Effects of Lead on Male Fathead Minnow Secondary Sex Characteristics and Behaviors

Anna Bogan

Abstract

Lead, a natural substance found within our environment, had been used in household items in the past. This was the case until it was discovered that lead was causing harm to humans through these everyday objects. The usefulness of the fathead minnow study comes from the data that indicates how much of an impact lead has on its exposed groups. It is important to understand this difference between lead exposed and non-lead exposed to see if lead exposure is a real contributor to the effects humans have seen when they have been exposed to lead. Over the course of two weeks, two tanks containing fathead minnow pairs were observed for male secondary sex characteristics and behavior. Each tank was sectioned off into three chambers, each chamber containing a male minnow, female minnow, and a semicircular PVC pipe. Each day of observation, three of the chambers were observed by a group for a duration of five minutes. It was found that the male fathead minnows that were exposed to lead were hyperactive, aggressive, and lacking in most secondary sex characteristics. These findings helped to support the idea that humans exposed to lead are actually being affected by the lead specifically because similar effects were shown in the lead exposed fathead minnows. Lead was found to be a true contributing factor to the abnormal characteristics seen in fathead minnows, as shown in the results of the study. These results supported the claim that humans are also affected by the exposure of lead due to the similarites in behavior and secondary sex characteristics seen in fathead minnows and humans.

Muskego High School

Introduction

Lead was and is still used and found in many common places and products. Not only is lead found naturally within the earth and soil, but can also be found in everyday products such as pipes and plumbing materials, gasoline, ammunition and batteries. Lead is used for these products because of its corrosion-resistant characteristic. Lead was used to help keep items such as pipes in houses from breaking down through the years, however, this element has proven harmful and sometimes even fatal to humans (1). Lead exposure in children is far more dangerous than any other age group because of their ability to absorb more of the element and their extreme sensitivity to it (2). Lead causes issues such as learning disabilities, delayed physical development, a lack in energy, and aggressive or unnatural behavior. This is because lead, when found in the body, can disrupt the production of blood cells along with absorb the calcium that the body is producing, leading to issues with muscles, bones, and nerves (3). Although lead exposure in children is considered to be the most dangerous, adults can also experience harmful effects of lead exposure. This may include low sperm count in men, mood disorders, high blood pressure, etc. (2). After discovering the true effects of lead, it has been banned from being used in many of the items it was once found in.

With information about the harmful effects lead has on humans, it was important to understand if these effects can also be found in other species, such as the fathead minnow. The use of the fathead minnow study was beneficial in understanding how lead affects different species and if any of these abnormalities are present in more than one of these species. Knowing that lead affects humans in ways such as reduced reproduction rates and behavioral issues, the male fathead minnows were studied based on these findings. With prior information on lead effects on humans, it was hypothesized that the lead exposed male fathead minnows would have

a lower rate of reproduction, act aggressively to those they shared a chamber with, and lack energy.

Materials and Methods

The materials used for the experiment include the following:

- Two tanks to hold the fathead minnows
- Six pairs of fathead minnows (one female and one male for each section)
- 4 dividers
- Water heaters
- Timer
- Data Sheet and writing utensil
- Six PVC pipes cut into semicircles
- Black bag covers for the tanks

Three out of the six pairs of the fathead minnows were exposed to a lead nitrate solution of 1 ppm for two weeks at the University of Wisconsin-Milwaukee before arriving at Muskego High School (4). Once the six pairs of minnows were transported to the school, they were separated into two tanks with dividers: one containing the lead exposed minnows and the other containing the non-lead exposed minnows, or the control group. All students conducting the study were unaware of which tank contained the lead exposed minnows to keep from obtaining biased results. Within each tank were three chambers separated with a perforated divider. Each chamber contained a semicircular PVC pipe, a male fathead minnow, and a female fathead minnow. The minnows were kept on a light cycle in which they were exposed to light for 16

hours and then covered with the black bag to keep the tanks dark for 8 hours (3). Over the course of two weeks, students observed the male secondary sex characteristics and behaviors of each fathead minnow within the tanks. The studied behaviors included nest prep, hover, patrol, chase, and spawn:

- A nest prep is recorded when the male fathead minnow brushes his head pad up against the roof of the semicircular PVC pipe. This is his way of preparing for the minnow eggs.
- A hover is recorded when the male fathead minnow swims/resides in the upper half of the semicircular PVC pipe.
- A patrol is recorded when the male fathead minnow swims/resides outside of the semicircular PVC pipe.
- A chase is recorded when the male fathead minnow goes after or tries to move the female fathead minnow.
- A spawn is recorded when the male and female move together in an attempt to reproduce.

