The Effect of UV Light Exposure on Embryological Development of *Danio Rerio*

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Contents

Background Information

During the development of zebrafish (*Danio rerio*) embryo, there are 3 stages of growth: epiboly, involution, and convergence(Strahle and Jesuthasan, 1993). Previous experiments showed that irradiation of zebrafish zygotes with ultraviolet light potentially impairs epiboly resulting in damage or death in zebrafish(Strahle and Jesuthasan, 1993). In a study by a researcher of Developmental Biology at Franklin and Marshall College, it was discovered that embryo treated with UV light progressed slower than those that were untreated. All of the control embryo (0 seconds of exposure) yielded normal epiboly movement. The importance of the mutation that occurred within each embryo was actually proportional with the level of UV exposure, proving that increasing UV light exposure does indeed yield to mutations in the development phase of zebrafish embryo(Alcaraz, 2001). However, rather than focusing on the intensity of UV exposure, this experiment focused on increasing the time increments of exposure to be able to identify the relationship between increased time increments of UV exposure and death/mutations within groups of zebrafish embryo. The hypothesis was as follows; if the amount of time an embryo was exposed to UV light increases, then the mutations and death of the embryo in the greater dish will increase because the UV light will have more of an opportunity to damage the stage of epiboly in the embryo that are exposed for longer periods of time. In other words, increasing the amount of time that an embryo is exposed to UV light would lead to an increase in the amount of deaths and mutations in said zebrafish embryo because of an impairment in the epiboly stage of development, thusly causing a halt in regular development.

The reason that researchers began to use fish (specifically zebrafish) in research in the 1960s is because the zebrafish embryo is fertilized and developed *outside* of the zebrafish body, thusly allowing for clear observation and more accurate depiction of the processes that occur within the egg sack. In addition to clarifying observations, zebrafish are much more cost effective than mice, as they are cost much less to maintain(“Why use the zebrafish in research?” 2014) Also, though zebrafish seem to be complete opposites to humans, a comparison to the human reference genome shows that approximately 70% of human genes have at least one obvious zebrafish orthologue (genes evolved from a common ancestral gene), proving them to be more similar to humans than previously thought (Kerstin Howe, 2013). Also, researchers studying the growth and development of these fish have generated zebrafish versions of a wide variety of human diseases(Lieschke and Currie, 2007).

Initially, the sole variable being measured and evaluated was the interval length that each of the 12 different dishes were to be exposed to UV light. However, upon collecting data, another variable worth noting and evaluating became apparent. This variable was the effect of multiple days of exposure on the embryo. Due to the

emergence of a second variable, both relationships to embryo death will be discussed and evaluated in the preceding pages.

The significance of UV light exposure on zebrafish embryo is quite recognizable. UV light has the potential to affect epiboly in zebrafish embryo(Strahle and Jesuthasan, 1993). The UV light (assumedly) targets microtubules. Due to how epiboly is initiated and driven by a pulling force dependent on microtubules in the yolk cytoplasmic layer, (Strähle & Jesuthasan, 1993), disrupting the microtubule creation with UV light would impair the growth of the embryo through epiboly and into involution.

In order to test the previously stated hypothesis, 12 petri dishes labeled 2, 4, 6… 35 seconds were filled with 2 dropperfuls of instant ocean and 5 zebrafish embryo that were in relatively the same stage of development. For 3 days, they were exposed to UV light once per day for their respective time periods within a controlled UV crosslinker box. The preset amount that each one of the dishes were exposed to was 120,000 microjoules per cm^2. After each day, the amount of deaths and hatchings were recorded, and multiple photos were taken of the embryo within each dish. The reason for using so many different intervals was to be as specific as possible when relating the results that increasing uv light had on the embryo. It was important to see not only the drastic changes that lengthening the interval had, but also the gradual impact that increasing the time by small increments had.

After collecting 3 days worth of data and observations regarding to the health of the fish, all of the fish after 10 seconds had died, leaving only the fish in the 00-08 second dishes living, as well as one in the 10 second dish. This not only allowed for a very positive correlation between increasing time increments exposed to UV light and zebrafish embryo death, but it also allowed a conclusion to be made regarding the critical point of the embryo(the point at which the embryo was unable to survive any longer), which would be approximately 10-11 seconds, due to the fact that after that point of exposure, all of the embryo died. In addition to that conclusion regarding the increasing time intervals, a conclusion can also be made in regards to the effect that multiple days of exposure had on the embryo. As the amount of days increased, the deaths of the embryo also increased, leading to a positive correlation between increasing days of exposure and fish death.

Abstract

The purpose of this experiment was to discover the effects of different periods of exposure to UV light on Zebrafish embryo. In order to find the results of said exposure, 11 different petri dishes were prepared with 5 embryo in each dish, each labeled with either 2, 4, 6, 8, 10, 12, 14, 16, 18, 25, or 35 seconds. For three days, the embryo were exposed to UV light using a UV Crosslinker once each day to their respective periods of time. Before exposing the embryo, one to two photos of the embryos were taken through a microscope in order to be able to accurately make judgements on the progression. After 3 days, and three periods of exposure for each dish, the qualitative and quantitative data proved that 10 seconds of exposure and under yielded living fish, though 4 seconds and up were fairly mutated. 10 seconds and up yielded all but one dead fish, proving that 10 seconds was the maximum amount of UV exposure that the embryo could sustain before death. There was one error. On the final day, rather than having 4 embryo remaining in the 10 second dish, there was only one left. This could have been a cause of accidental bumping and spilling, but whatever the case, there was only one to record. In addition to the fact that there was only one, the heartbeat was impossible to make out, so it was very difficult to determine whether or not it was living. Due to this, all of the data/statistics including the 10 second embryo will be done twice, once for if the fish had been alive, and once for had the fish been dead.

Upon comparing the means of each of the days to each other, the comparison of day 0 to day 3 (when looking at death of zebrafish embryo) proved to be very if not *extremely* statistically significant, proving the effect that prolonged UV light exposure has on zebrafish embryo OVER MULTIPLE DAYS is indeed detrimental. In other words, the more often the zebrafish are exposed, the more deaths there will be.

However, the main purpose of this experiment was to discover the effects that *different time periods* of exposure had on zebrafish embryo, so in addition to the previously stated data collection and comparison over the period of 3 days (comparing the mean of each day), the amount of fish dead and alive within the 2, 4, 6, 8, 10, 12, 14, 16, 18, 25, And 35 second intervals in comparison to the control (0 second group) on one specific day (day 2) were compared in order to discover the relationship of UV light exposure and death of zebrafish while not looking at the multiple day factor/confounding variable. After observational and statistical data was collected, the statistical significance was undeniable.

Materials and Methods

Materials

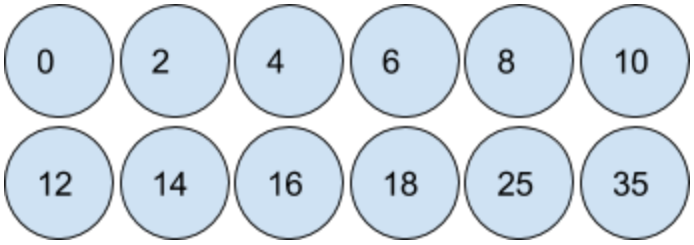
1. 12 translucent petri dishes with lids (approximately 2 inches in diameter)
2. 60 living zebrafish embryo
3. Approximately 500 ml Instant ocean solution
4. 2 beakers (one for waste, one for instant ocean solution)
5. Approximately 8 plastic pipets (2 per day + 2 extra)
6. Paper towel (5 pieces, white preferably)
7. One incubator
8. One FB UVXL-1000 UV Crosslinker (Preset to be at 120,000 microjoules per cm^2)
9. 3 electrical outlets (one for microscope, one for UV crosslinker, one for incubator)
10. 1 Microscope

Safety

- Wear hair up and out of eyes in order to keep it from falling into any of the dishes
- Wash hands before and after experimentation
- Do not stare at the UV machine when it is at work, though there is a protective layer of glass shielding the experimenter from the damaging rays, it would not be advised to spend too much time too close.
- Wear shirts that do not have intrusive sleeves

Method

1. Collect materials
2. Carefully use the dropper to pick up 5 embryos and place them into a petri dish that has approximately 2 pipets full of instant ocean coating the bottom. Make sure that each of the 5 chosen embryo are alive and well, not far from each other in regards to the developmental stages that they are all in. Do this for 12 different petri dishes, labeling them as follows:



