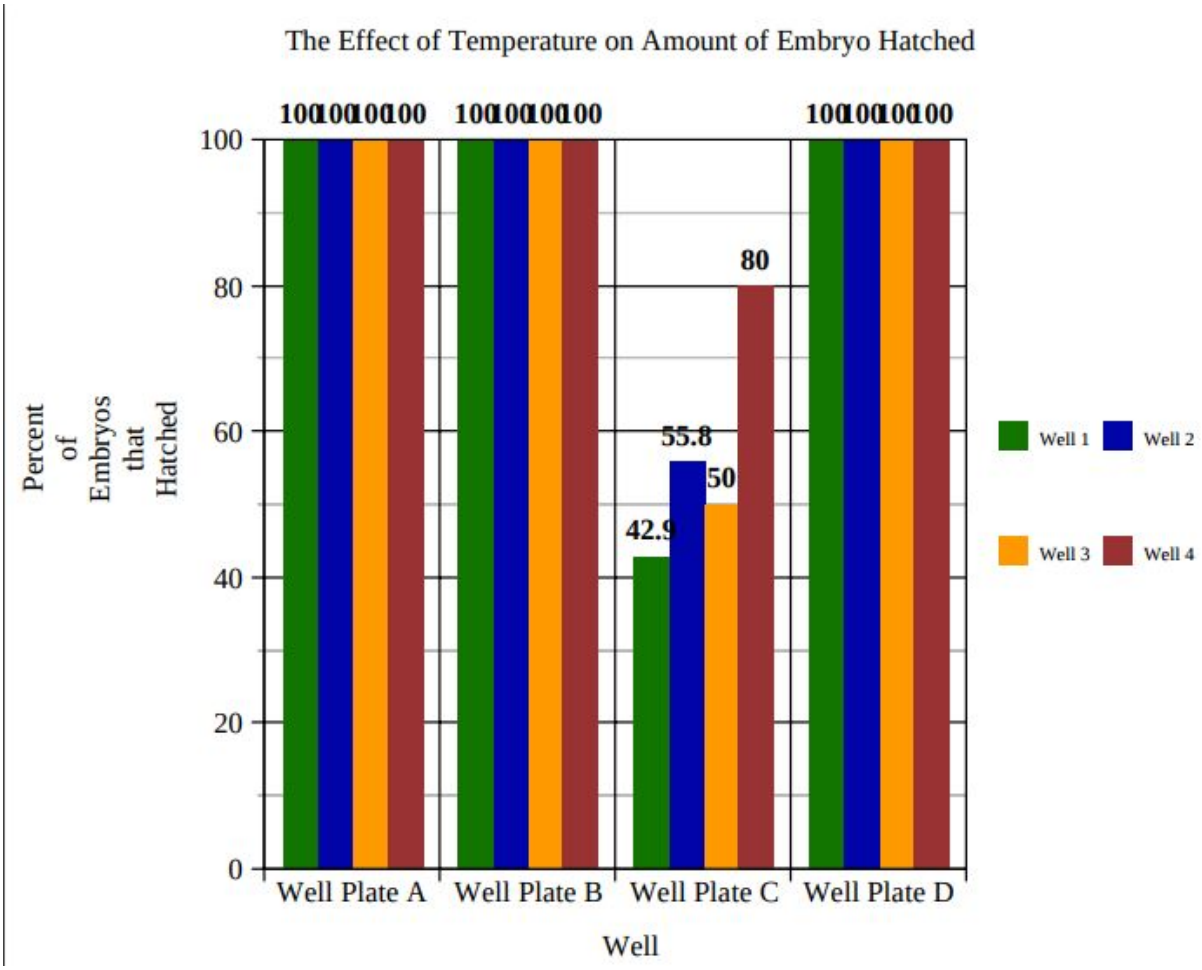
Well Plate D (27 degrees celsius)

Well	Number of embryos alive (day 1)	Number of embryo death (day 1)	Number of embryos hatched (day 1)	Number of embryos alive (day 3)	Number of embryo deaths (day 3)	Number of embryos hatched (day 3)	Number of embryos alive (day 5)	Number of embryo deaths (day 5)	Number of embryos hatched (day 5)
1	10	0	0	8	2	4	8	2	8

