Abstract

In recent years, there has been research conducted about the harmful effects of lead, which is a naturally found element, but has also been used in plumbing, paint, and glass. Although this research shows lead exposure is harmful it fails to show the differences in the effects in the natural environment. Fathead minnows were observed in a blind study over two weeks with two control tanks and two experimental tanks. One control tank had non lead exposed fish in a tank with dividers, one control tank had non lead exposed fish without dividers, the other tank had lead exposed fish in a tank with dividers, and the last tank had lead exposed fish with no dividers. The data collected suggests that exposure to lead decreases both secondary sex characteristics and reproductive behaviors. This research suggests that adverse effects can arise from lead exposure in natural environments, but more research should be done to determine the spectrum of effects.

Introduction

Previously, the use of lead was common in everyday items such as paint, gasoline, inexpensive metal jewelry, and drinking water pumped through leaded pipes. It is found naturally in the soil, and research has shown the detrimental effects lead exposure has on humans. Children under the age of six are especially at risk to the effects of lead, because their growing bodies absorb the metal more than adults. The increase of lead exposure can lead to developmental issues, such as a decreased ability to learn, behavioral problems, speech and language delays, and seizures (“About Lead Exposure”). Additionally, there has been research on the effects of fish exposed to lead. Research has found that fathead minnows exposed to lead

have a decrease in reproduction overall. The lead accumulated in the testes and ovaries, which decreased spermocyte production and the ovaries were smaller in size compared to control fish. Therefore, the number of eggs deposited decreased compared to the fish not treated with lead. Furthermore, male fathead minnows exposed to lead were found to display fewer secondary sex characteristics than the males that were not exposed (Weber, 1993).

Lead can be exposed to waterways in various different ways. The lead used in gasoline can be emitted through the fuel emission, and it ends up in the environment. Soil that is in close proximity to highways or frequently used streets contain more lead because of the exposure to lead dust, which can stay in the environment indefinitely. Crop enhancers, improperly disposed batteries, and machinery that contains lead can cause lead to leak into the soil and surrounding environment ("How Does Lead Affect Our Environment?"). Fathead minnows live in ponds, lakes, and streams in the environment, so it is important to study the effects that exposure to lead has on them. An experiment was designed using *Pimephales promelas*—commonly known as fathead minnows—to test the effects on lead exposure on fish contained within dividers and fish not contained within dividers. Fathead minnows are known to be socially facilitated breeders, which means they tend to breed more when in groups ("Integrating Physiology and Behavior"). Therefore, it was hypothesized that male fathead minnows exposed to lead would display fewer reproductive behaviors, and less breeding would occur in both the fish in tanks with and without dividers.

Materials and Methods

The materials used in this experiment include the following:

- 12 pairs of fathead minnows
- 4 ten gallon tanks
- 4 dividers
- 12 semicircle pieces of PVC
- 4 water heaters for aquarium use
- 8 filters intended for aquarium use
- Timers
- Data sheet and pencil
- 4 black covers

Twelve pairs of adult fathead minnows were exposed to lead for two weeks at the Aquatic Animal Facility of the University of Wisconsin-Milwaukee Children's Environmental Health Sciences Center. The minnows were exposed to a lead chloride solution at a concentration of 1 ppm. The minnows were kept on a photoperiod of 15 hours of lights on, and 9 hours of lights off. The fish were then transported from the University of Wisconsin-Milwaukee to Muskego High School, and allowed to acclimate for three days before being observed. The fathead minnows were placed into four different tanks, two of which did not have dividers to separate the pairs (Fig. 1 & 2). All four tanks had three PVC breeding chambers, one aquatic heater, and two filters. Two of the tanks contained lead exposed fish, but only one tank of the non exposed and lead exposed had dividers. The tanks that had dividers had one pair of minnows in each divided section, one male and one female. The tanks without dividers had three males and three females in total all together. The minnows were on a regulated exposure of 16 hours of

light, and 8 hours of dark. This was maintained by the use of the black tank covers. This was a blind study, as none of the observers knew which tanks contained the lead exposed fish. The class then observed the fathead minnows for two weeks to observe any male reproductive behavior and male secondary sex characteristics. The male reproductive behaviors recorded included nest prep, hover, spawn, patrol, and the chasing of female minnows:

- A nest prep is where a male minnow uses his head pad to prepare the top of the breeding chamber.
- A hover is when the male is in the top half of the breeding chamber.
- A spawn is when a male flips over a female in an attempt to breed.
- A patrol is where a male leaves the breeding chamber to eat, or to swim around.
- A female chase is when the male minnow attempts to move the female into the breeding chamber.