Each time a chamber was observed, these behaviors were observed and recorded over a period of five minutes. At the end of the study, the number of times each behavior was observed was averaged, the standard error of the mean was calculated, and a graph was created. In addition to this, a Fisher Exact Test was done to determine whether the male secondary sex characteristics observed between the lead and non-lead exposed minnows were statistically significant. These calculations were done for both the lead exposed and non-lead exposed fathead minnows.

The male secondary sex characteristics include head pad, dorsal fin spot, side bars, and tubercles. Each time a chamber was observed, these characteristics were recorded. The percent of

fathead minnows displaying each characteristic was calculated and put into a graph. These calculations were done for both the lead exposed and non-lead exposed fathead minnows. A diagram showing these characteristics is shown below in **Fig 1.**

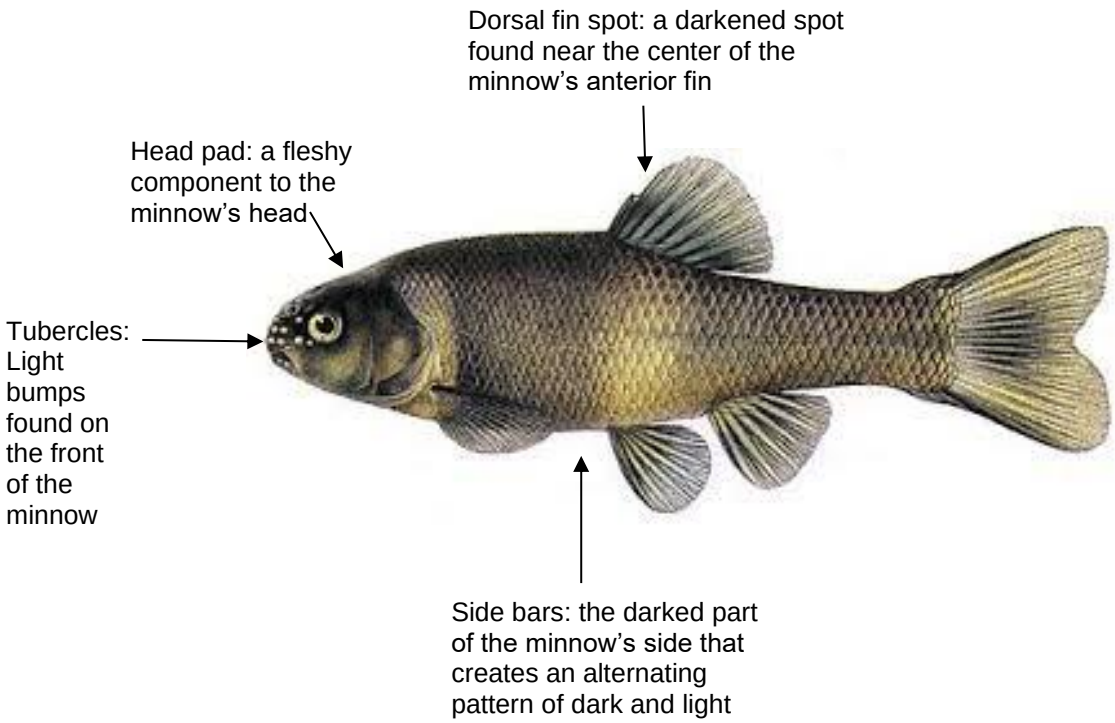

Fig 1.

Results

Tank number one in this study contained the fathead minnows that were exposed to the lead nitrate solution two weeks prior at the University of Wisconsin-Milwaukee. Tank number two in this study contained the fathead minnows that were not exposed to lead and were considered the control group of the experiment. The control group was crucial to the experiment because it allowed the two groups to be compared later. The independent variable was the exposure of lead to the minnows while the dependent variables were the display of male secondary sex characteristics and the behaviors observed during the five minute intervals. The independent and dependent variables are related because the data collected showed that on

avereage, the lead exposed minnows displayed fewer than or an equal number of secondary sex characteristics than the fathead minnows that were not exposed to lead. The overall idea of the experiment was to identify if there was a connection between lead exposed male fathead minnows and their secondary sex characteristics and reproductive behaviors.