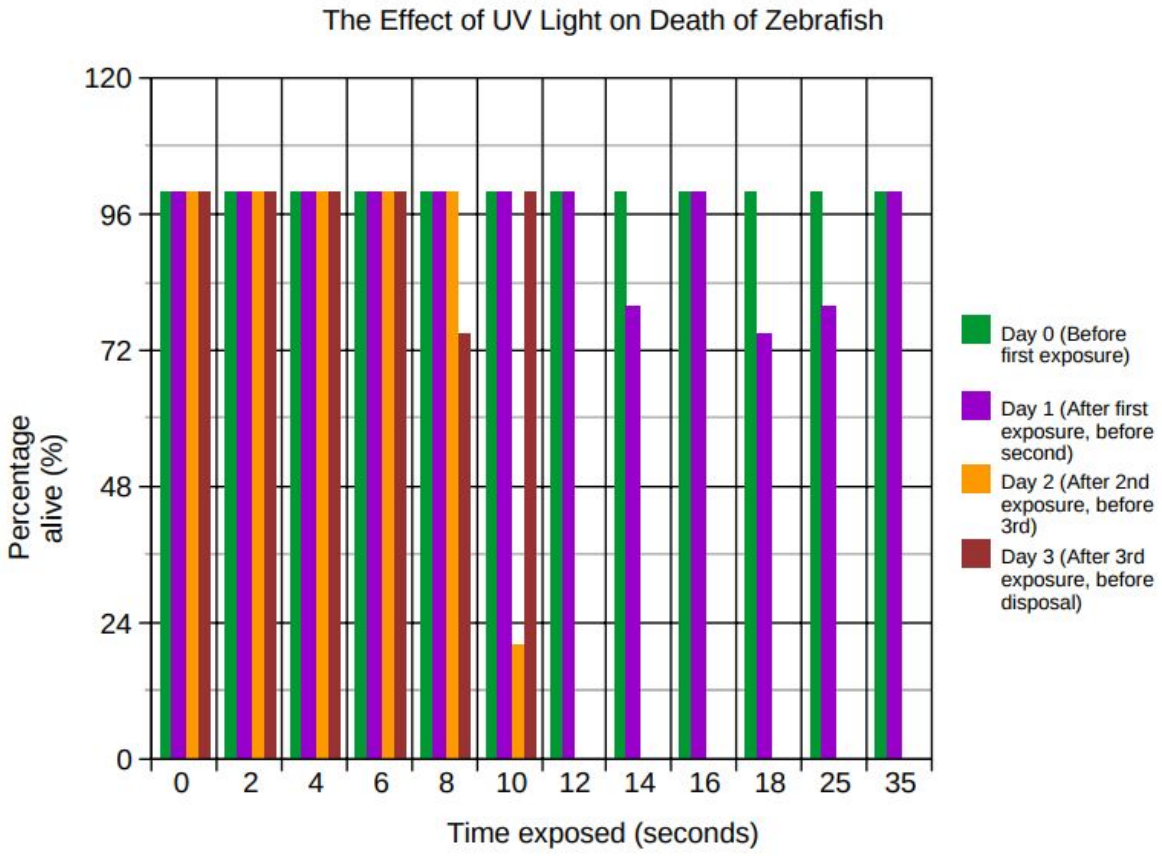
3. Move the dishes over to the UV CrossLinker, and place the second (2 seconds) dish into the center of the compartment, atop a piece of white paper towel. Make sure that it closes securely.
4. After inserting the dish, activate the power switch.
5. Press “time” and set the CrossLinker to the labeled period of time
6. Click start (do not worry about setting the intensity, the machine is already preset at 120,000 microjoules per cm^2)

7. After the timer has gone off, remove the petri dish, place the cover on top, and put it into the incubator.
8. Repeat steps 3-9 with each petri dish
9. Repeat step 9 once per day for 3 days, taking photos through the microscope BEFORE each daily treatment, and recording the observed results/effects of the UV light.

For this experiment, the independent variable is the amount of time that each dish will be exposed to the UV light. The dependent variable is the effect that said exposures has on said embryos. The control group is going to consist of 5 embryos that will not be exposed to UV light at all. The experimental group will consist of all of the other embryos that are in fact going to be exposed to the UV radiation. The constants are room temperature, environment size and shape (petri dish), type of embryo, and relative age of embryo. The sample size consists of 60 embryos.

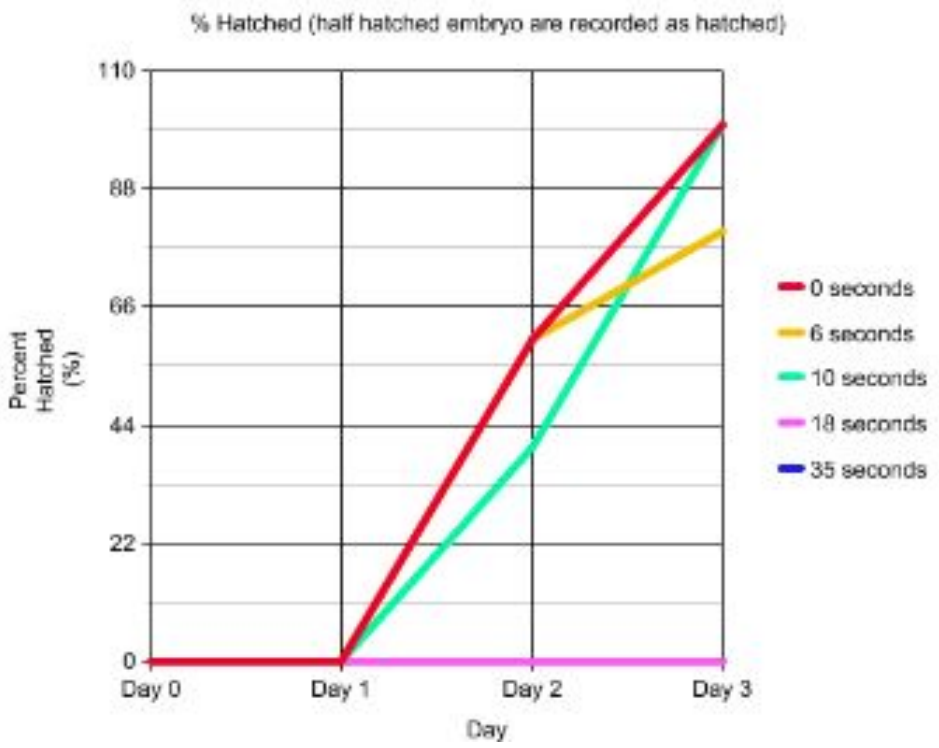
Results (Graph form)

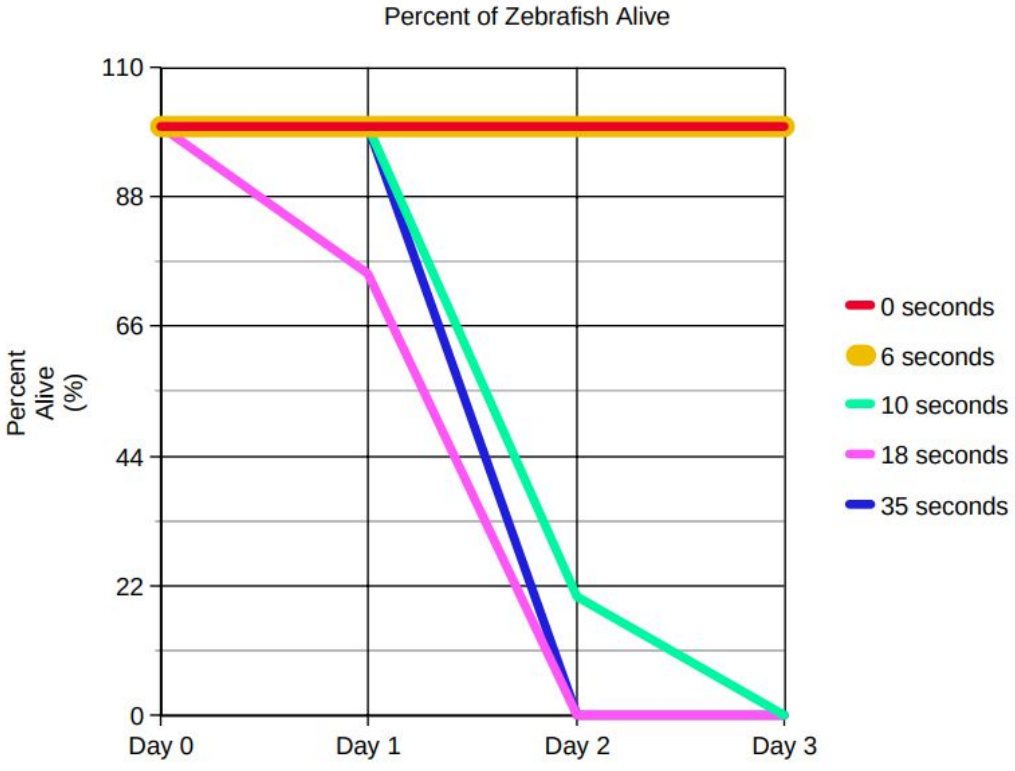
This graph was made under the conditions that the single embryo in dish 10 was alive on day 3



