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The Effect of Embryonic Arsenic Exposure on the Sensorimotor Behavior of Zebrafish (Danio Rerio)

Laura Paye
University of Maine

THE EFFECT OF EMBRYONIC ARSENIC EXPOSURE ON THE SENSORIMOTOR BEHAVIOR OF ZEBRAFISH (*DANIO RERIO)*

By

Laura Paye

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Advisory Committee:

Rebecca J. Van Beneden, Professor of Biochemistry and Marine Science
Mary Scarlett Tudor, Research Assistant for the Animal Health Lab, Cooperative Extension and Aquaculture Research Institute
Nishad Jayasundara, Assistant Professor of Marine Science
Thane Fremouw, Chair, Undergraduate Coordinator and Associate Professor of Psychology
Melissa Ladenheim, Associate Dean of the Honors College

ABSTRACT

The goal of this study is to determine the effect of arsenic exposure on vision in zebrafish (*Danio rerio)*. The optic system of *D. rerio* is ideal for examining visual defects. Their eyes are similar to eyes of humans and can therefore be useful models in studies of human eye disease. Their optic system functions similarly to humans, so it is beneficial to observe how zebrafish are affected by contaminants in the environment. Arsenic is ubiquitous in groundwater, due to its natural presence in bed rock, but is elevated by human activities. In order to see any immediate effects on vision by arsenic, a behavioral assay was used. Due to accumulation in eye tissues, we predict that visual acuity will decrease with increased exposure to arsenic. Zebrafish embryos were exposed to 0, 10, 50, and 500 parts per billion of arsenic. At five days post hatch, a striped, rotating cylindrical drum created a pattern designed to elicit the optomotor response in zebrafish. Time spent following and going against the striped pattern was calculated to determine if the optomotor response was evoked in the zebrafish. A positive value would indicate an optomotor response, and a negative value would indicate no response. The control group did not behave as expected, as they showed a negative value in response to the cylindrical drum. Statistical analyses revealed a batch effect in this data set, and the total distance travelled showed a significant difference in activity level between batch one and batches two and three. Batch one showed an unexpected positive trend in optomotor response with increased arsenic exposure, with 50 ppb arsenic treatment group following the rotational pattern of the striped drum for a longer amount of time relative to the control. Batches two and three showed no significant differences between arsenic treatments and the control group. Changes in experimental design may result in the

expected control behavior, and further replication would be necessary to determine any effects of arsenic on vision in zebrafish.

ACKNOWLEDGEMENTS

I first and foremost would like to thank Dr. Rebecca Van Beneden, who gave me my first opportunity to work in scientific research and started me on this zebrafish-filled journey. I would also like to thank The Maine IDeA Network of Biomedical Research Excellence (NIH INBRE), and the University of Maine Honors College, for selecting me for two of their fellowships, which funded this research. Along with Becky, I would like to acknowledge Torey Bowser and Remy Babich, for all of their input and assistance throughout this research project and my time in the Van Beneden lab. I would like to thank Torey Bowser again, along with Dr. M. Scarlett Tudor for all of their help processing and presenting the data in this thesis. My committee members Dr. Thane Fremouw and Dr. Nishad Jayasundara provided insight and fresh perspective to me when I needed it most. This honors thesis would not have reached its fullest potential without them. I would also like to thank Dr. Melissa Ladenheim for going through the lengthy process of providing edits on my reading list, which shows my intellectual and personal journey throughout my time at the University of Maine. I would lastly like to thank a few of my friends, namely Cassandra Steele and Michaela Murray, for providing additional edits and insights into my writing, and being supportive friends throughout this research project.

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INTRODUCTION

It has become increasingly apparent that arsenic (As) is detrimental to human health. Many individuals, in the United States as well as other countries, are exposed to arsenic levels above the standard via drinking water from unregulated private wells (Tyler and Allan, 2014). The World Health Organization and the US Environmental Protection Agency lowered the maximum allowable level of arsenic in drinking water from 50 parts per billion (50 ppb) to 10 parts per billion (10 ppb) in 2004 (Hallauer et al., 2016). Even with these regulations, arsenic has been found in Maine groundwater in concentrations up to 500 ppb (Nielsen et al., 2010). Chronic exposure to arsenic can cause neurological damage, skin lesions, hypertension, cancer, and heart disease in humans (Hallauer et al., 2016). Arsenic has been linked to negative effects on perceptual reasoning and memory, particularly in children. Children exposed to greater than or equal to 5 μg/L of arsenic in Maine had IQs 6 points below those exposed to less than 5 μg/L (Wasserman et al., 2014). This is shown in other studies, where there are negative cognitive impacts due to arsenic accumulation in the brain of children and adults (Tyler & Allen, 2014). The growing list of arsenic's impacts on human health make it an important contaminant to study.

Arsenic is naturally found in soils, rocks, and living organisms (DeClementi, 2013). The presence in soil and rock allows for leaching into water. All bodies of water will naturally contain some arsenic. Human activities like the mining and smelting of metals and burning of fossil fuels drastically increases the amounts in soil, air, and water (DeClementi, 2013). Inorganic arsenic is present in two valence forms, +3 and +5 (Flora et al., 2007). Under reduced conditions, As III is dominant, while in oxygenated

conditions, As V is the dominant form (Uphadhyay et al., 2019). Similarly, arsenic is found in waterways in the less toxic form of As V and is reduced in the cell to As III, arsenite (Castro et al., 2009). As III is reactive with thiol-containing proteins, including important transcriptional factors and metabolic enzymes that may have cascading effects in organisms that are exposed to it (Hallauer et al., 2016). This could have a wide range of impacts on the health of living organisms, not all of which are currently understood.

Zebrafish (*Danio rerio)* have been used as models for human exposure (Hallauer et al., 2016). 70% of human genes have at least one obvious zebrafish orthologue (Howe et al., 2013). Zebrafish may also accumulate contaminants in tissues similarly to humans. Arsenic enters directly through the gills in zebrafish and, like in humans, is transported by the aquaporins into cells (Dipp et al., 2018). Previous studies have shown that arsenic accumulates significantly in the liver, skin, and eyes of zebrafish. These accumulations may also have an effect on their behavior (Hamdi et al. 2009; Lee and Freeman, 2014; Hallauer et al., 2016). Specifically, arsenic accumulating in the eye and liver shows increased oxidative stress in zebrafish (Hallauer et al., 2016).

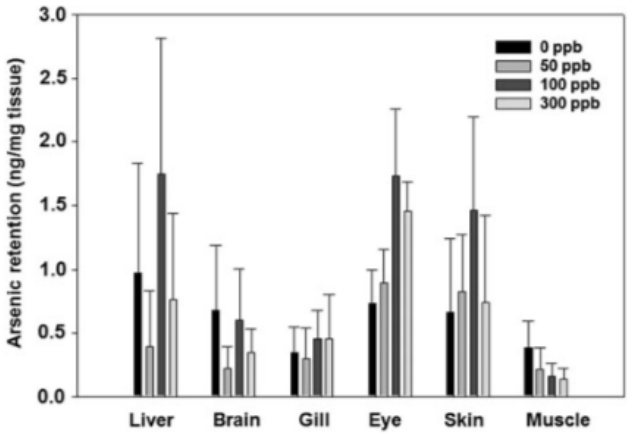

Figure 1 Retention of arsenic in zebrafish tissue after chronic exposure from fertilization to six months. N=5. Error bars are standard error of the mean (from Hallauer et al., 2016).