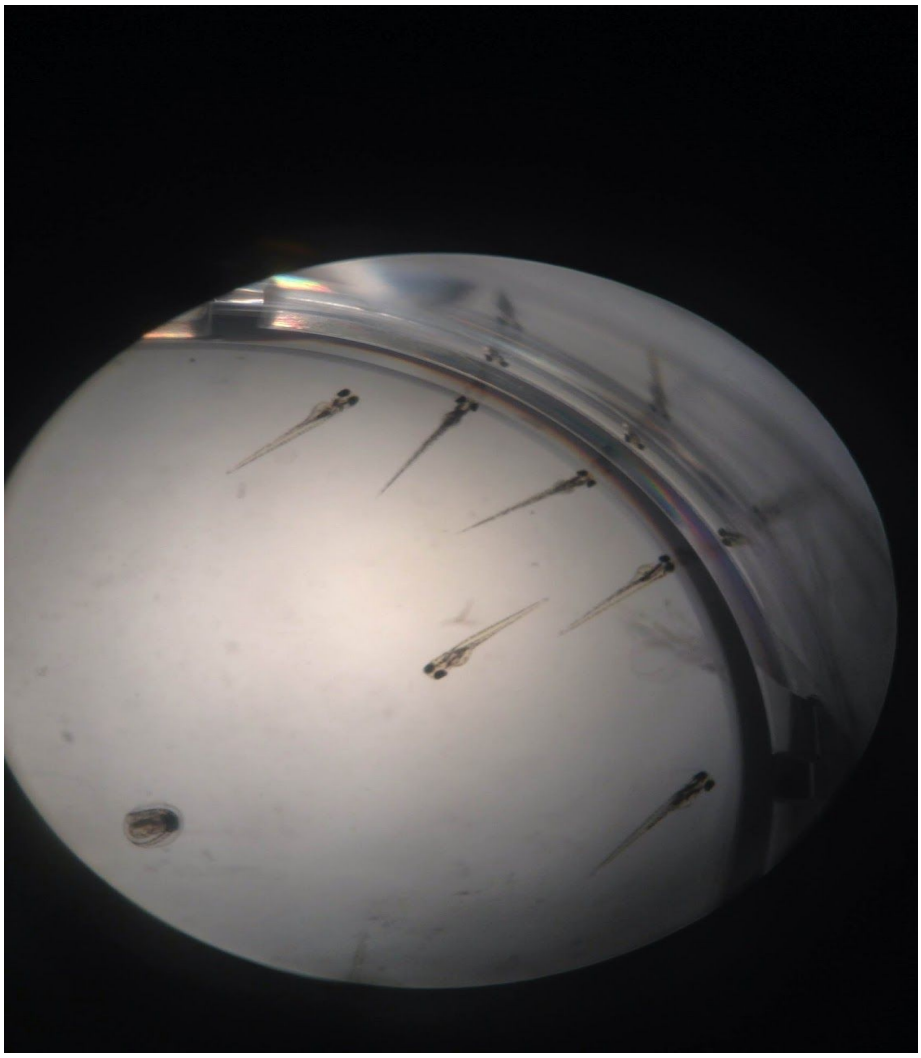
2	9	0	0	9	0	1	9	0	9
3	11	0	0	8	3	1	8	3	8
4	7	0	0	2	5	0	2	5	2



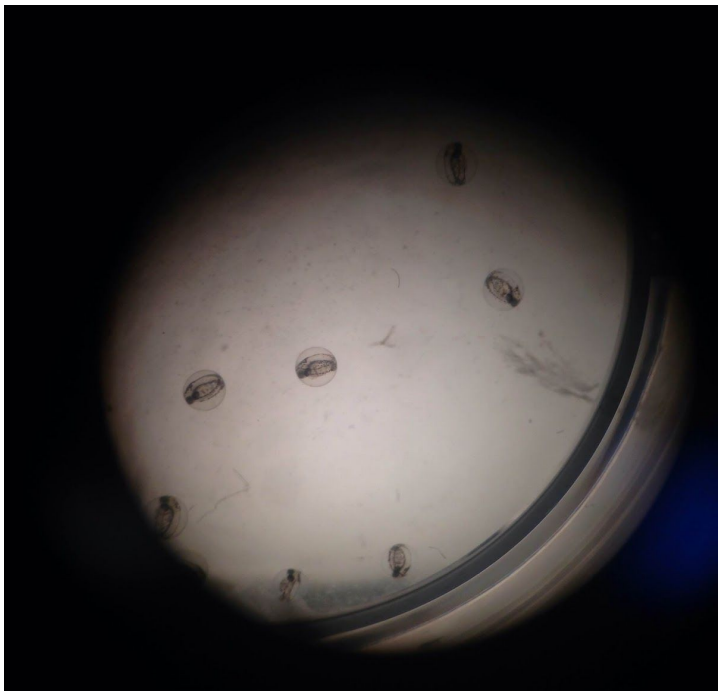
Graph of percent of total embryo that died in each well.