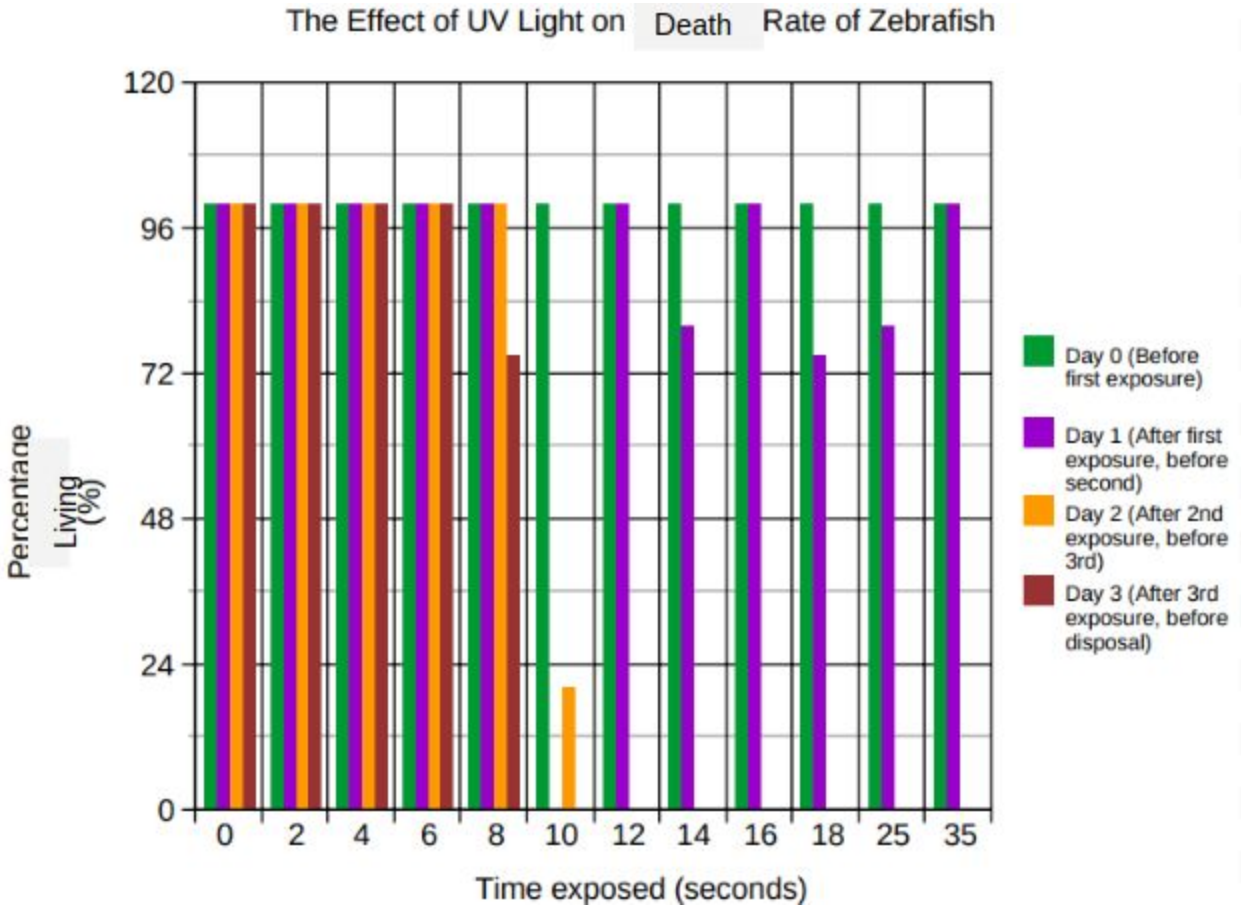
NOTE: On the third and final day of exposure, there was only one fish present within the 10 second dish. This can be attributed to accidental splashing. So, when 100% appears, it is important to note that 100% stands for 1/1.

Trend: As the 3 days progressed, the dishes that were exposed to a longer period of UV light had more deaths than those being exposed for a shorter period of time.


% Hatched (half hatched embryo are recorded as hatched)
110
88
66
44
22
0
Percent Hatched (%)
Day 0
Day 1
Day 2
Day 3
Day
0 seconds
6 seconds
10 seconds
18 seconds
35 seconds

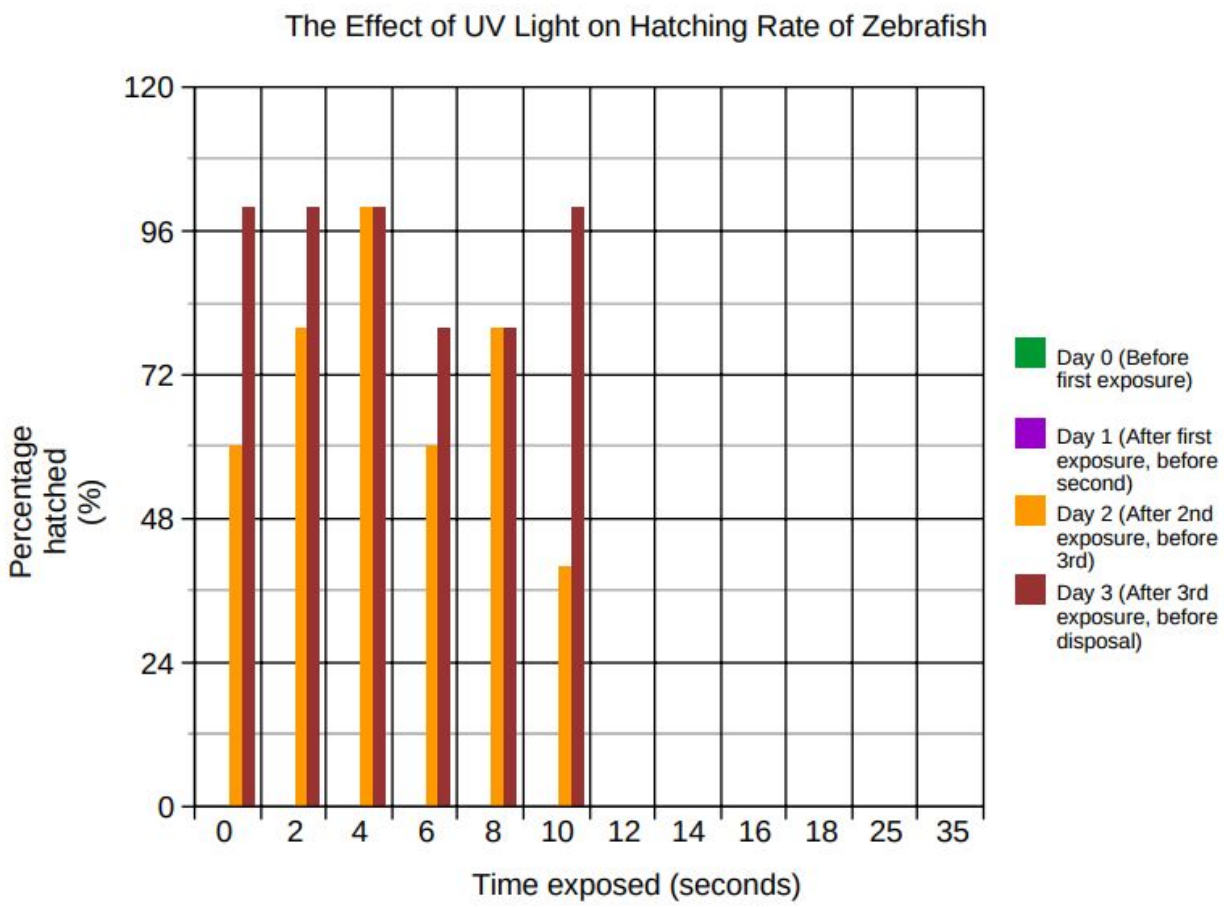

Percent of Zebrafish Alive
110
88
66
44
22
0
Percent Alive (%)
Day 0
Day 1
Day 2
Day 3
0 seconds
6 seconds
10 seconds
18 seconds
35 seconds

This graph was made under the conditions that the single embryo in dish 10 was dead on day 3



NOTE: On the third and final day of exposure, there was only one fish present within the 10 second dish. This can be attributed to accidental splashing. So, when 100% appears, it is important to note that 100% stands for 1/1.