Recent studies in our laboratory have shown that embryonic arsenic is linked to thinning in the retinal pigmented epithelium (RPE), through changes in gene expression which have the potential to impact the visual system (Babich and Van Beneden, 2018; Figures 2 & 3). Studies with cadmium and nickel exposure in zebrafish have shown negative effects in visually guided behaviors (LeFauve and Connaughton, 2017).

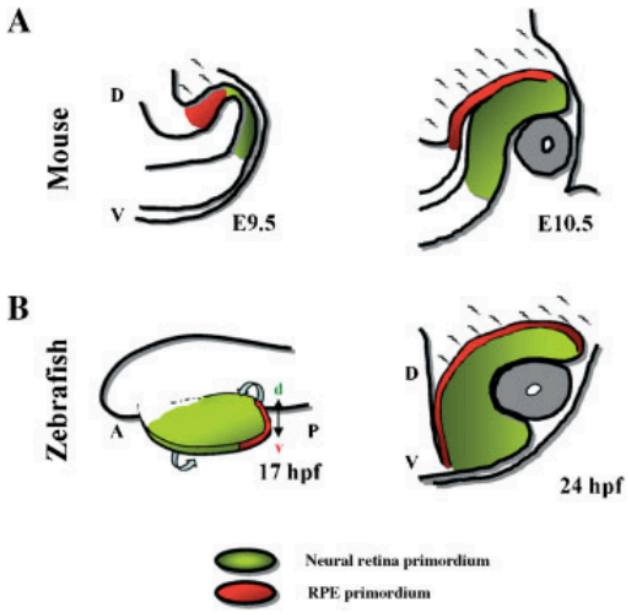

Figure 2: Development of the eye in two common vertebrate model organisms, the mouse and the zebrafish. Note the RPE in B, the zebrafish, shown in red, which acts as the blood-retina barrier, transports nutrients, ions and water, absorbs light to prevent photooxidation, prevents entry of free radicals, and maintains the renewal of photoreceptors (Martinez-Morales et al., 2004).

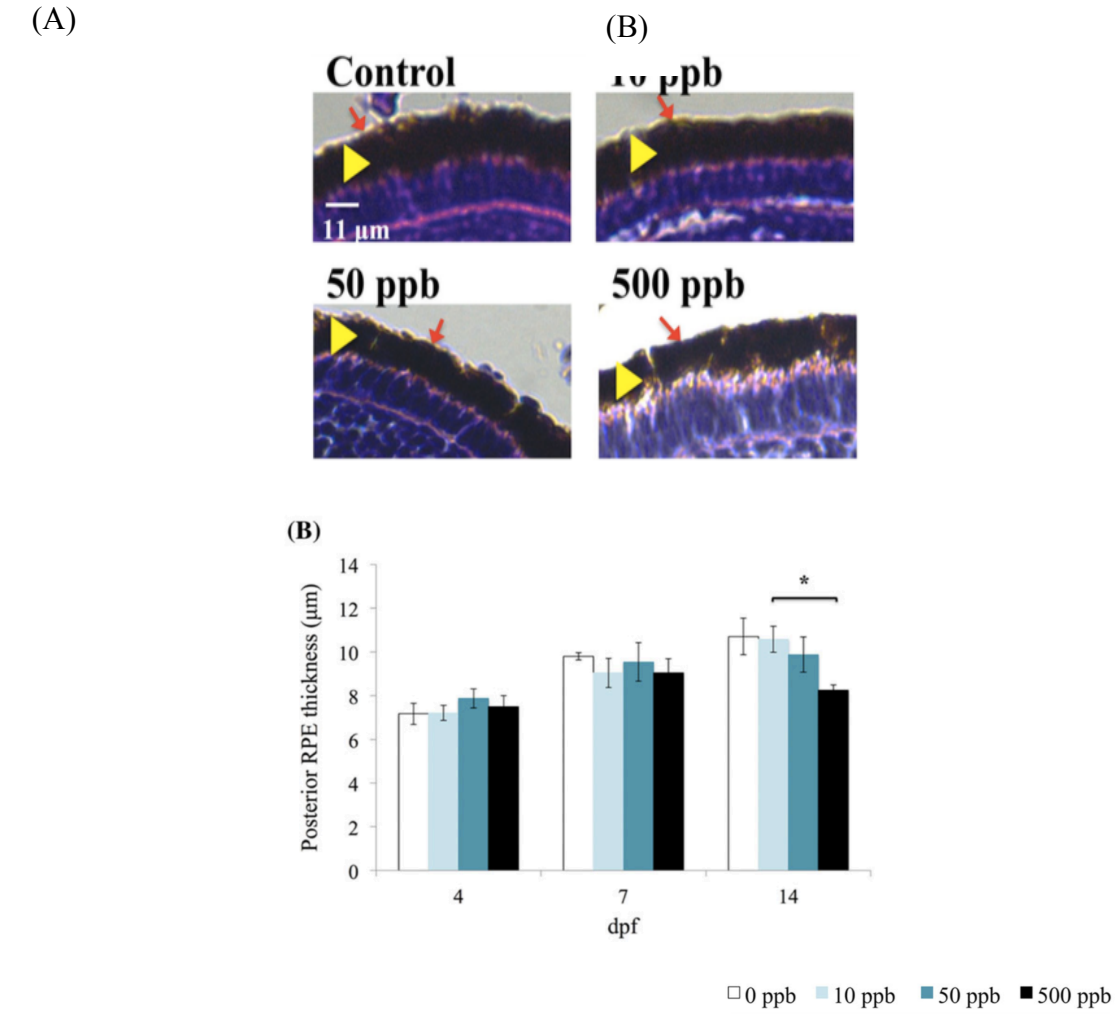

Figure 3. (A) Stained sections of a zebrafish eye at 14 days post fertilization, after being exposed to arsenic in embryo. Thinning of the retinal pigmented epithelium (RPE) with increased arsenic concentration, is indicated by the yellow triangle. (B) Shows the decrease in RPE thickness (µm) with increasing arsenic treatment at 14 days post fertilization (from Babich and Van Beneden, 2018).

There are several advantages to studying the optic system of zebrafish. Retinal cells appear in zebrafish at 28 hours post fertilization, followed by the optic tectum and cones at 48 hours post fertilization. The inner retina is fully mature at 5 days post fertilization (Huang & Neuhauss, 2008). The optokinetic response, the response of the eye to visual stimuli, can be detected at 72 hours post fertilization, when the eyes are fully developed and embryos hatch (Easter & Nicola, 1996). Morphological studies have

shown accumulation of arsenic in zebrafish eyes (Hamdi et al., 2009), and previous studies in our lab have shown changes in gene expression during visual development that result in changes in morphology (Babich and Van Beneden, 2018). There has been little research on the behavioral effects. Behavioral tests are a way to observe any impacts arsenic may have on visual acuity.