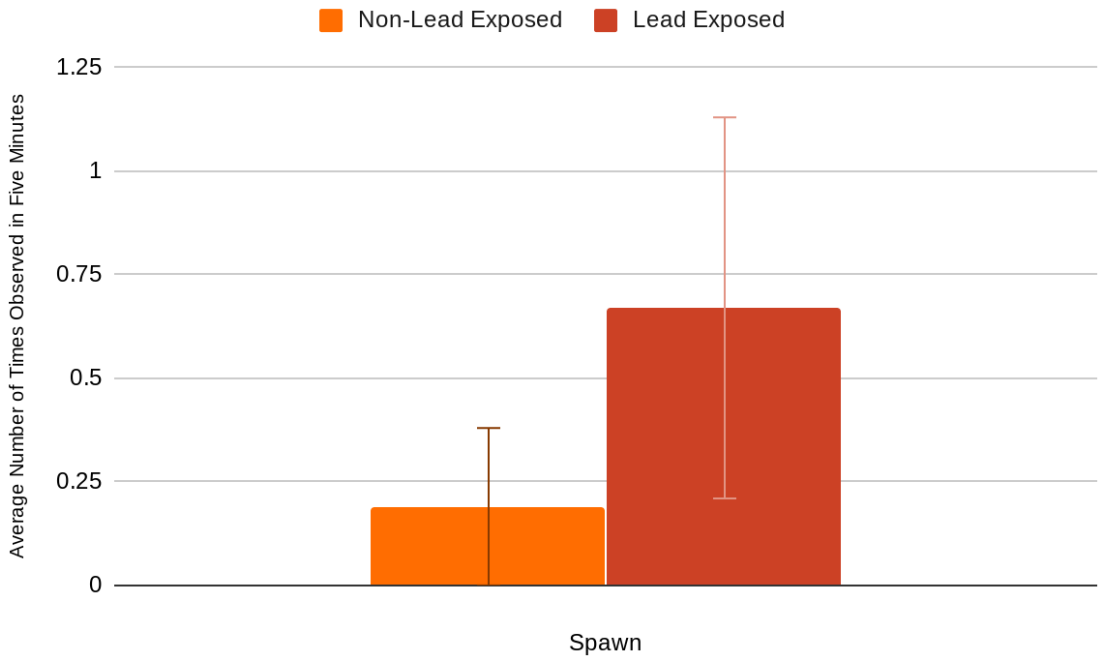

Fig 2.

Fig 2. This graph demonstrates the relationship between the average number of times the lead exposed and non-lead exposed minnows spawned. The error bars overlap, demonstrating that there is not a significant difference between the lead and non-lead exposed fathead minnows that display this behavior.