Trend: As the 3 days progressed, the dishes that were exposed to a longer period of UV light had more deaths than those being exposed for a shorter period of time.



NOTE: On the third and final day of exposure, there was only one fish present within the 10 second dish. This can be attributed to accidental splashing. So, when 100% appears for the 10 second dish on the third day, it is important to note that 100% stands for 1/1. Also, if embryo were “half-hatched”, they are represented on the graph as “hatched”.

Trend: As the 3 days progressed, the amount of fish in the containers being exposed for 0 seconds through 10 seconds had the amount of hatched fish increase. However, the embryo in the 12-35 second exposure groups did not hatch at all.

% Hatched (embryo that were half hatched are recorded as hatched)

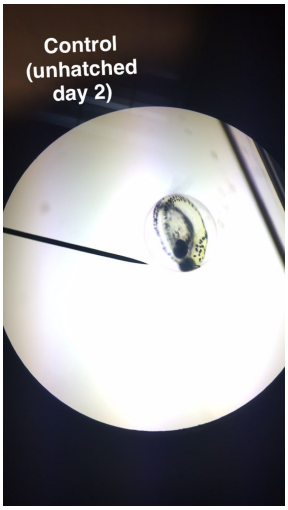
	Day 0 (before first exposure)	Day 1 (Before 2nd exposure, after 1st)	Day 2 (Before 3rd exposure, after 2nd)	Day 3 (after 3rd exposure, before disposal)
0 seconds	0/5 = **0%**	0/5 = **0%**	3/5= **60%**	5/5 = **100%**
2 seconds	0/5 = **0%**	0/5 = **0%**	4/5= **80%**	5/5 = **100%**
4 seconds	0/5 = **0%**	0/5 = **0%**	5/5= **100%**	5/5 = **100%**
6 seconds	0/5 = **0%**	0/5 = **0%**	3/5=**60%**	4/5 = **80%**
8 seconds	0/5 = **0%**	0/5 = **0%**	4/5=**80%**	4/5 = **80%**
10 seconds	0/5 = **0%**	0/5 = **0%**	2/5=**40%**	1/1=? (error, others accidentally misplaced)
12 seconds	0/5 = **0%**	0/5 = **0%**	All dead	All dead
14 seconds	0/5 = **0%**	0/5 = **0%**	All dead	All dead
16 seconds	0/3 = **0%**	0/3 = **0%**	All dead	All dead
18 seconds	0/4 = **0%**	0/4 = **0%**	All dead	All dead
25 seconds	0/5 = **0%**	0/5 = **0%**	All dead	All dead
35 seconds	0/5 = **0%**	0/5 = **0%**	All dead	All dead

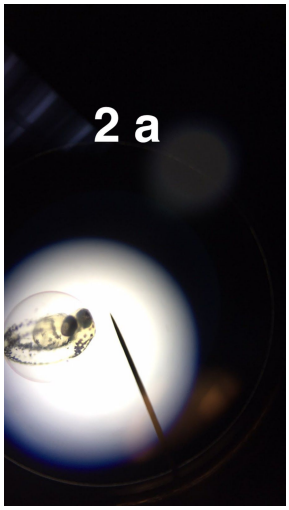
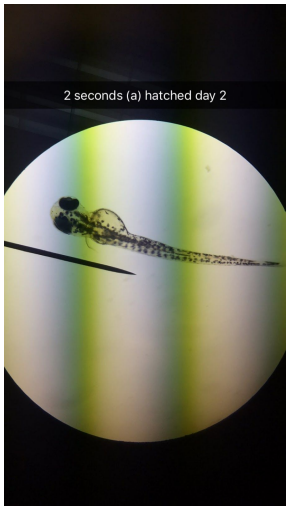
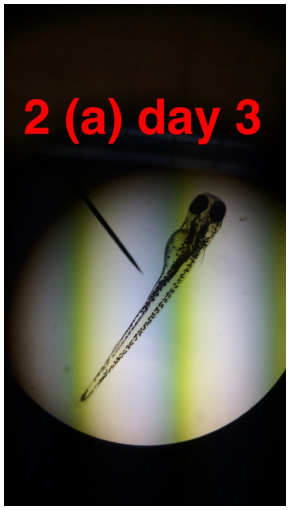
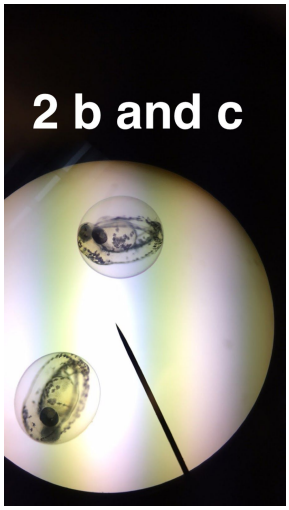
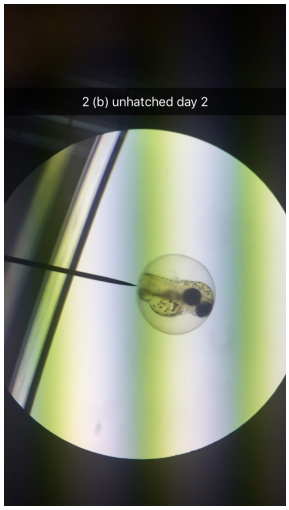
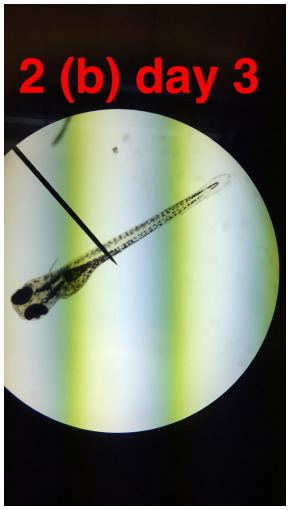
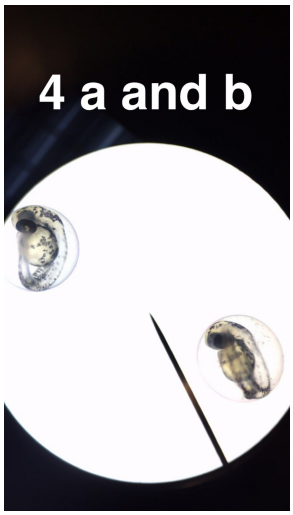
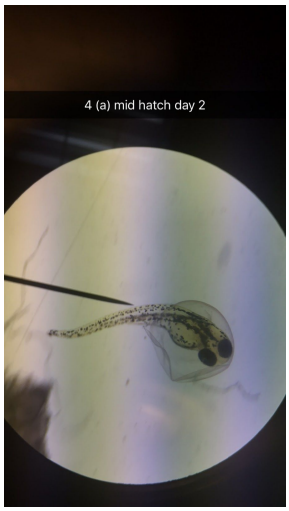
% Alive