Graph of percent of total embryos hatched in each well. In well plates A, B, and D there was a consistent result of 100% hatch rate. C showed a lowering hatching rate compared to the other wells.



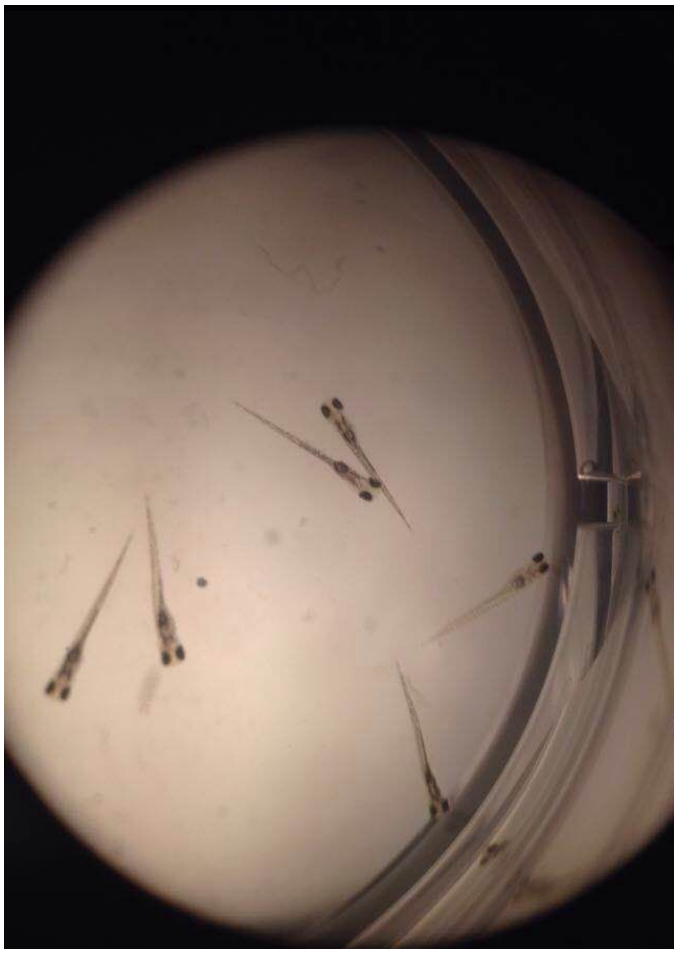
Day 4; Well Plate B, Well 3. Hatched embryo are in view, One unhatched embryo is in view.



Day 4; Well Plate C, Well 2. Unhatched embryo are in view.

Day 5 ; Well Plate B, Well . All embryo are hatched.

Day 5; Well Plate C, Well 1. 2 embryo are hatched. 3 embryo remain unhatched.



Day 5; Well Plate D, Well 3. All of the embryo in the well are hatched.

T-tests Results

Well B (28.5 degrees celsius) vs Well A (29 degrees celsius)
Day 3 Percent of Embryo Death Comparison
P value - .5162
Significant - Not statistically significant

Group	28.5	29
Mean	0.11250	0.06075
SD	0.13150	0.07231
SEM	0.06575	0.03616
N	4	4

Day 3 Percent of Embryo Hatched Comparison

P value - .1914
Significant - Not statistically significant

Group	28.5	29
Mean	0.06675	0.18750
SD	0.08179	0.14219
SEM	0.04089	0.07109
N	4	4

Day 5 Percent of Embryo Death Comparison
P value - .1751
Significant - Not statistically significant

Group	28.5	29
Mean	0.16250	0.06075
SD	0.11087	0.07231
SEM	0.05543	0.03616
N	4	4

Day 5 Percent of Embryo Hatched Comparison
P value - N/A, perfect data

Well Plate B (28.5 degrees celsius) vs Well Plate C (24 degrees celsius)
Day 3 Percent of Embryo Death Comparison
P value - .7199
Significant - Not statistically significant