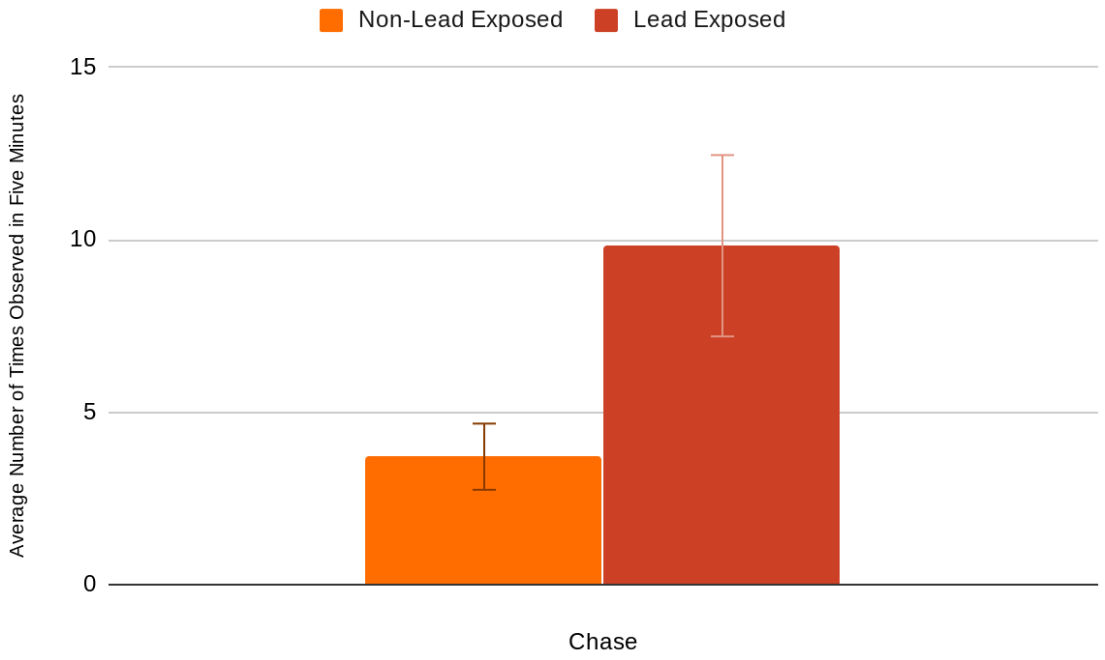

Fig 3.

Fig 3. This graph demonstrates the relationship between the average number of times the lead exposed and non-lead exposed minnows chased the female minnow in their chamber. It is shown that the average number of chases by the lead exposed minnows is over two times greater than the average number in the non-lead exposed minnows. The error bars on the graph show the statistical significance in this behavior.

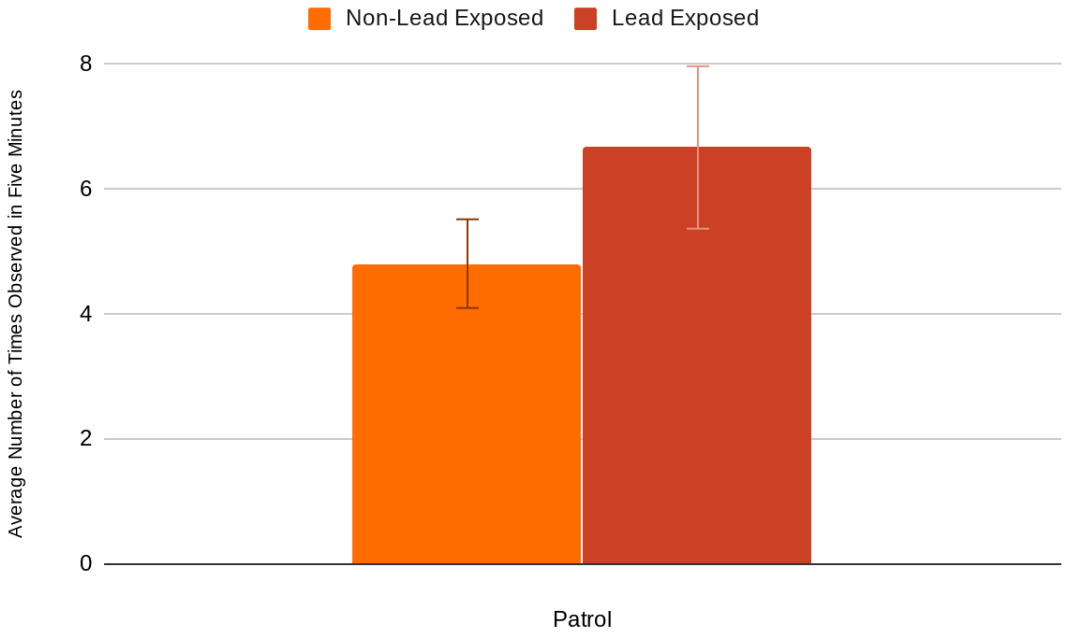

Fig 4.

Fig 4. This graph demonstrates the relationship between the average number of times the lead exposed and non-lead exposed minnows patrolled outside of their provided semicircular PVC pipe. The error bars on the graph show that the difference is not statistically significant for this behavior.