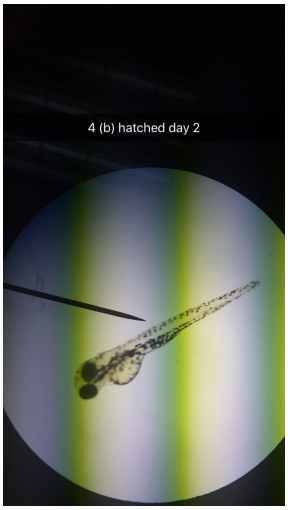
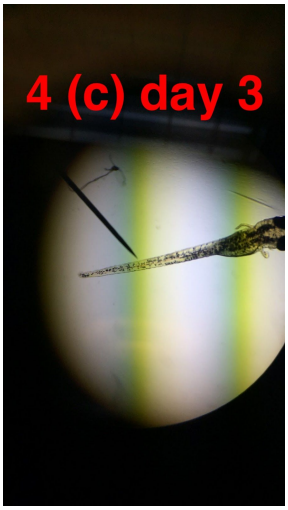
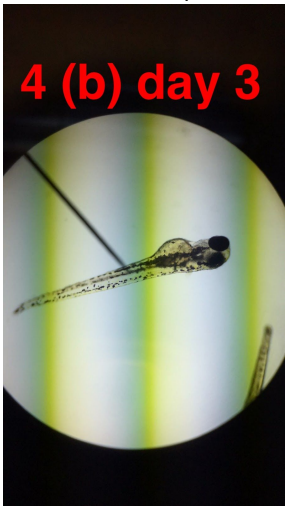
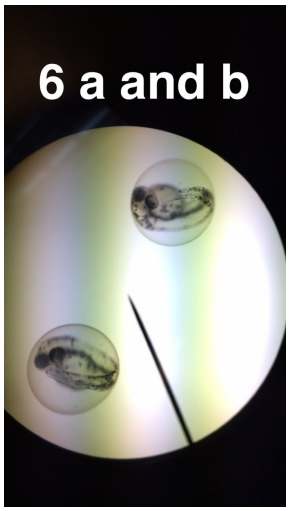
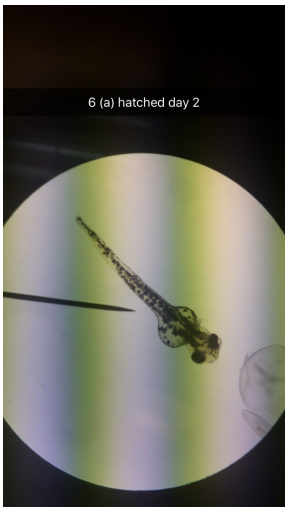
	Day 0 (Before 1st exposure)	Day 1 (Before 2nd exposure, after 1st)	Day 2 (Before 3rd exposure, after 2nd)	Day 3 (After 3rd, before disposal)
0 seconds	5/5 = **100%**	5/5 = **100%**	5/5 = **100%**	5/5 = **100%**
2 seconds	5/5 = **100%**	5/5 = **100%**	5/5 = **100%**	5/5 = **100%**
4 seconds	5/5 = **100%**	5/5 = **100%**	5/5 = **100%**	5/5 = **100%**
6 seconds	5/5 = **100%**	5/5 = **100%**	5/5 = **100%**	5/5 = **100%**
8 seconds	5/5 = **100%**	5/5 = **100%** (one forced out of sack - removed)	4/4 = **100%**	3/4 = **75%** (Hearts barely beating)
10 seconds	5/5 = **100%**	5/5 = **100%**	1/5 = **20%**	1/1 (Error) Unsure.
12 seconds	5/5 = **100%**	5/5 = **100%**	0/5 = **0%**	0/5 = **0%**
14 seconds	5/5 = **100%**	4/5 = **80%**	0/5 = **0%**	0/5 = **0%**
16 seconds	3/3 = **100%**	3/3 = **100%**	0/5 = **0%**	0/5 = **0%**
18 seconds	4/4 = **100%**	3/4 **=75%**	0/5 = **0%**	0/5 = **0%**
25 seconds	5/5 = **100%**	4/5 **=80%**	0/5 = **0%**	0/5 = **0%**
35 seconds	5/5 = **100%**	5/5 = **100%**	0/5 = **0%**	0/5 = **0%**

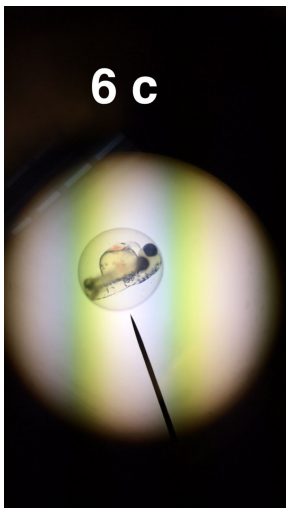
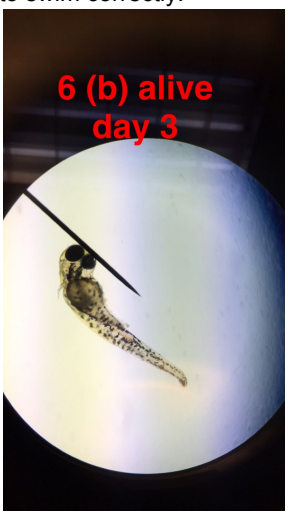
Images as experiment progressed

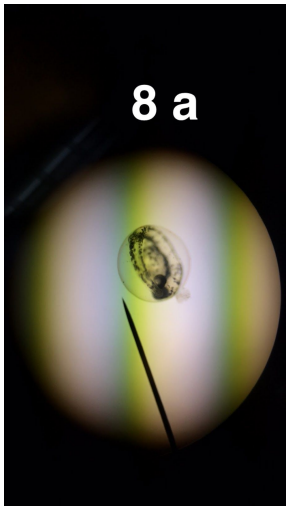
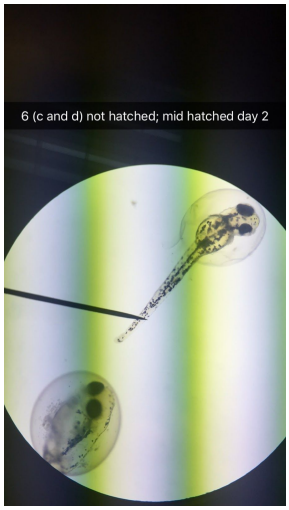
NOTE: Letters such as A and B were used solely to be able to ensure repeats were not incorporated into the table. In other words, A of day 2 is not necessarily the same fish as A of day 3.

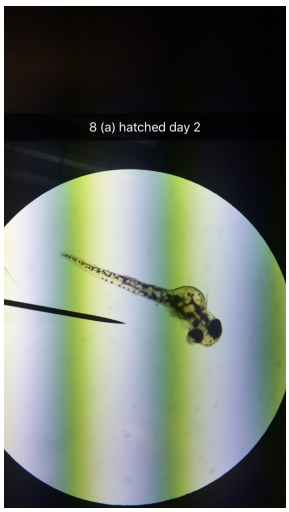
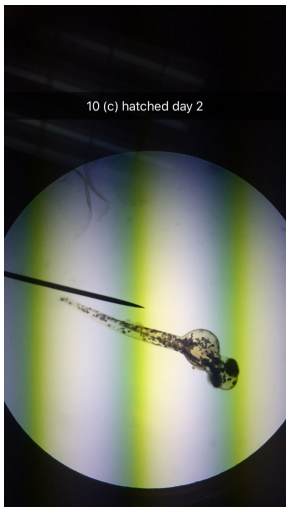
	Day 1 (Day after first exposure)	Day 2 (Day after second exposure)	Day 3 (Day after third exposure)
Control (0 seconds)		 	 

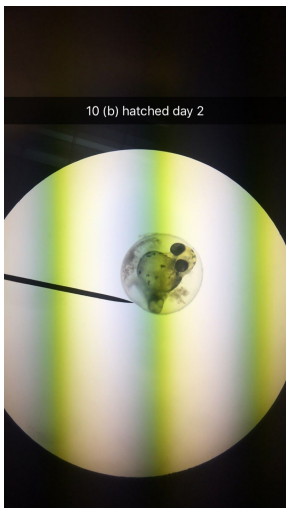
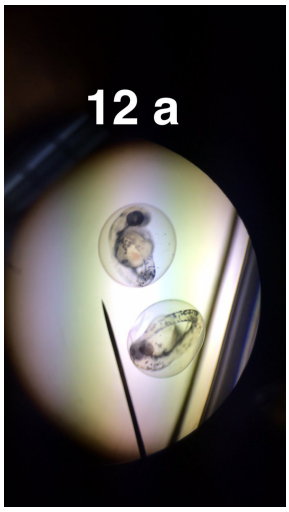
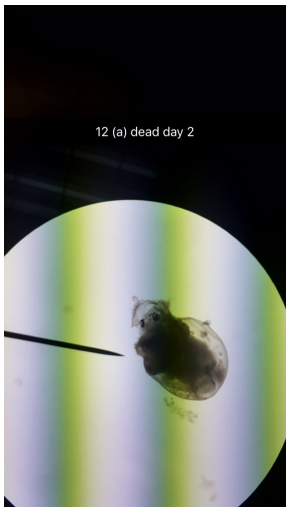
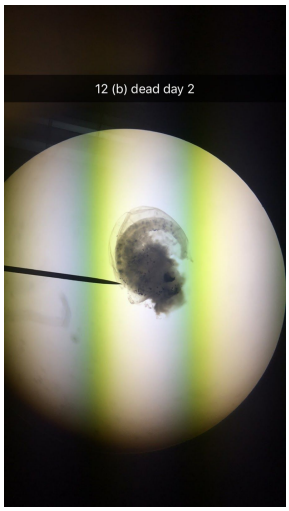
2 seconds			
			
4 seconds	 Unhatched, functioning normally.	 Clear curvature of the spine, mid-way hatched	

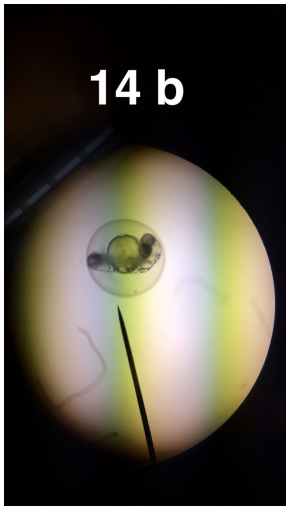
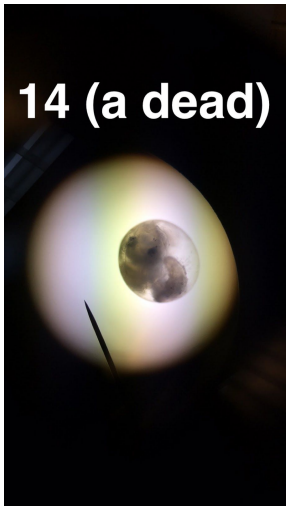
			 Deformation of fins, slight curvature of the spine.  Slight curvature of tail and spinal formation.
6 seconds			

			Severe curvature of the spinal formation, inability to swim correctly.  Severe curvature of the spine, inability to swim correctly  Severe curvature of the tail

			 Still unhatched, somewhat cloudy inside egg sack.
8 seconds		 	 Death post-hatching. Mutation of the tail as well as clouding around the body.