These behaviors were recorded in pairs over the span of five minutes. Each behavior was then averaged and the standard error of the mean (+/- 1 SEM) was calculated to see if there was statistical significance between the lead exposed fish and non exposed fish in both the divided and undivided tanks. The male secondary sex characteristics were also observed. These include tubercles, the dorsal fin spot, side bars, and a head pad (Fig. 3). Occasional pictures were taken to observe any changes in sex characteristics or unusual behavior (Fig. 4 & 5).

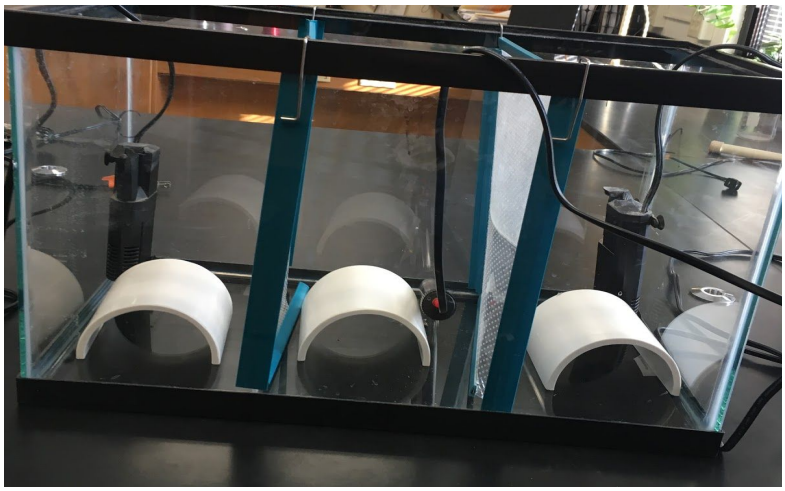

Fig. 1. The tank setup with dividers before the water and minnows were added.

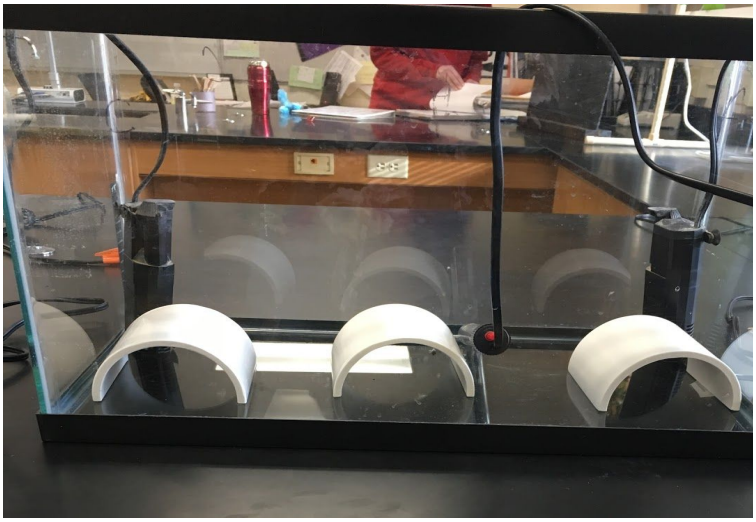

Fig. 2. The tank setup without dividers before the water and minnows were added.