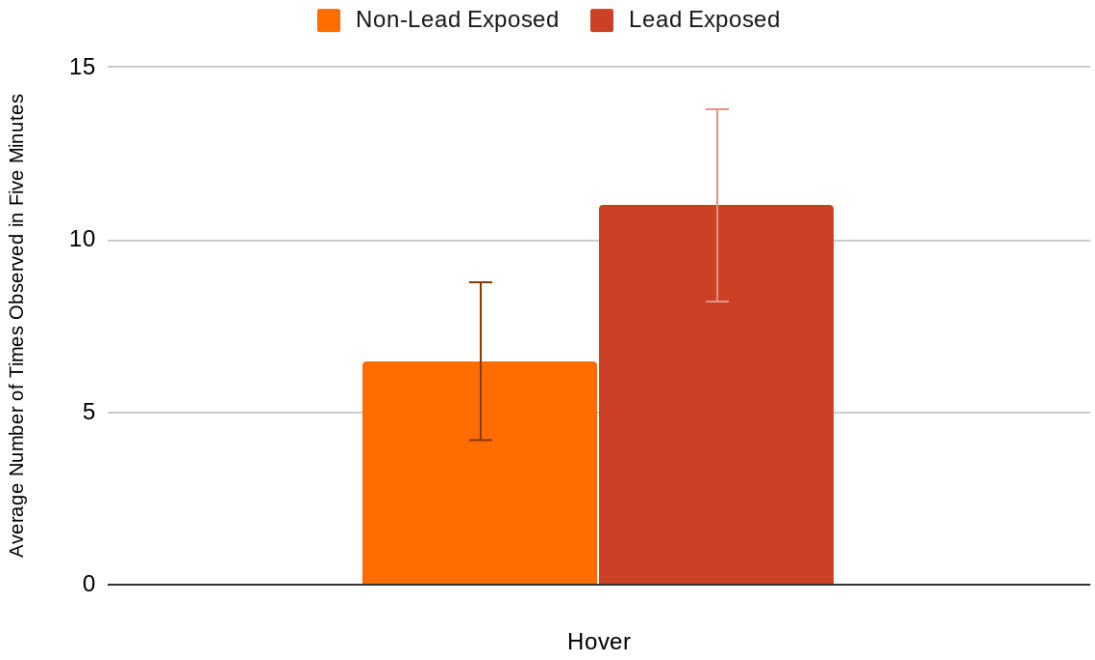

Fig 5.

Fig 5. This graph demonstrates the relationship between the average number of times the lead exposed and non-lead exposed minnows hovered within their provided semicircular PVC pipe. The error bars demonstrate that the difference is not statistically different for this behavior.

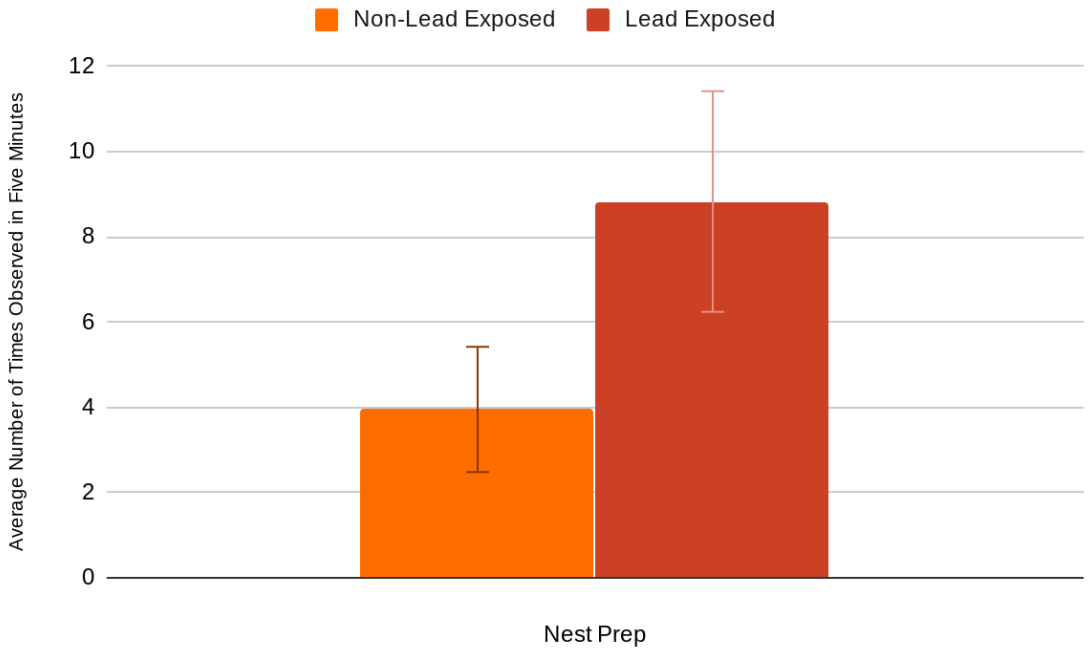

Fig 6.