			 Slight curvature of the spine, somewhat discolored, eyes deformed.
10 seconds		 	

			
12 seconds		 	(all dead and disposed)

14 seconds	 		(all dead and disposed)
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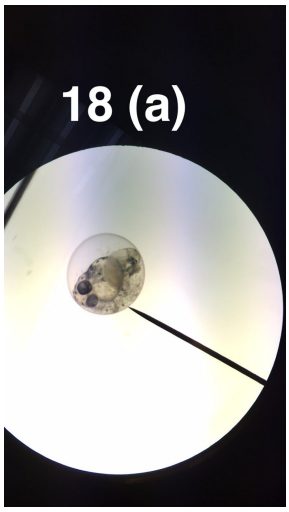
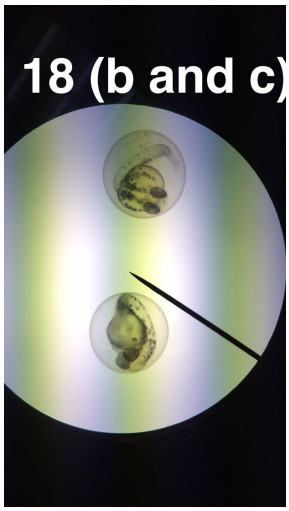
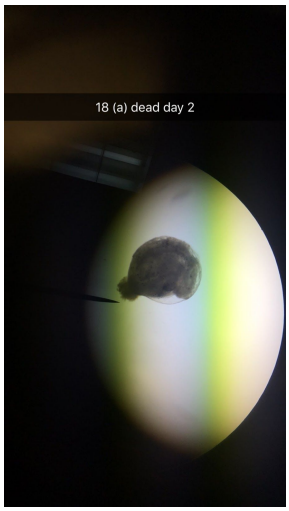
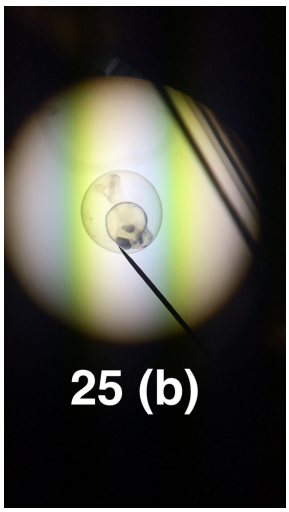
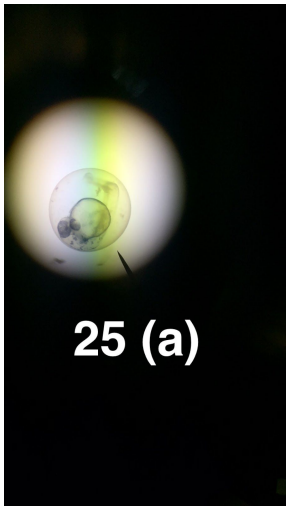
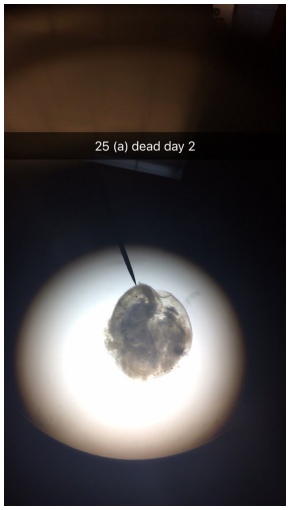
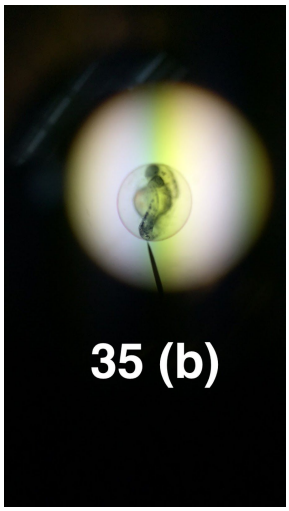
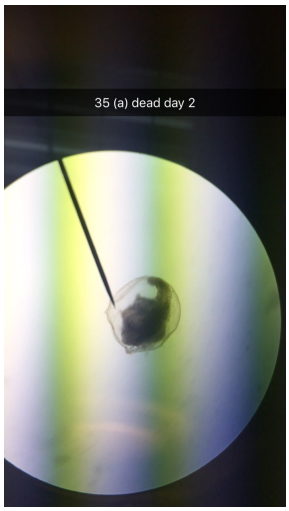
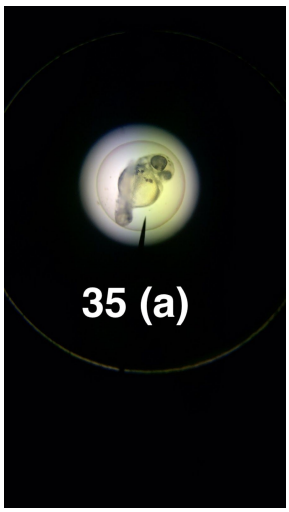
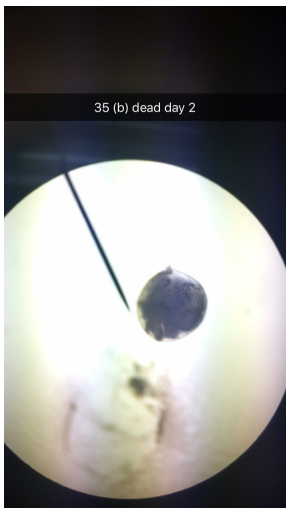
18 seconds	 		(all dead and disposed)

25 seconds	 	 	(all dead and disposed)
35 seconds			(all dead and disposed)

Statistical significance of Multiple days (First Variable)

NOTE: The following data significance is involving the effect that multiple days of exposure had on the zebrafish embryo. Note that this particular data is not assessing the effect that increasing each time increment has on the zebrafish embryo, it was collected by comparing the mean of dead or alive fish of each DAY, not of each time increment. In other words, the following data is revealing the statistically significant correlation between increasing time increments of UV light exposure (the 2nd variable) and zebrafish death/hatching.

Comparing Day 0 to Day 3 (after 3 rounds of exposure) - Death v. Life (if 10 was alive)

Group 1 = Day 0
Group 2 = Day 3

P value and statistical significance:
The two-tailed P value equals 0.0017
By conventional criteria, this difference is considered to be very statistically significant.

Confidence interval:
The mean of Group One minus Group Two equals 52.08
95% confidence interval of this difference: From 21.84 to 82.33

Intermediate values used in calculations:
t = 3.5714
df = 22
standard error of difference = 14.583

Group	Group One	Group Two
Mean	100.00	47.92
SD	0.00	50.52
SEM	0.00	14.58
N	12	12

Comparing Day 0 to Day 3 (after 3 rounds of exposure) - Death v. Life (if 10 was dead)

Group 1 = Day 0
Group 2 = Day 3

P value and statistical significance:
The two-tailed P value equals 0.0003
By conventional criteria, this difference is considered to be extremely statistically significant.

Confidence interval:
The mean of Group One minus Group Two equals 60.42
95% confidence interval of this difference: From 30.85 to 89.98

Intermediate values used in calculations:
t = 4.2383
df = 22
standard error of difference = 14.255

Group	Group One	Group Two
Mean	100.00	39.58
SD	0.00	49.38
SEM	0.00	14.25
N	12	12

Statistical significance of increasing intervals (Second variable)

NOTE: The following data significance involves Comparing each time interval to the control (Day 2: Before 3rd exposure, but after 2nd) In reference to total deaths. Viewing how increasing the time interval affects the amount of deaths present within the initially living embryo. In other words, the following data is revealing the statistically significant correlation between increasing time increments of UV light exposure (the 2nd variable) and zebrafish death.