Behavioral tests can be used to observe motor function in fish from auditory, olfactory, or visual stimulants (Tierney, 2011). One such behavior ruled by visual function is the optomotor response. The optomotor response occurs when animals move their eyes relative to surroundings, regardless of changes in body or head orientation (Tauber et al., 1968). Fish will turn their heads towards a stimulus to reduce retinal movement (LeFauve & Connaughton, 2017). There are several behavioral assays used to elicit the optomotor response in zebrafish (*Danio rerio*). One commonly used assay is the rotating cylindrical drum, where the larvae swim in a container surrounded by a moving striped drum (Figure 4). The striped pattern is typically created by using a dark material with slits cut into it with a lighted background, typically LED lights, to create shadows. This drum rotates around an arena in which the fish sits (Rock and Smith, 1986).

Figure 4: Apparatus to test optomotor response (Neuhauss, 2003)

There are different methods to achieve this grated pattern, such as a computer image that moves to create the pattern (LeFauve and Connaughton, 2017). This test was originally used to screen for zebrafish mutants with visual system defects (Brockerhoff et al., 1995).

The cylindrical drum test has been optimized through several different studies (Schaerer and Neumeyer, 1996; Bilotta, 2000; Darland and Dowling, 2001; Krauss and Neumeyer, 2003). Studies have determined that normal light/dark cycles are best for visual acuity, and that visual acuity increases with age (Bilotta, 2000). Zebrafish will follow a pattern up to 20 rotations per minute under normal operating conditions (Krauss and Neumeyer, 2003). This test has also been used to ensure optimal lighting conditions (Schaerer and Neumeyer, 1996). This assay has been used to screen for effects of cocaine and lidocaine on vision in zebrafish (Darland and Dowling, 2001), as well as how embryonic exposure to ethanol affects vision in zebrafish (Bilotta, 2002) (Figure 5).

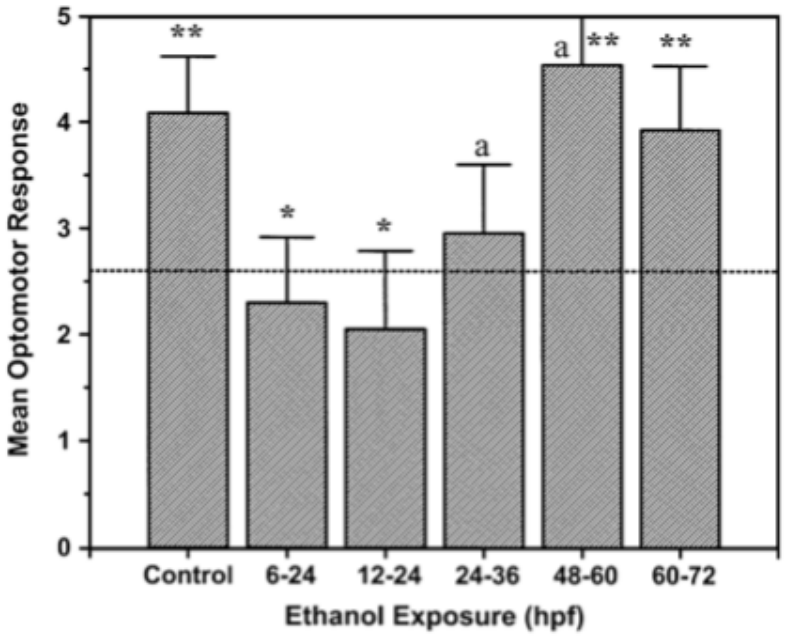

Figure 5 The optomotor response score for zebrafish. Control subjects (n=23) and test subjects exposed to 1.5% ethanol at 6–24 (n=17), 12–24 (n=7), 24–36 (n=16), 48–60 (n=23), and 60–72 (n=18) hours post fertilization (hpf). Groups designated with a single asterisk (*) indicate that these groups are significantly different from groups with a double asterisk (**); groups designated with the letter 'a' are significantly different from one another. All significance levels are P < .05. (from Bilotta, 2002).

In the current study, we use the cylindrical drum test to elicit an optomotor response in zebrafish to see any effects of arsenic on visual acuity. While there have been morphological studies looking at accumulation of arsenic in the eyes of zebrafish (Hallauer et al., 2016), there has been little behavioral research measuring whether this has negative consequences on visual acuity. Accumulation of arsenic in zebrafish eyes upon embryonic exposure is expected to reduce the optomotor response, with the decrease correlated with increased exposure. To study this, we calculated the time spent following and going against the pattern of the cylindrical drum test, designed to elicit the optomotor response, in 4 different treatments of arsenic: 0 ppb (control), 10 ppb, 50 ppb, and 500 ppb. Three different batches of zebrafish embryos were exposed to arsenic in this experiment, which revealed variation within batches. In both, the control did not elicit an

optomotor response, shown by a negative number which means they spent more time swimming against the pattern of the cylindrical drum. In one batch, we saw an increase in optomotor response in the 50 ppb arsenic treatment group when compared with the control, and no other differences in treatment groups. In the other pooled batches, we saw no differences in activity between treatments. These results show the need for increased replication of this experiment to determine any effects of arsenic on vision in zebrafish.

METHODS

Animals

Zebrafish embryos were obtained from the University of Maine Zebrafish Facility. Zebrafish embryos were exposed from 1 to 72 hours post fertilization to 4 treatments of $AsNaO_2$ (Krauss and Neumeyer, 2003): 0 ppb, a control; 10 ppb, the current water standard; 50 ppb, the previous water standard; and 500 ppb, which is the upper environmentally relevant threshold in Maine (Ayotte et al., 2003; World Health Organization). Embryos were held in egg water (60 μg/mL of Instant Ocean®,St. Blacksburg, VA, USA, sea salts in distilled water), in 100 mm x 15 mm petri dishes, with 20 embryos per petri dish. 50% of the water was changed daily. Larvae were transferred to 100mL clean egg water in 500mL beakers at 3 days post fertilization (Figure 6). 20 fish were used per treatment, with three batches (Krauss and Neumeyer, 2003) (Appendix 1). Tests began at five days post hatch, when the eye is fully developed (Easter and Nicola, 1996). Zebrafish were handled and disposed of according to IACUC protocol number A2017-05-04.

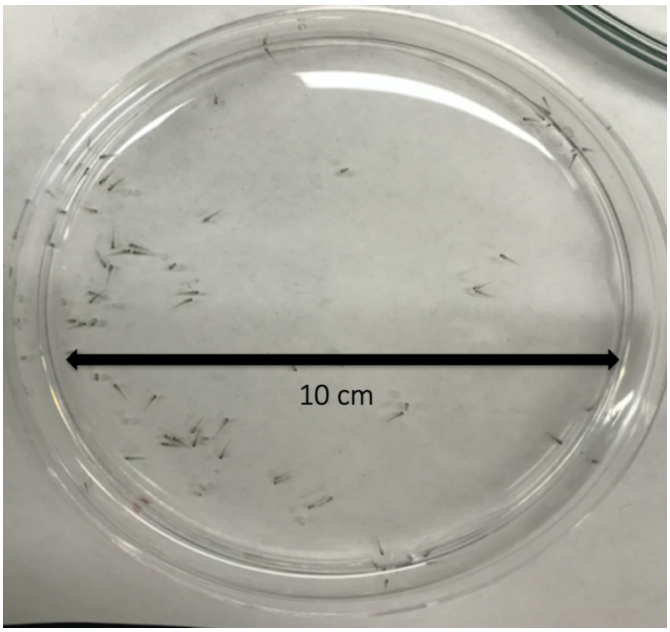

Figure 6 Zebrafish at 3 days post-fertilization before transfer to beakers.