Fig 6. This graph demonstrates the relationship between the average number of times the lead exposed and non-lead exposed minnows prepared their nest within their provided semicircular PVC pipe. It is shown here that the average number of nest preps by the lead exposed minnows is over two times greater than the average number of hovers by the non-lead exposed minnows. The difference in this behavior is statistically significant as shown by the error bars on the graph.

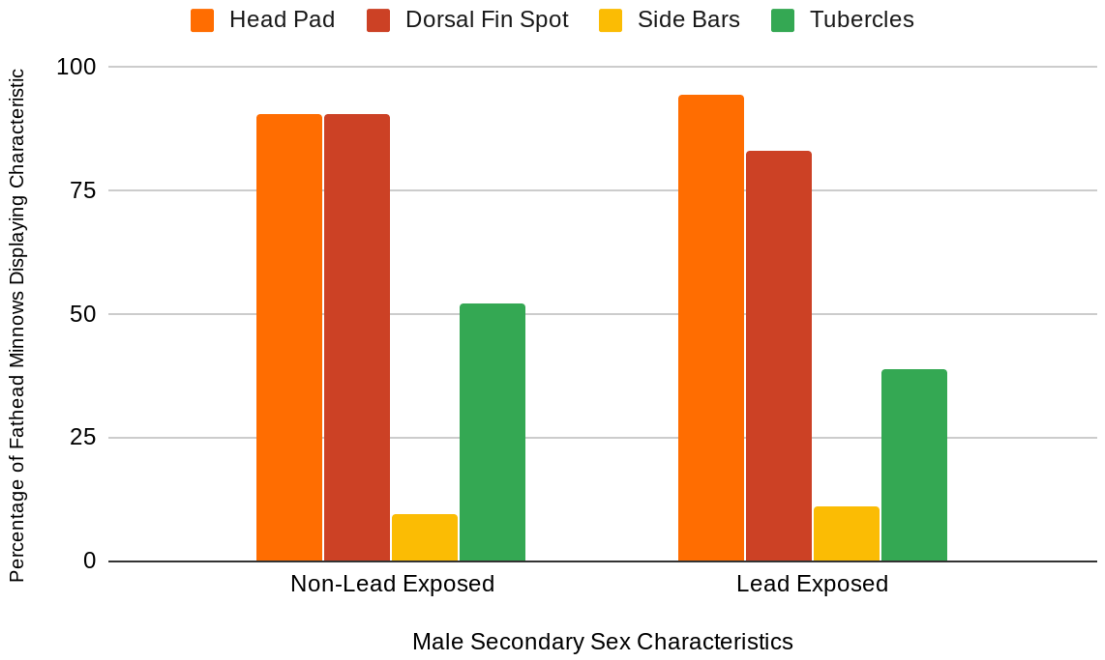

Fig. 7

Fig 7. The graph above shows the percentage of male fathead minnows displaying the secondary sex characteristics in both the lead exposed and non-lead exposed male minnows. The head pad was seen to be slightly more prominent in the lead exposed male minnows. On the other hand, the rest of the secondary sex characteristics, dorsal fin spot, side bars, and tubercles, were observed either nearly the same or a greater number of times in the non-lead exposed male minnows. Although some characteristics were observed more in the non-lead exposed tanks than the lead exposed tanks, the Fisher Exact Test done demonstrated that there is no statistical significance in any of the characteristics.

	Head Pad	No Head Pad	Total
Non-Lead Exposed	19	2	21
Lead Exposed	17	1	18
Total	36	3	39

Fisher's exact test

The two-tailed P value equals 1:0000
The association between rows (groups) and columns (outcomes) is considered to be not statistically significant.

	Dorsal Fin Spot	No Dorsal Fin Spot	Total
Non-Lead Exposed	19	2	21
Lead Exposed	15	3	18
Total	34	5	39

Fisher's exact test

The two-tailed P value equals 0:6466
The association between rows (groups) and columns (outcomes) is considered to be not statistically significant.