*NOTE

In order to discover the statistical significance of the differences in time intervals in comparison to the mean, the data was entered into the T test calculator as such.
Say the 10 second interval was to be compared to the 0 second control on day 2. Only 1/5 fish remained alive within the 10 second dish, while 5/5 remained alive in the 0 second control dish.

The data would be entered as follows:

0 second interval (control)	**10 second interval**
1	1
1	0
1	0
1	0
1	0

Control vs. 2 Seconds

Perfect data, both remained with 5/5 alive.

Control vs. 4 Seconds

Perfect data, both remained with 5/5 alive.

Control vs. 6 Seconds

Perfect data, both remained with 5/5 alive.

Control vs. 8 Seconds

Perfect data, both remained with 5/5 alive.

Control vs. 10 Seconds

Unpaired *t* test results

P value and statistical significance:
The two-tailed P value equals 0.0039
By conventional criteria, this difference is considered to be very statistically significant.

Confidence interval:
The mean of Control minus 10 Seconds equals 0.80
95% confidence interval of this difference: From 0.34 to 1.26

Intermediate values used in calculations:
t = 4.0000
df = 8
standard error of difference = 0.200

Group	Control	10 Seconds
Mean	1.00	0.20
SD	0.00	0.45
SEM	0.00	0.20
N	5	5

Control vs. 12 Seconds

Perfect data, Control remained with 5/5 alive, 12 seconds remained with 0/5 alive.

Control vs. 14 Seconds

Perfect data, Control remained with 5/5 alive, 14 seconds remained with 0/5 alive.

Control vs. 16 Seconds

Perfect data, Control remained with 3/3 alive, 16 seconds remained with 0/3 alive.

Control vs. 18 Seconds

Perfect data, Control remained with 4/4 alive, 18 seconds remained with 0/4 alive.

Control vs. 25 Seconds

Perfect data, Control remained with 5/5 alive, 25 seconds remained with 0/5 alive.

Control vs. 35 Seconds

Perfect data, Control remained with 5/5 alive, 35 seconds remained with 0/5 alive.

NOTE: The following data significance involves comparing each time interval to the control (Day 2: Before 3rd exposure, but after 2nd) In reference to total hatched. Viewing how the time interval affects the amount of embryo that hatched.

*NOTE

In order to discover the statistical significance of the differences in time intervals in comparison to the mean, the data was entered into the T test calculator as such.
Say the 10 second interval was to be compared to the 0 second control on day 2. In the 10 second dish, ⅖ eggs were hatched, while in the control, ⅗ were hatched.
Also, embryos that were (for lack of a better word) “Half-hatched”, will be considered as hatched.
This data is only considering whether or not the embryos hatched, it does not include life v. death.

The data would be entered as follows:

0 second interval (control)	**10 second interval**
1	1
1	1
1	0
0	0
0	0

Control vs. 2 Seconds

P value and statistical significance:
The two-tailed P value equals 0.5447
By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:
The mean of Control minus 2 Seconds equals -0.20
95% confidence interval of this difference: From -0.93 to 0.53

Intermediate values used in calculations:
t = 0.6325
df = 8
standard error of difference = 0.316

Group	Control	2 Seconds
Mean	0.60	0.80
SD	0.55	0.45
SEM	0.24	0.20
N	5	5

Control vs. 4 Seconds

P value and statistical significance:
The two-tailed P value equals 0.1411
By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:
The mean of Control minus 4 Seconds equals -0.40
95% confidence interval of this difference: From -0.96 to 0.16

Intermediate values used in calculations:
t = 1.6330
df = 8
standard error of difference = 0.245

Group	Control	4 Seconds
Mean	0.60	1.00
SD	0.55	0.00
SEM	0.24	0.00
N	5	5

Control vs. 6 Seconds

P value and statistical significance:
The two-tailed P value equals 1.0000
By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:
The mean of Control minus 6 Seconds equals 0.00
95% confidence interval of this difference: From -0.80 to 0.80

Intermediate values used in calculations:
t = 0.0000
df = 8
standard error of difference = 0.346

Group	Control	6 Seconds
Mean	0.60	0.60
SD	0.55	0.55
SEM	0.24	0.24
N	5	5

Control vs. 8 Seconds

P value and statistical significance:
The two-tailed P value equals 0.5447
By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:
The mean of Control minus 8 Seconds equals -0.20
95% confidence interval of this difference: From -0.93 to 0.53

Intermediate values used in calculations:
t = 0.6325
df = 8
standard error of difference = 0.316

Group	Control	8 Seconds
Mean	0.60	0.80
SD	0.55	0.45
SEM	0.24	0.20
N	5	5

Control vs. 10 Seconds

P value and statistical significance:
The two-tailed P value equals 0.5796
By conventional criteria, this difference is considered to be not statistically significan

Confidence interval:
The mean of Control minus 10 Seconds equals 0.20
95% confidence interval of this difference: From -0.60 to 1.00

Intermediate values used in calculations:
t = 0.5774
df = 8
standard error of difference = 0.346

Group	Control	10 Seconds
Mean	0.60	0.40
SD	0.55	0.55
SEM	0.24	0.24
N	5	5

Control vs. 12 Seconds

P value and statistical significance:
The two-tailed P value equals 0.0400
By conventional criteria, this difference is considered to be statistically significant.

Confidence interval:
The mean of Control minus 12 Seconds equals 0.60
95% confidence interval of this difference: From 0.04 to 1.16

Intermediate values used in calculations:
t = 2.4495
df = 8
standard error of difference = 0.245

Group	Control	12 Seconds
Mean	0.60	0.00
SD	0.55	0.00
SEM	0.24	0.00
N	5	5

Control vs. 14 Seconds

P value and statistical significance:
The two-tailed P value equals 0.0400
By conventional criteria, this difference is considered to be statistically significant.

Confidence interval:
The mean of Control minus 14 Seconds equals 0.60
95% confidence interval of this difference: From 0.04 to 1.16

Intermediate values used in calculations:
t = 2.4495
df = 8
standard error of difference = 0.245

Control vs. 16 Seconds

P value and statistical significance:
The two-tailed P value equals 0.1158
By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:
The mean of Control minus 16 Seconds equals 0.60
95% confidence interval of this difference: From -0.20 to 1.40

Intermediate values used in calculations:
t = 1.8371
df = 6
standard error of difference = 0.327

Group	Control	16 Seconds
Mean	0.60	0.00
SD	0.55	0.00
SEM	0.24	0.00
N	5	3

Control vs. 18 Seconds

P value and statistical significance:
The two-tailed P value equals 0.0676
By conventional criteria, this difference is considered to be not quite statistically significant.

Confidence interval:
The mean of Control minus 18 Seconds equals 0.60
95% confidence interval of this difference: From -0.06 to 1.26

Intermediate values used in calculations:
t = 2.1602
df = 7
standard error of difference = 0.278

Group	Control	18 Seconds
Mean	0.60	0.00
SD	0.55	0.00
SEM	0.24	0.00
N	5	4

Control vs. 25 Seconds

P value and statistical significance:
The two-tailed P value equals 0.2415
By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:
The mean of Control minus 25 Seconds equals 0.40
95% confidence interval of this difference: From -0.33 to 1.13

Intermediate values used in calculations:
t = 1.2649
df = 8
standard error of difference = 0.316

Group	Control	25 Seconds
Mean	0.60	0.20
SD	0.55	0.45
SEM	0.24	0.20
N	5	5

Control vs. 35 Seconds

P value and statistical significance:
The two-tailed P value equals 0.0400
By conventional criteria, this difference is considered to be statistically significant.