Experimental Design

To test visual response, zebrafish were placed individually in a 60 x 15 mm petri dish, which was mounted on top of a platform in the form of a black bucket. The platform provided a way to record the fish from below, using a portable USB microscope camera, with 1080P HD and 50-1000x zooming capabilities (Figure 7). A hinged lid, covering the arena, allowed for removal and replacement of each fish. Following a 3-minute acclimation period, fish activity was recorded for 1 minute in a baseline test, in the arena with no visual stimulus. Immediately following the baseline test period, the fish went through a cylindrical drum test, the visual stimulus, in the same arena. A striped cylindrical drum was attached to the hinge, which would rotate around the arena at 18 rotations per minute via a DC power source, which is enough to elicit a visuomotor response according to Krauss and Neumeyer (2003) (Figure 8).

The cylindrical drum itself was made with a two 11-cm diameter metal rings of, wrapped with a cardboard structure with cuts providing two-centimeter-wide stripes,

painted black for contrast against LED string lights, which together created the striped pattern. The cylindrical drum was mounted onto the movable arm, which was lowered to cover the arena during testing, and surrounded by a stationary white bucket onto which white LED string lights were mounted (Figure 9). The size of the drum was determined following the procedure of Krauss and Neumeyer (2003). This illuminated the arena and provided the striped pattern necessary to induce the optomotor response. Zebrafish exhibiting a normally functioning visual response are expected to move in the direction of the light pattern (Springer et al., 1977; Bilotta, 2000; Neuhauss, 2003). Any deviation from this pattern would suggest visual defects.

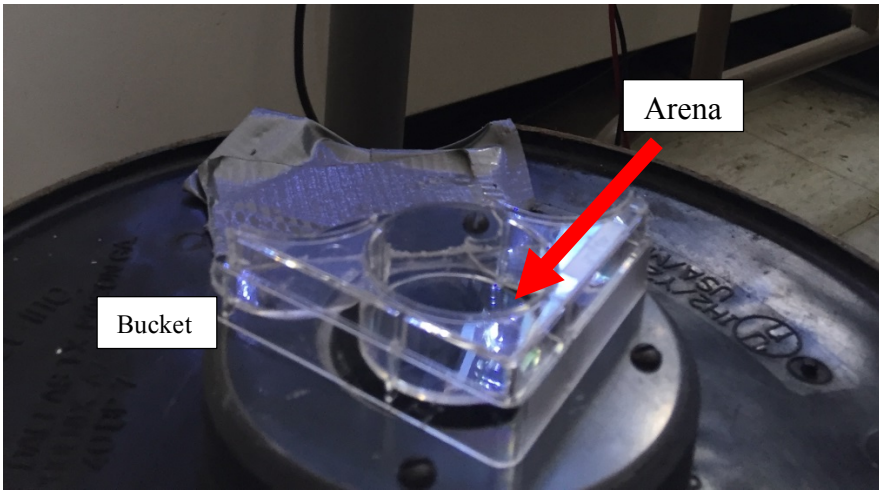

Figure 7 Arena (60x15 mm petri dish) mounted on apparatus, on top of a bucket. The USB microscope camera was placed directly under the arena which rests on top of a hole in the bucket, allowing the camera a clear image. The fish was placed in this dish with about 50mL of egg water, for all tests.

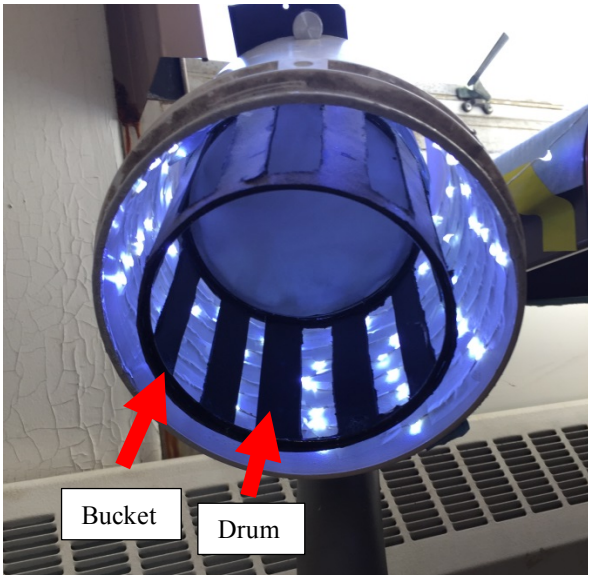

Figure 8 *Cylindrical drum (11-inch diameter) mounted on a hinge and illuminated by LED string lights attached to a white bucket (13-inch diameter) provided the visual stimulus.*

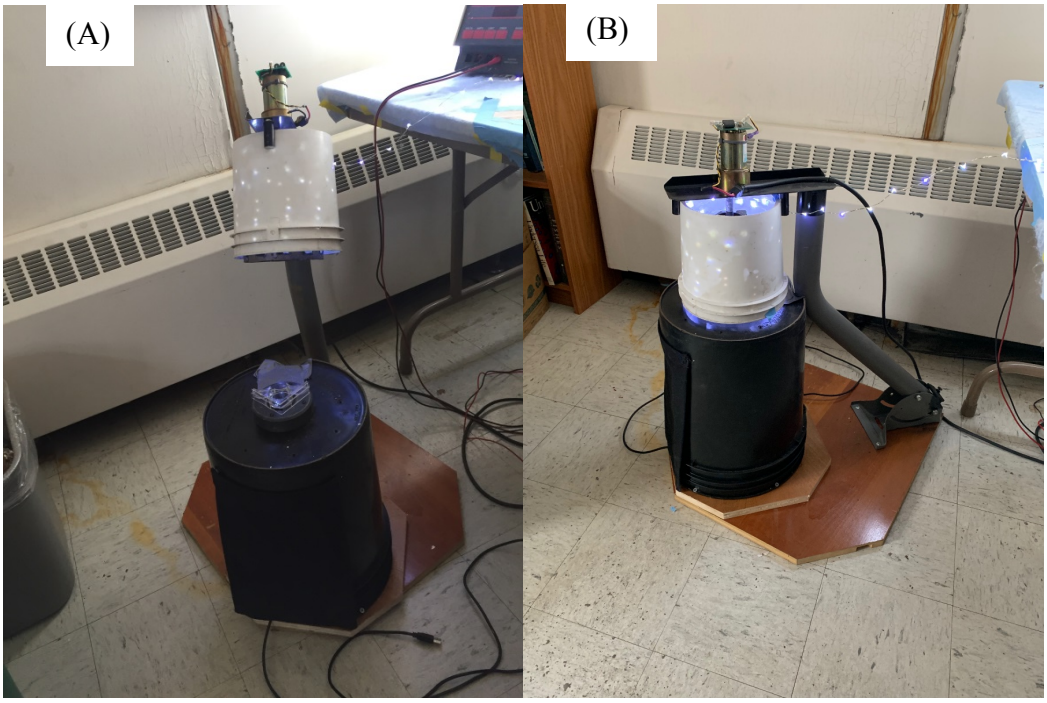

Figure 9 *(A) Experimental set-up connected to the power source for the spinning drum in the top right corner. (B) shows entire set-up during testing, with the white bucket containing the cylindrical drum lowered over the fish in its arena.*