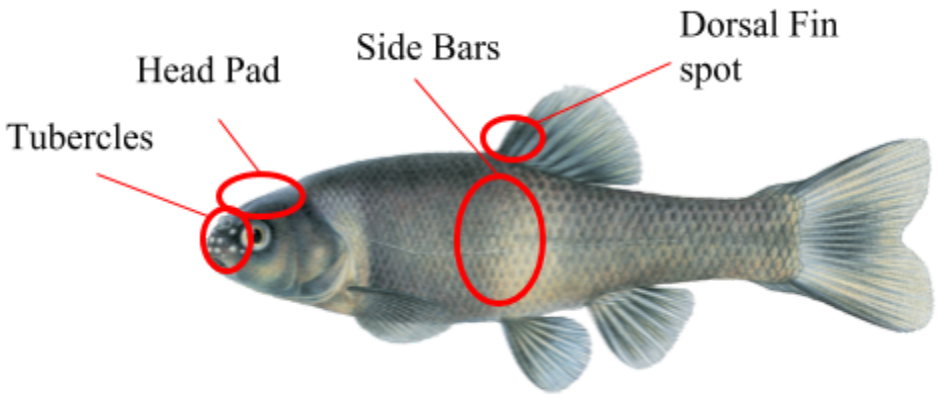

Fig 3. A diagram of a male fathead minnow displaying prominent secondary sex characteristics.

Fig 4. A male fathead minnow with prominent secondary sex characteristics from the non lead exposed tank with no dividers.

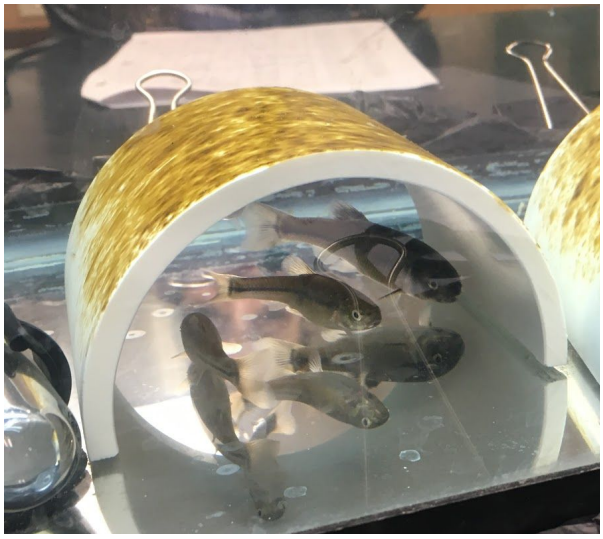

Fig. 5. A male fathead minnow in a breeding chamber with two female minnows. This tank was exposed to lead and had no dividers.

Results

The first tank was not exposed to lead and had dividers. The second tank was exposed to lead as lead chloride at 1 ppm for two weeks before being observed and had dividers. The third tank was not lead exposed and did not have dividers. The last tank was exposed to lead as lead chloride at 1 ppm for two weeks before being observed with no dividers.

The independent variables in this experiment were the lead exposure and the dividers being present in the tanks. The dependent variables were the number of times the male secondary sex characteristics were observed and how many times the male reproductive behaviors were observed. The reproductive behavior and secondary sex characteristics observed from the first tank can be compared to the tanks that were lead exposed with and without dividers, and the non lead exposed tank without dividers. The aim of this experiment was to test the effects of lead exposure on reproductive behavior of socially facilitated breeders.

The graphs below show the average number of times the male secondary sex characteristics were observed over the course of two weeks, and the average number of times each of the male reproductive behavior was observed over the course of five minutes. The graph that displays secondary sex characteristics shows that the minnows exposed to lead had a significant decrease in side bars and tubercles. However, both the lead exposed and non lead exposed fish displayed head pads and the dorsal fin spot. The graphs that show the individual male reproductive behaviors showed a difference in only some behaviors between the lead exposed and non lead exposed.