Group	28.5	24
Mean	0.1125	0.0750
SD	0.1315	0.1500
SEM	0.0657	0.0750
N	4	4

Day 3 Percent of Embryo Hatched Comparison
P value - .1537
Significant - Not statistically significant

Group	28.5	24
Mean	0.06675	0.00000
SD	0.08179	0.00000
SEM	0.04089	0.00000
N	4	4

Day 5 Percent of Embryo Death Comparison
P value - .3843
Significant - Not statistically significant

Group	28.5	24
Mean	0.1625	0.0750
SD	0.1109	0.1500
SEM	0.0554	0.0750
N	4	4

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Day 5 Percent of Embryo Hatched Comparison
P value - .0018
Significant - Very statistically significant

Group	28.5	24
Mean	1.00000	0.57175
SD	0.00000	0.16105
SEM	0.00000	0.08053
N	4	4

Well Plate B (28.5 degrees celsius) vs Well Plate D (27 degrees celsius)
Day 3 Percent of Embryo Death Comparison
P value - .3050
Significant - Not statistically significant

Group	28.5	27
Mean	0.11250	0.29675
SD	0.13150	0.30115
SEM	0.06575	0.15058
N	4	4

Day 3 Percent of Embryo Hatched Comparison
P value - .3527
Significant - Not statistically significant

Group	28.5	27
Mean	0.06675	0.18400
SD	0.08179	0.21796
SEM	0.04089	0.10898
N	4	4

Day 5 Percent of Embryo Death Comparison
P value - .4348
Significant - Not statistically significant

Group	28.5	27
Mean	0.16250	0.29675
SD	0.11087	0.30115
SEM	0.05543	0.15058
N	4	4

Day 5 Percent of Embryo Hatched Comparison
P value - N/A, perfect data

VI. Discussion

As previously stated, the hypothesis suggested that if the temperature is altered from natural temperature, then the development of the embryos will be altered or halted because the temperature change may cause denaturing in the process of development.The results of the experiment were not sufficient enough to draw conclusions on the hypothesis. The differences in the percent of total embryo death for each well were not drastic enough to show any real trend, and in comparison to the control, none of the results proved to be statistically significant. The percent of total embryo hatched were identical (at 100%) for wells in the 27(D), 28.5(B), and 29(A) degree celsius plates. The embryo in well plate C (24 degrees celsius) were very delayed in hatching, beginning to hatch on day 4 (about 54-78 hours post fertilization) compared to the other wells that hatched on day 3 (around 30-54 hours post fertilization). Though the difference in hatching totals of well plate C compared to the control was statistically significant (per T-test results), conclusions can not be from that data due to many factors; The data sample size was too small, with only four wells tested in each temperature there is not enough sufficient data to make definite conclusions. In addition to the small sample size, another limiting factor of the data is that there were not multiple trials run for each temperature. Had there been multiple water baths for each temperature that showed similar results, the data could have better shown that the delayed hatching (in well plate C) was due to temperature of incubation, and not other outside factors. There were also many parts of the lab process that could of provided incorrect or errored data. The well plates were kept in plastic bins that floated on top of a water bath and there was no thermometer inside the plastic bin, this may have led to a misjudgement of incubations temperature. There also may have been agitation to the embryo when the instant ocean solution was changed. That may have cause some embryo death, affecting the overall death percentages and decreasing the reliability of the data.

Though the data from the experiment was inconclusive and the delayed hatching rate of the 24 degrees celsius was too small of a data set, there are multiple sources that support the hypothesis that altering incubation temperatures can affect fish embryo development.Thépot and Jerry (2015) ran a lab where *Lates calcarifer* (asian seabass) embryo were incubated and developed under temperatures ranging from 26-36 degrees celsius. In their findings they found that the rate of embryonic development had a positive correlation with an increase in incubation

temperature and that its thermal tolerance ranged from 28 to 34 degrees celsius (2015). The findings support the data in showing that the higher incubation temperature results in a higher embryonic development rate and respectively lower incubation temperature results in a lower embryonic development rate. To successfully obtain results similar to those in the works of Thépot and Jerry, the experiment would need to be run at a larger scale. Multiple trials would needed to be tested in a larger range of temperatures (to also possibly test the thermal limits of the embryo). All embryo should be kept in a standard incubator (not a water bath) and multiple plates should be tested at each temperature. Additional experiments could be run to discover fish embryo's thermal range and survival rate in temperature changes. Data could be used to set standard fish temperatures.

VII. References

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