Data Analysis

Fish movement was measured as the time (in seconds) spent moving with and against the pattern created by the cylindrical striped drum. The cylindrical drum moved in a clockwise rotational pattern, so the fish moving clockwise would be with the pattern, and counterclockwise would be against the pattern. By calculating the difference in these values, a positive number indicates an optomotor response, and a negative number indicates no optomotor response. Three batches of each of four treatments were done, and if, according to this analysis, the data were statistically the same they could be pooled (Appendix 1). The Kruskal-Wallis test for ranks was used to determine any batch effects. If there was any batch effect, the Mann-Whitney U test could then be performed to determine where that significant difference lies (Appendix 2).

Total distance travelled was calculated and analyzed as a measure of activity level. To do so, videos were converted into pictures at 5 frames per second, and the fish were manually tracked by point analysis in MATLAB. This provided coordinates, from which total distance travelled for each test was calculated by converting the number of pixels travelled in an image to centimeters. Looking at the total distance travelled for the baseline test period, batch one was significantly different ($p>0.05$) from batches two and three according to the Mann-Whitney U test so they could not be pooled for analysis.

If the time analysis data had significantly different distributions according to the Kruskal-Wallis test, the Mann-Whitney U test was applied. This would determine if there were any significant differences between the control and the three arsenic treatments. All statistical analyses were performed in SPSS (V25.0) on a basis of 95% confidence ($p<0.05$).

RESULTS

Batch one was significantly different from batches two and three in time spent moving in a clockwise rotational movement (Mann-Whitney U: batch 1 and 2 $Z = -2.58$, $p = 0.01$; batch 1 and 3 $Z = -1.78$, $p = 0.075$; batch 2 and 3 $Z = -1.187$, $p = 0.235$; Table 1). Batch one had a significantly higher activity level (total distance travelled) than either batches two & three during the baseline testing period (Mann-Whitney U: $Z = -8.151$, $p<0.01$; Figure 10).

Due to the significant difference between batch one and batches two and three, or a batch effect, data were analyzed separately to look at treatment effects. The Kruskal-Wallis test revealed a significant difference in the continuous data for the arsenic treatment groups (Table 2). In batch one, the control showed a negative value in time spent following the stimulus. This indicates that the fish in the control group spent more time going against the pattern of the stimulus meant to elicit the optomotor response (Figure 11). The control was not significantly different from the 10 ppb or 500ppb arsenic treatment group (Mann-Whitney U for 10ppb: $Z = -1.226$, $p = 0.22$; Mann-Whitney U for 500ppb: $Z = -1.694$, $p = 0.09$) (Table 3 and Figure 11). There was a significant difference where the 50ppb group had more time following the striped drum pattern than the 0ppb group (Mann-Whitney U: $Z = -3.43$, $p = 0.001$; Table 3).

In batches two and three, the control did not show a positive value that would indicate an optomotor response (Figure 12). There was no difference in time spent following the pattern of the cylindrical striped drum, the visual stimulus, among arsenic

treatment groups (Kruskal-Wallis H = 3.161, p = 0.367; Table 4). Time spent following the pattern of the cylindrical striped drum, the visual stimulus, did not differ among arsenic treatment groups (Figure 12).

Grouping Variable: Batch 1 vs. Batches 2 & 3	Statistical values on Distance Traveled (cm) in Baseline Test
Mann-Whitney U	1975
Wilcoxon W	11845
Z	-8.151
p-value	<0.01

Table 1 Mann-Whitney U results for batch one (N=82) versus batches two and three (N=140), including all treatments. These are statistics done on the total distance traveled (cm) in one minute in the baseline testing period, recorded immediately before the cylindrical drum test was performed to stimulate the optomotor response in the zebrafish. This shows a significant difference between batch one versus batches two and three, which required a separate analysis for arsenic treatment affects.

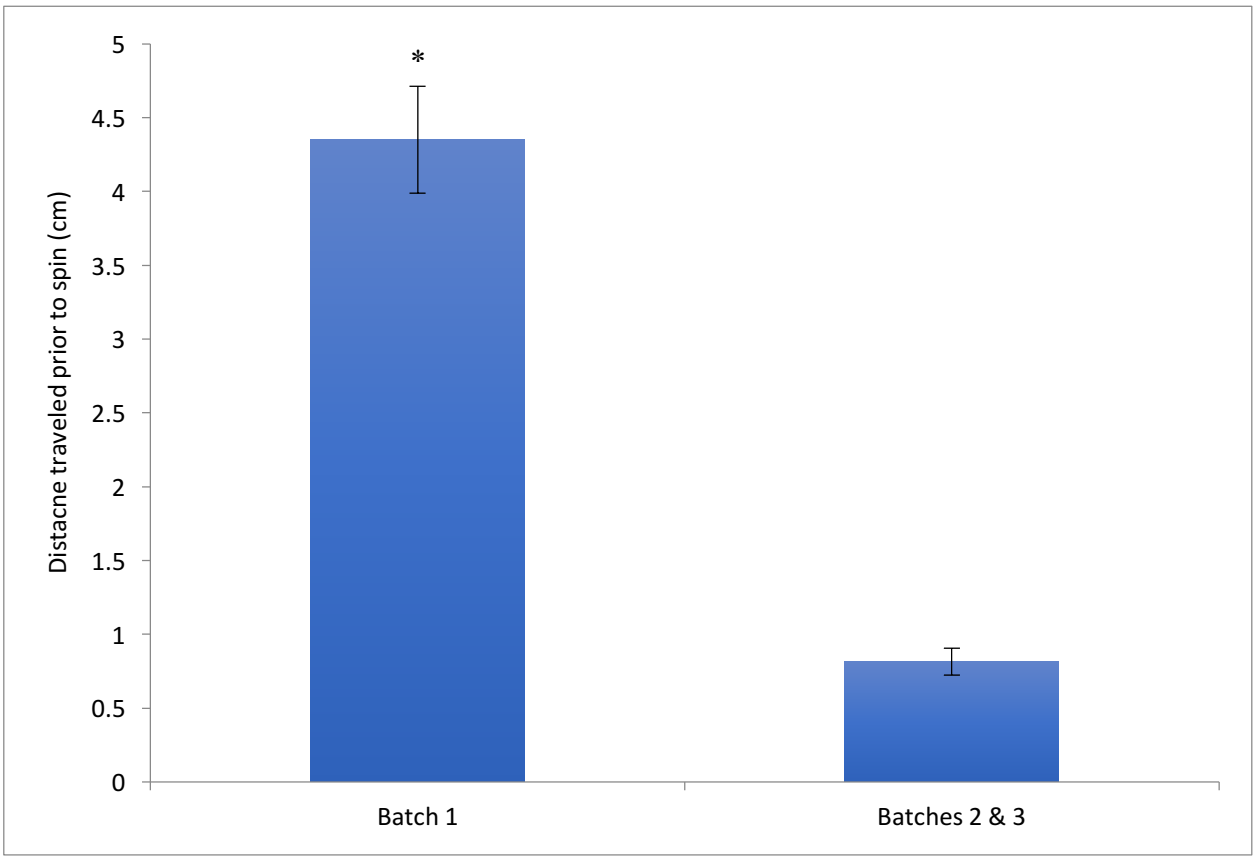

Figure 10 Distance travelled in the baseline test. This shows the difference in activity level in batch one (N=82) compared with batches two and three (N=140) of zebrafish testing period. All treatment groups are included; error bars are standard error. An asterisk (*) indicates a significant difference between groups ($p<0.05$)