Confidence interval:
The mean of Control minus 35 Seconds equals 0.60
95% confidence interval of this difference: From 0.04 to 1.16

Intermediate values used in calculations:
t = 2.4495
df = 8
standard error of difference = 0.245

Group	Control	35 Seconds
Mean	0.60	0.00
SD	0.55	0.00
SEM	0.24	0.00
N	5	5

Discussion

Two variables were being tested during this experiment; (1) the effect of multiple days of UV exposure on Zebrafish embryo, and (2) the effect of increasing periods of time exposed to UV light on zebrafish embryo. The (statistically significant) trend of the first variable mentioned was that as the amount of days increased, more embryo died. This proved the existence of a strong positive correlation between number of days exposed and death in zebrafish. The statistically significant trend of the second variable mentioned was that, as the period of time increased from 0 seconds to 35 seconds, the embryo died. However, this death was not gradual. For the time period 2 - 8 seconds, there was no difference in the death rate in comparison to the control (0 Seconds). Due to this, no correlation could be drawn between extended UV light exposure and death rate. However, upon reaching the 10 second mark, there was a very statistically significant drop in the amount of living embryo in said dish in comparison to the control (0 second) dish with a two tailed P value of (0.0039). Following the 10 second dish, the remaining embryo in the 12, 14, 16, 18, 25 and 35 second dishes all perished, revealing a very strongly significant correlation between extended UV light exposure and death in zebrafish embryo. Due to how the T Test was unable to compare this “perfect data”, it is difficult to draw exact statistical significance from the data. However, due to the fact that after 10 seconds, the initial data and the final data were complete opposites, by definition, there is a very statistically significant pattern apparent.

Due to the death of every fish after the 10 second mark, the critical point can be inferred to be just over 10 seconds. This means that after 10 seconds of continuous exposure to a set amount of UV light, approximately all zebrafish embryo will perish.

In regards to the effect that increasing the amount of time exposed had on hatching, the dishes being exposed from 2 to 10 seconds did not show statistical significance in regards to the number hatched in comparison to the control (0 seconds) dish. However, upon reaching 12 and 14 seconds, the differences in the amount hatched in said dishes in comparison to the control (0 seconds) dish was indeed statistically significant. However, after 14 seconds, the statistical significance fluctuated as the time period increased, showing a slight correlation between hatching and UV light exposure. There is reason for such a minor connection. Due to how the fish that were exposed to longer periods of UV light, they died upon receiving the treatment before they had the chance to hatch. Due to that, the fish that received 16 or more seconds of treatment would not have been able to show progress, disregarding the occasional fish that managed to get out of its sack by error (perhaps by being shook too violently) such as in the 35 second dish that was indeed out of its sack, but dead and definitely not fully developed, leading to the conclusion that it was accidentally forced out of its egg sack.

Due to the fluctuation in statistical significance and the lack of a pattern, it would be difficult to create conclusions in regards to hatched or unhatched based on this evidence. However, in regards to overall death of zebrafish embryo, the hypothesis of this experiment was proven to be correct, as increasing the increment of time that embryo are exposed did increase the amount of deaths that occurred.

Though this was a very strictly controlled experiment, there were a variety of sources of error. For example, the most significant source of error was the situation that occurred with the 10 second interval dish on the third day. Upon examining the embryo in the dish, there was only one remaining of the previous 4, meaning that somewhere in the transportation from table to incubator, the other three had splashed out due to what can be assumed was slightly jerky transportation. In addition to the lack of embryo to observe, the final remaining embryo was very difficult to classify as dead or alive due to the faintness of it's heartbeat. Even through a microscope, the trained eye could not make out a heartbeat, though there may very well have been one. In order to keep the data as accurate as possible, two sets of statistical significance calculations and two sets of graphs were made ; one had 10 been dead, and one had 10 been alive. A second source of error was that it was impossible to pick out embryo that were all in the exact same stage of embryological development to begin the experiment with. Due to this, the hatching times could have been different due not to UV light exposure, but due to differences in their initial development. And lastly, one final source of error was that the UV Crosslinker box, upon being activated, took about one second to "heat up", meaning that the embryo may have been subjected to one second less of UV light than intended.

References

Alcaraz, D. (2001, August, 2). Ultraviolet irradiation impairs epiboly via microtubules in Zebrafish embryos. Retrieved December 10, 2016, from https://www.swarthmore.edu/NatSci/sgilber1/DB_lab/Fish/ZF_UV.html

Strähle, U., & Jesuthasan, S. (1993, November 1). Ultraviolet irradiation impairs epiboly in zebrafish embryos: Evidence for a microtubule-dependent mechanism of epiboly. *The Company of Biologists Limited, 119*(No.3), 909-919. Retrieved December 10, 2016, from Ultraviolet irradiation impairs epiboly in zebrafish embryos: evidence for a microtubule-dependent mechanism of epiboly.

Why use the zebrafish in research? (2014, November 17). Retrieved December 10, 2016, from http://www.yourgenome.org/facts/why-use-the-zebrafish-in-research

Kerstin Howe, Matthew D. Clark, Carlos F. Torroja, James Torrance, Camille Berthelot, Matthieu Muffato, John E. Collins, Sean Humphray, Karen McLaren, Lucy Matthews, Stuart McLaren, Ian Sealy, Mario Caccamo, Carol Churcher, Carol Scott, Jeffrey C. Barrett, Romke Koch, Gerd-Jörg Rauch, Simon White, William Chow, Britt Kilian, Leonor T. Quintais, José A. Guerra-Assunção, Yi Zhou, Yong Gu, Jennifer Yen, Jan-Hinnerk Vogel, Tina Eyre, Seth Redmond, Ruby Banerjee, Jianxiang Chi, Beiyuan Fu, Elizabeth Langley, Sean F. Maguire, Gavin K. Laird, David Lloyd, Emma Kenyon, Sarah Donaldson, Harminder Sehra, Jeff Almeida-King, Jane Loveland, Stephen Trevanion, Matt Jones, Mike Quail, Dave Willey, Adrienne Hunt, John Burton, Sarah Sims, Kirsten McLay, Bob Plumb, Joy Davis, Chris Clee, Karen Oliver, Richard Clark, Clare Riddle, David Elliott, Glen Threadgold, Glenn Harden, Darren Ware, Sharmin Begum, Beverley Mortimore, Giselle Kerry, Paul Heath, Benjamin Phillimore, Alan Tracey, Nicole Corby, Matthew Dunn, Christopher Johnson, Jonathan Wood, Susan Clark, Sarah Pelan, Guy Griffiths, Michelle Smith, Rebecca Glithero, Philip Howden, Nicholas Barker, Christine Lloyd, Christopher Stevens, Joanna Harley, Karen Holt, Georgios Panagiotidis, Jamieson Lovell, Helen Beasley, Carl Henderson, Daria Gordon, Katherine Auger, Deborah Wright, Joanna Collins, Claire Raisen, Lauren Dyer, Kenric Leung, Lauren Robertson, Kirsty Ambridge, Daniel Leongamornlert, Sarah McGuire, Ruth Gilderthorp, Coline Griffiths, Deepa Manthravadi, Sarah Nichol, Gary Barker, Siobhan Whitehead, Michael Kay, Jacqueline Brown, Clare Murnane, Emma Gray, Matthew Humphries, Neil Sycamore, Darren Barker, David Saunders, Justene Wallis, Anne Babbage, Sian Hammond, Maryam Mashreghi-Mohammadi, Lucy Barr, Sancha Martin, Paul Wray, Andrew Ellington, Nicholas Matthews, Matthew Ellwood, Rebecca

Woodmansey, Graham Clark, James D. Cooper, Anthony Tromans, Darren Grafham, Carl Skuce, Richard Pandian, Robert Andrews, Elliot Harrison, Andrew Kimberley, Jane Garnett, Nigel Fosker, Rebekah Hall, Patrick Garner, Daniel Kelly, Christine Bird, Sophie Palmer, Ines Gehring, Andrea Berger, Christopher M. Dooley, Zübeyde Ersan-Ürün, Cigdem Eser, Horst Geiger, Maria Geisler, Lena Karotki, Anette Kirn, Judith Konantz, Martina Konantz, Martina Oberländer, Silke Rudolph-Geiger, Mathias Teucke, Christa Lanz, Günter Raddatz, Kazutoyo Osoegawa, Baoli Zhu, Amanda Rapp, Sara Widaa, Cordelia Langford, Fengtang Yang, Stephan C. Schuster, Nigel P. Carter, Jennifer Harrow, Zemin Ning, Javier Herrero, Steve M. J. Searle, Anton Enright, Robert Geisler, Ronald H. A. Plasterk, Charles Lee, Monte Westerfield, Pieter J. de Jong, Leonard I. Zon, John H. Postlethwait, Christiane Nüsslein-Volhard, Tim J. P. Hubbard, Hugues Roest Crollius, Jane Rogers & Derek L. Stemple. (2013, April 17). The zebrafish reference genome sequence and its relationship to the human genome. *Nature, International Weekly Journal,496*(7446), 498-503. Retrieved December 10, 2016, from http://www.nature.com/nature/journal/v496/n7446/full/nature12111.html

Lieschke, G., & Currie, P. (2007, May). Animal models of human disease: Zebrafish swim into view. Nature Reviews Genetics, 353-367. Retrieved December 10, 2016, from http://www.nature.com/nrg/journal/v8/n5/full/nrg2091.html