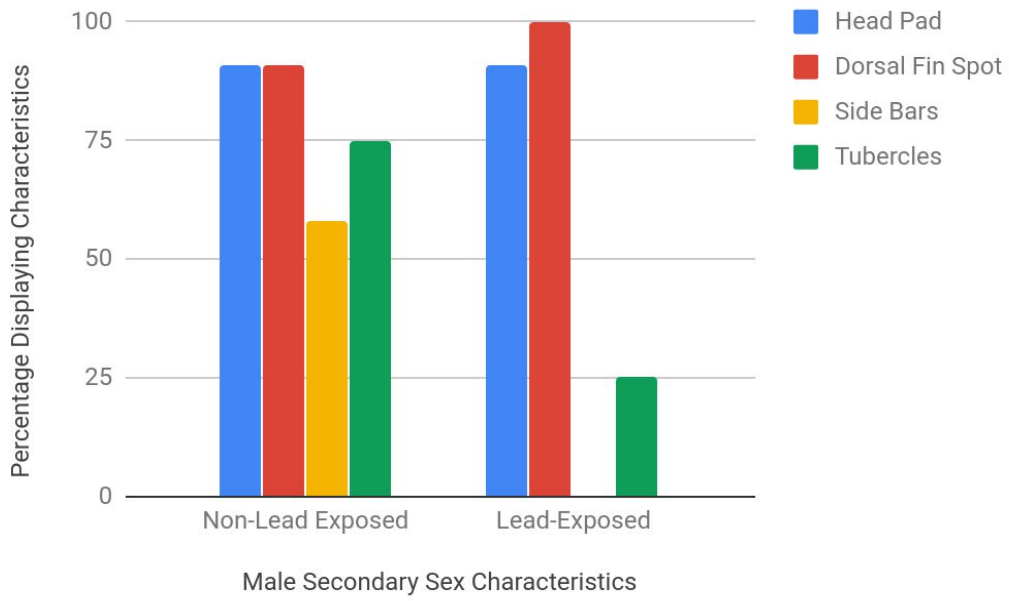

Fig. 6. This graph shows the percent number of male minnows that displayed the characteristics. There is about a 50% difference rate in the number of minnows exposed to lead and non exposed minnows displaying both side bars and tubercles. The percentage of minnows displaying head pads and dorsal fin spots were about equal between non lead exposed and lead exposed fish.