Arsenic Treatment Effects in Batch 1	
Kruskal-Wallis H-value	11.931
DF	3
p-value	0.008

Table 2. Results of the Kruskal-Wallis test of ranks for batch one, sorted by treatment, shows a significant difference ($p<0.05$) between treatments.

Control versus Arsenic Treatment Groups	10 ppb	50 ppb	500 ppb
Mann-Whitney U	171.5	84.5	153
Wilcoxon W	424.5	337.5	406
Z	-1.226	-3.43	-1.694
p-value	0.22	0.001	0.09

Table 3 Mann-Whitney U test results when the control is compared to the arsenic treatment groups within batch one. There is no significant difference between the control and 10 ppb or 500 ppb arsenic treatment groups. There is a significant difference between the control and 50 ppb arsenic treatment group ($p<0.05$).

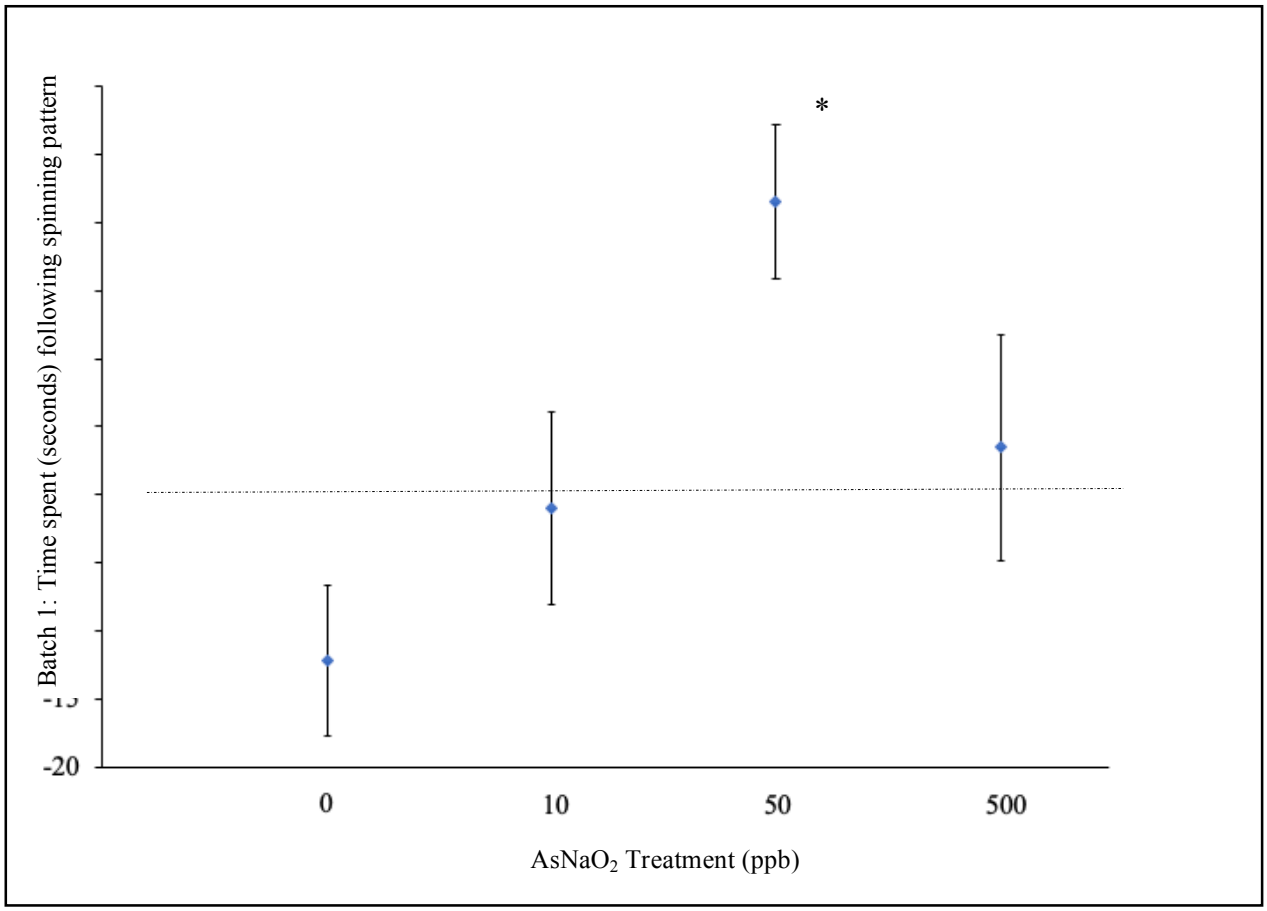

Figure 11 The time spent, in seconds, following the spinning pattern of the visual stimulus, a cylindrical striped drum. On the y-axis, positive numbers indicate the zebrafish followed the pattern of the visual stimulus, negative numbers indicate zebrafish swam against the pattern of the visual stimulus. The horizontal axis shows the 4 different arsenic treatments: 0 ppb (N=22), 10 ppb (N=20), 50 ppb (N=20), and 500 ppb (N=20). An asterisk (*) indicates a significant difference from the control ($p<0.05$). Error bars are standard error of the mean.

Arsenic Treatment Effects in Batches 2 & 3	
Kruskal-Wallis H-value	3.161
DF	3
p-value	0.367

Table 4 Results of the Kruskal-Wallis test of ranks for batches two & three, shows no significant differences ($p>0.05$) between the controls and arsenic treatments.