	Side Bars	No Side Bars	Total
Non-Lead Exposed	2	19	21
Lead Exposed	2	16	18
Total	4	35	39

Fisher's exact test

The two-tailed P value equals 1:0000
The association between rows (groups) and columns (outcomes) is considered to be not statistically significant.

	Tubercles	No Tubercles	Total
Non-Lead Exposed	11	10	21
Lead Exposed	7	11	18
Total	18	21	39

Fisher's exact test

The two-tailed P value equals 0:5233
The association between rows (groups) and columns (outcomes) is considered to be not statistically significant.

Fig 8. The images shown above are the calculations from a Fisher Exact Test. Each characteristic was calculated to determine if there was a statistical significance in the male secondary sex characteristics between the lead and non-lead exposed groups. It is shown that for each characteristic there is no statistical significance.

Discussion

At the beginning of the study, it was hypothesized that the lead exposed male minnows would, on average, display fewer secondary sex characteristics than the male minnows that were not exposed to lead. It was also hypothesized that the male minnows that were exposed to lead would have a decrease in their sexual drive, act aggressively towards their mates, and lack energy. At first glance, the graph displaying the secondary sex characteristics seemed to show that the characteristics were overall more prominant in the non-lead exposed minnows. However, after a Fisher Exact Test was done, it was shown that none of the secondary sex charcteristics were statistically significant between the two groups. One piece of the hypothesis that matched the results was the thought that the male minnows would be aggressive. This trait was shown

through the “chase” behavior. The males exposed to lead had a high average of chases after their female companion during observation, which explains the idea that the males would show high levels of aggression when exposed to lead. The part of the hypothesis that didn’t match the results was the idea that the minnows would lack sexual maturity. In fact, the opposite was observed. It was noted in qualitative information that the minnows exposed to lead were “flinchy” and on “high alert.” Although this outcome did not match that portion of the hypothesis, it matches research that was discussed earlier such as intellectual development issues and general behavior issues. There would have to be more research done on the fathead minnows to conclude that being “flinchy” and on “high alert” would match abnormal characteristics and behavior found in humans exposed to lead.

Though the study matched some of the hypothesis, there were a few errors that could have led to skewed data. One of these errors comes from not recording perfectly accurate data. Some of the male minnows moved quickly and performed several behaviors in sequence, which made it difficult to tell which behaviors they were displaying when observing. It is possible that each behavior was counted more or less times than it should have been counted. Another limitation of this experiment is that of the number of male fathead minnows observed. This study could have easily been made more accurate with more time to study a larger number of male minnows. More minnows studied means a greater variability and more accuracy when it comes to averages and percentages that are being compared.

The reason for studying fathead minnow behaviors when exposed to lead is very relevant today. In recent years, it has been found that lead exposure in humans can prove very harmful. Lead was being found in common household items that was causing intellectual and developmental issues in infants and children along with causing serious health problems in

adults. Not only did this study show scientists that lead exposure can affect many types of animals, but also showed that if lead can affect a small and seemingly unharmable creature, such as the fathead minnow, the presence of lead in humans would also prove detrimental. The issue with lead exposure on various species has been highlighted through this important study.

References

1 "Lead - Element Information, Properties and Uses: Periodic Table." *Lead - Element Information, Properties and Uses | Periodic Table*, 2020, www.rsc.org/periodic-table/element/82/lead.

2 "Learn about Lead." *EPA*, Environmental Protection Agency, 12 Aug. 2019, www.epa.gov/lead/learn-about-lead.

3 "Lead Poisoning (for Parents) - Nemours KidsHealth." Edited by Kate M. Cronan, *KidsHealth*, The Nemours Foundation, Mar. 2019, kidshealth.org/en/parents/lead-poisoning.html.

4 "Integrating Physiology and Behavior: Using Fathead Minnows to Model the Effects of Environmental Agents." National Institute of Environmental Health Sciences (NIEHS), 2012.

5 "Introduction: The Fathead Minnow." *Digital Atlas of Fathead Minnow Histology: RESPIRATORY SYSTEM*, aquaticpath.phhp.ufl.edu/fhm/intro.html.