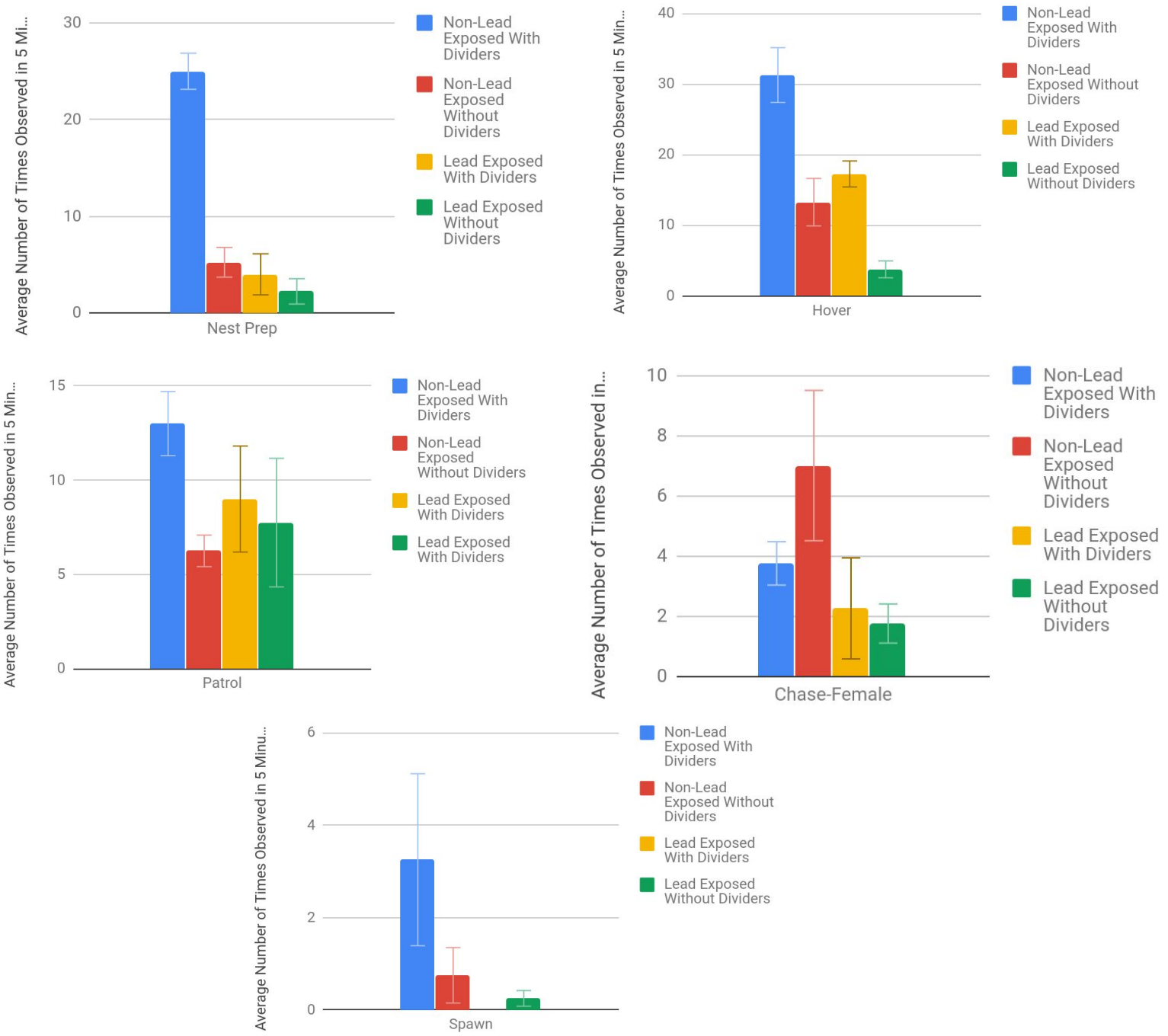

Fig. 7. These graphs show the average number of times these behaviors were observed within a five minute observation period with error bars that signify +/- 1 SEM, with a 68%

confidence interval. The graph that displays the nest prep behavior shows that the differences in the lead exposed with dividers and the non lead exposed with dividers is statistically significant. The differences between the minnows in the non lead exposed and the lead exposed tanks without dividers are also statistically significant. The graph that displays the hover behavior demonstrates that the lead exposed without dividers and the non lead exposed tanks without dividers is statistically significant. The graph also shows the average number of times the non lead with dividers displayed this behavior was a significant amount above the non lead exposed with dividers. The graph that displays the spawning behavior shows that the there is a significant increase in the number of times the behavior was observed in the lead exposed without dividers as opposed to the tank that was not exposed with dividers. Again, it is also shown that the average number of times displayed in the non lead exposed tank with dividers is statistically more than the non lead exposed tank without dividers. The graph that shows the patrol behavior did have overlaps due to the fact that patrolling is not as impacted by testerone levels. In the final graph, which shows the average number of times the male minnows chased a female there was no data that was statistically significant comparing the lead exposed groups or the non lead exposed groups together.

Discussion

Overall, the fish that were exposed to lead and in a tank without dividers showed the fewest number of reproductive behaviors. The lead exposed fish also displayed around 50% less of the secondary sex characteristics of the side bars and tubercles. The non lead exposed fish that were in tanks with dividers showed the highest number of times observed for the reproductive behaviors. Therefore, this reflects the hypothesis that fish that were exposed to lead would

display fewer of the secondary sex characteristics and the male reproductive behaviors in both the tanks with and without dividers as opposed to the non lead exposed fish. While both groups of fish that were in tanks without dividers displayed fewer reproductive behaviors, the fish that were lead exposed showed fewer behaviors than the non lead exposed fish.

Although this experiment had results that supported the hypothesis, many errors and limitations could have been fixed for further replication. A major error that this experiment had was that three fish observed from the lead exposed group died, and one fish observed from the non lead exposed group also died. In order to have accurate results, the same fish should have been observed throughout the whole span of two weeks. The experiment should have been restarted when new fish arrived to allow for accurate results and observations. Another limitation that this experiment had was the observation period. The fish in each tank were only observed every two days, for five minutes each. This may not have allowed for comprehensive data, as the fish at any time could be displaying different behaviors than the five minutes they were observed.

Although there has been research done on how lead impacts humans and their development, there haven't been many studies done on how lead impacts a socially facilitated breeder. As lead exposure becomes an increasing problem for those around the world, it's important to remember the lead exposure is not limited to humans. The data demonstrated by this experiment suggests that those exposed to lead demonstrate a decrease in both sex characteristics and typical male behavior, which may lead into insight about how lead affects humans. Further experiments should be conducted in order to gain a better understanding of how lead exposure effects socially facilitated breeders when placed in a group.

References

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