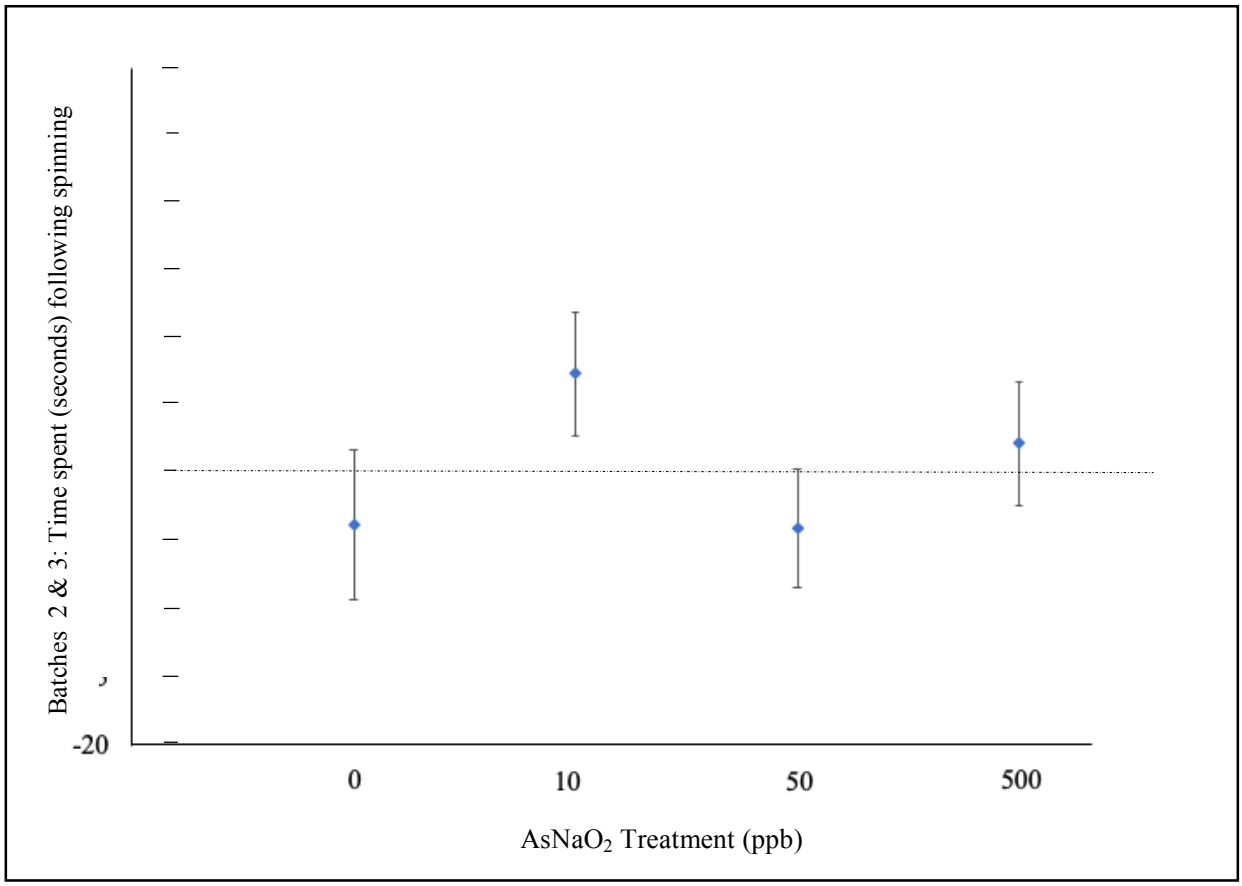

Figure 12 The time spent, in seconds, following the spinning pattern of the visual stimulus, a cylindrical striped drum. On the y-axis, positive numbers indicate the zebrafish followed the pattern of the visual stimulus, negative numbers indicate zebrafish swam against the pattern of the visual stimulus. The horizontal axis shows the 4 different arsenic treatments: 0 ppb (N=40), 10 ppb (N=20), 50 ppb (N=40), and 500 ppb (N=40). There are no significant differences among treatment groups. Error bars are standard error of the mean.

DISCUSSION

The purpose of this study was to examine the effects of embryonic arsenic exposure on vision in zebrafish. To do so, we used a behavioral assay to elicit the optomotor response, a reaction to a visual stimulus that results in stereotypic swimming patterns. The initial hypothesis was that increased exposure to arsenic would decrease the ability of zebrafish to respond to the visual stimulus. Here, we will discuss the results and how they contradicted this hypothesis, and any confounding factors that may have had an influence.

The first factor to address would be the significant difference in batches, and any variations that occurred with them. One difference between batch one compared with batches two and three are the collection dates from the Zebrafish Facility at the University of Maine (Figure 10). Batch one was collected on March 21st, 2018 and tested on March 29th, 2018. I attempted to collect batch two the following day, but after one day I found all zebrafish embryos dead in every treatment, including the control. The next dates that I could collect from the facility were March 28th and 29th for batches two and three, respectively, which put their testing dates on April 5th and 6th. The variations resulting in the batch effect are most likely due to differences in mating pairs at the time of embryo collection.

In batch one, the control did not exhibit the expected positive optomotor response. Possible explanations for this could be that the fish were not developed enough to exhibit this response, or that they did not recognize a visual stimulus in the form of the striped cylindrical drum. Previous studies have used the optomotor response as early as 3 days

post fertilization (Bilotta et al., 2002). Studies in our lab, however, have not shown morphological changes due to contaminants until 14 days post fertilization (Babich and Van Beneden, 2018). Batch 1 shows no significant differences between the control, the 10 ppb, and the 500 ppb treatments (Figure 11). In regard to the drum itself, previous research has shown that zebrafish elicit the optomotor response between a range of 4 and 20rpm (Krauss and Neumeyer, 2003). This speed was the lowest setting available from our power source at 18rpm, however, it may have been too fast for some zebrafish to follow. Some studies have also suggested that the introduction of small, cylindrical post in the middle of the arena helps orient zebrafish to follow this pattern more closely (Krauss and Neumeyer, 2003). As for the increased optomotor response in zebrafish exposed to 50 ppb arsenic, this is opposite to the initial postulation, that arsenic would inhibit visual function, theoretically showing the strongest optomotor response in the control. Since the control did not respond to the stimulus as expected, the increase in the 50 ppb treatment group may be due to the arsenic's effect on behavior. Some contaminants invoke anxiety-like behavior in zebrafish, which can cause darting movements in an attempt to escape, which may explain the increased movement in the 50 ppb treatment group (Kalueff et al., 2012). Zebrafish are also spontaneous swimmers, which makes their swimming behavior variable (Krauss and Neumeyer, 2003).

Batches two and three showed no differences in optomotor response among arsenic treatments (Figure 12). One reason for this may be that the zebrafish did not recognize the cylindrical drum, therefore eliciting no optomotor response. Previous research shows that the optomotor response in zebrafish may not be sensitive enough to pick up a difference between the control and the groups treated with toxicants in embryo

(Bilotta et al., 2002). Using the cylindrical drum method to test optomotor response, Bilotta et al., (2002) demonstrated that when zebrafish were exposed to ethanol, there was no difference in motor activity between controls and treatment groups, yet electroretinographs showed a significant difference in optokinetic responses. There were no differences between controls and arsenic treatment groups, which may be due to the optomotor response itself.

CONCLUSION

The goal of this study was to determine any effects of embryonic arsenic on vision in larval zebrafish. The significant differences between batch one and batches two and three show varying trends, and therefore make it difficult to make any decisive conclusions on how arsenic affects vision. The variances in batches could be due to a number of reasons, including different mating pairs on different collection dates. The control did not behave as expected in batch one, which may be due to the age of zebrafish at the time of testing, or the drum set-up. The 50 ppb treatment group in batch one exhibited the strongest optomotor response, which could be from an anxiety-like response to arsenic or just due to variable swimming behavior. In batches two and three, the optomotor response may not have been evoked in the zebrafish, thereby not revealing any impacts of arsenic on vision. The cylindrical drum may still be a viable method of looking at visual defects by contaminants, like arsenic, but it may not have been effective in these conditions. Arsenic's impact on vision may also not be quantifiable by the optomotor response, as it may not be sensitive enough to show obvious signs of effects by arsenic.

FUTURE DIRECTIONS

The lack of response in the control may be due to measurement of optomotor response itself. A more sensitive visual assay, such as the optokinetic response may be able to pick up on nuances in vision in zebrafish and may be a more viable option for examining responses to contaminants. The optokinetic response could be tested in conjunction with the optomotor response. The optokinetic response requires a microscope with high enough resolution to see eye movements in zebrafish but would be powerful enough to determine if arsenic effects vision in zebrafish. An increase in batches and replicates of this experiment could also help minimize variations once the experimental design was optimized. Increasing the number of zebrafish in this experiment as well as batches could help detect any effects on vision by arsenic. This could also validate any findings from this experiment, such as the increased movement with increased arsenic in batch one, or the lack of optomotor response in all arsenic treatments in batches two and three. Testing zebrafish later in development may also reveal any effects from arsenic, since morphological changes occurred later in development than examined in this study.

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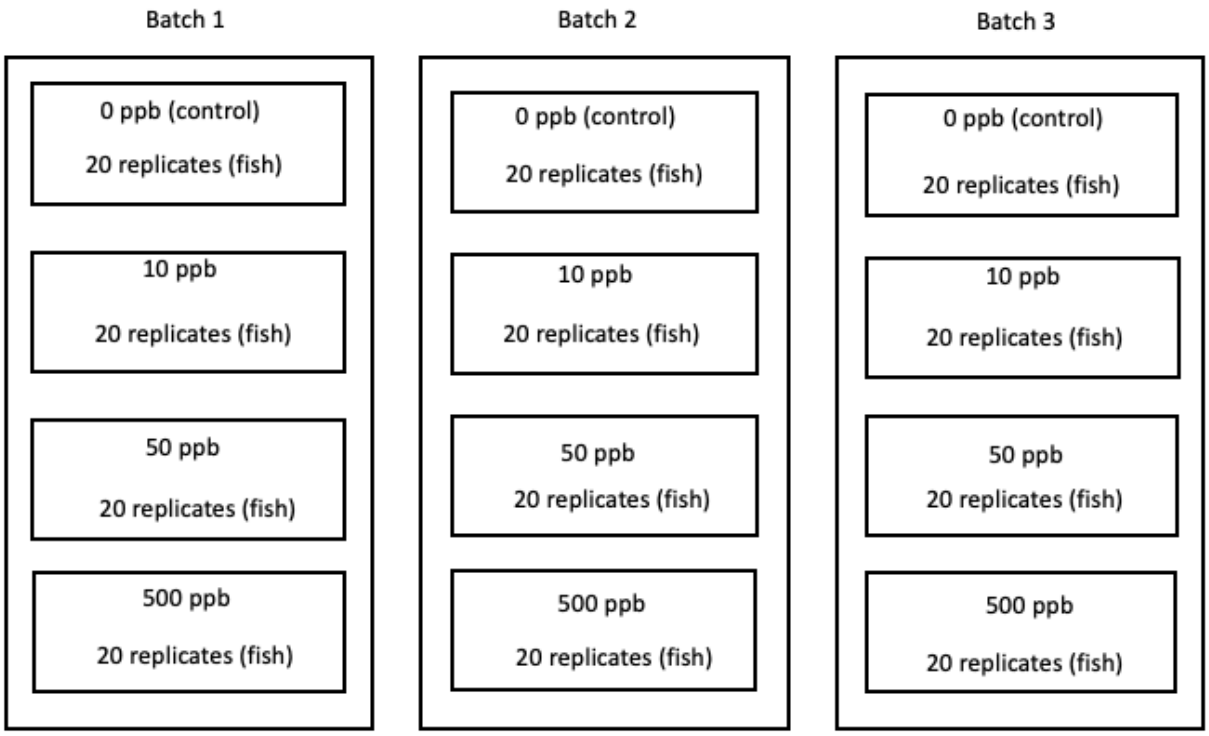
APPENDIX 1

Experimental organization: showing what is meant by the terms 'batch' and 'replicate'



APPENDIX 2

All statistical analyses were performed in SPSS (V25.0) on a basis of 95% confidence ($p<0.05$). All batches failed to meet Shapiro-Wilks test for normality ($p<0.05$), so non-parametric tests were performed that do not require an assumption of normality. The first step in statistical analysis was to determine if there was a batch effect (see figure in appendix 2 for clarification). In order to determine a batch effect, the Kruskal-Wallis test of ranks would show any significant differences in batches. All treatments were pooled, and the three batches went through this test, where each value would be assigned a rank, to determine if the batches have a similar distribution. If this value was less than 0.05, there was a significant difference between batches and other tests can be performed to find this difference.

In order to test for where the batch effect is, the Mann-Whitney u test can determine a significant difference between two groups. It is essentially a non-parametric t-test. Each batch was compared to the other, until the difference shown in the Kruskal-Wallis test was determined. This happened to be a significant difference between batch 1 and batch 2, and batch 1 and batch 3. Batches 2 and 3 were not significantly different, and thus could be pooled.

Once the batch effect was determined, treatment effects of arsenic within these batches could be determined. The same methodology was applied, where the Kruskal-Wallis test of ranks was applied to an entire batch, and if less than 0.05 there was a significant difference somewhere within the treatment groups. In Batch 1, the Kruskal-Wallis p-value was less than 0.05, meaning that there was a treatment effect in the batch. Each treatment was compared to the control using the Mann-Whitney u test, and a

difference between the control and the 50 ppb arsenic treatment group was found. The Kruskal-Wallis p-value was greater than 0.05 for batches 2 and 3, so there were no treatment effects within those batches.

AUTHOR'S BIOGRAPHY

Laura Paye grew up in Western Massachusetts, in the town of Westfield. She grew up with a father who's love for the outdoors inspired her to pursue a career that allows her to spend as much time outside as she can. Her family's frequent trips to Maine made her familiar with the state, enough to attend college there, leaving her twin sister, parents, and two dogs in Massachusetts. At the University of Maine, she is pursuing a dual degree in Marine Science and Ecology and Environmental Science. To achieve honors along with her two degrees, she is completing this honors thesis project, which she was fortunate to have the opportunity to do as a part of the Van Beneden lab. Laura has had a fulfilling undergraduate career at the University of Maine, where she took advantage of several opportunities that exposed her to different areas of science. She began her research at the Maine Center for Research in STEM Education (RiSE), where she completed a project looking at how gender influences students' interests in STEM fields. She then received an INBRE fellowship to start her research on how arsenic effects vision in zebrafish. During the summer of 2018, she got the opportunity to do research on a NASA flight project in California, so she took a break from her zebrafish research to drive across the country and study nitrous oxide in the San Joaquin Valley of California. After an exciting summer, she returned home to UMaine, and finished this honors thesis for your reading pleasure.