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The Effects of Lead on Male Fathead Minnow Reproductive Behaviors

Abstract

Lead is a natural metal that is found in many different products and sources that humans utilize, which then makes it a cause for harmful neurological and bodily impacts. Many advances have been made to reduce the impacts of lead poisoning, including the development of DMSA, which greatly aids in the extraction of lead from the body. In order to observe the effects of these two substances on reproductive behaviors, our class performed an experiment utilizing fathead minnows. Four different tanks involved four different extents of exposure: only DMSA, only lead, DMSA with previously exposed individuals, and neither substance. Over two weeks, every other day, we observed the reproductive characteristics and behaviors of male fathead minnows under these conditions. Based on the collected data, I can conclude that lead has a detrimental effect on the reproductive behaviors of fathead minnows. The observations showed that actions such as nest preparation, hovering, and spawning were more common in controlled tanks, while patrols and chases were more prevalent in experimental tanks. These results can be connected to the health concerns that lead implicates, and how important preventative measures against lead poisoning are in today's society.

Introduction

Lead is a naturally occurring metal that is used in processes such as mining and manufacturing, as well as products such as car batteries, pipes, paints, and ammunitions. Because of the widespread accessibility to these items, lead can easily find its way into an organism's body. By inhaling lead particles or ingesting contaminated food or water, lead can accumulate in a person's organs and bones (World Health Organization 2016). From there, lead can affect the nervous system, the production of hemoglobin and myoglobin, and the urinary tract. In children, the nervous system is the most vulnerable, and lead can cause significant behavioral and developmental issues, such as decreased IQ and cognition.

Other effects across generations include lead's interference with vitamin D production and iron utilization in the production of heme. Although lead has been found to be detrimental to reproductive capabilities in various fish species, including fathead minnows (Weber 1993), the impact of lead on human reproductive systems has not been widely or effectively studied (Lowry 2010).

The effects of lead poisoning can be fatal, but there are effective measures that are currently being taken to reduce these effects. The most preferred method for reducing the impact of lead poisoning is known as chelation therapy, in which chelating agents attach to metals to form complex structures and are excreted from the body. One of the most common and effective agents of chelation therapy is known as meso-2, 3-dimercaptosuccinic acid, or DMSA for short. This agent is the most effective in attaching to and removing toxic metals from the body through the urinary tract, and it has low toxicity and side effects (Flora and Pauchari 2010).

Various studies have been performed to analyze the effects of lead on the nervous system, but not much is known about lead's possible impacts on the mental and physical features of the reproductive system in humans. Although legislations have been passed within the United States that limits the usage of lead in accessible materials, such as gasoline, paint, and plumbing, there are still areas, including older homes in urban areas, that run the potential risk of exposing prospective parents and children to lead (Environmental Protection Agency 2016). Therefore, it is important to consider and analyze the possible effects that lead could have on the reproductive systems of people. Through this rationale, an experiment was performed using fathead minnows, a good representative organism in experiments, to analyze the effects of lead on male reproductive behaviors. It was hypothesized that if one group of fathead minnows are exposed to lead and another group is exposed to DMSA, then the fathead minnows within DMSA will show improved reproductive behaviors because the chelating agent would release lead from the system and allow the recovery of reproductive habits.

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Materials and Methods

For this experiment, we utilized the following:

- 24 fathead minnows (12 males and 12 females; one half of these should be exposed to lead at least two weeks before the experiment)
- DMSA
- 4 water tanks with filters, divided into 3 sections, equipped with one breeding chamber each
- Data sheets (to keep track of physical features and breeding behaviors)
- Timer

Each tank in this experiment contained one of four different variables: lead, DMSA, DMSA on previously exposed fish, or neither. The minnows that were exposed to lead prior to the experiment (six males and six females) were contained in water that held 1 ppm of lead nitrate for two weeks. In the DMSA tanks, 20mL of 10Mm DMSA (within 0.1M NaOH in 20gal of water) were added every day. A male and female minnow pair were placed in three different sections of the tank, as well as a breeding chamber for reproductive behaviors to occur. The fish were fed at the same time every day, and the tanks were covered when observations were not occurring in order to maintain sixteen hours of light and eight hours of darkness daily (Weber et al. 2013) . Since harmful chemicals were being utilized, our teacher took care of tank maintenance. Every other day for two weeks, students were assigned into groups of four, in which they would split into partner groups and each observed three different tank sections a day. On data tables, the male characteristics (dorsal fin spot, head pad, side bars, tubercles) and reproductive behaviors (nest preparation, hover, patrol, chase, spawn) would be counted for five minutes (Weber et al. 2013). After the assigned three tanks were observed, the partner groups would exchange their data. This process would be repeated until all the tanks were observed three times over the course of two weeks. Reproductive characteristics and behaviors were compiled, averaged, and inserted into tables and graphs.

Standard error was also used to determine the statistical significance of the data. However, these calculations may have varied between groups.

Results

Tank red 1 was exposed to DMSA only. It served as a control tank to see if DMSA alone would have any affect on minnows who were not exposed to lead previous to the experiment. Tank red 2 was not exposed to lead or DMSA; therefore, it also served as a control tank to show how frequent reproductive behaviors occurred in natural settings. Tank blue 1 was also exposed to DMSA, but the minnows were under the influence of lead previous to the experiment. This tank thus served as an experimental tank to see if DMSA would improve the rate of reproductive behaviors in previously exposed male minnows. Finally, tank blue 2 was exposed to lead; thus it was also an experimental tank that observed the normal effects of lead on male minnow reproductive behaviors.

The independent variables were the mediums of exposure in the tanks (lead, DMSA, both, neither). The dependent variable was the average number of reproductive behaviors that were performed during the duration of the experiment. The controls included the tanks that were exposed to DMSA (tank red 1) and neither lead nor DMSA (tank red 2). These controls were used to compare the reproductive behaviors under normal circumstances with those in impactful substances.

The results that follow include the total reproductive behaviors of each tank section and the average behaviors of each tank. The graph shows the compiled averages of each tank, including error bars to show the significance of the data. The graph shows that actions such as nest preparation, hovering, and spawning began to decrease as the presence of lead grew. However, other behaviors, such as chase and patrol, varied greatly in all four tanks and seemed to be most prevalent in the lead-exposed tanks. No major differences in the male physical characteristics were observed.

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Key	DMSA Only (Red 1)	Control (Red 2)	DMSA and Lead (Blue 1)	Lead Only (Blue 2)

	Red 1a	Red 1b	Red 1c	Red 2a	Red 2b	Red 2c	Blue 1a	Blue 1b	Blue 1c	Blue 2a	Blue 2b	Blue 2c
Dorsal Fin Spot	X	X	X	X	X	X	X	X	X	X	X	X
Head Pad	0	0	X	X	X	X	X	X	X	X	X	X
Side Bars	X	X	X	X	X	X	0	0	0	0	X	0
Tubercles	X	X	X	X	X	X	0	X	X	0	0	0

These are the observed male characteristics for all the tank sections that were observed during the experiment. X's show the presence of a characteristic, while O's show the absence of a characteristic.

	1a	1b	1c	2a	2b	2c	1a	1b	1c	2a	2b	2c
Nest Prep	67	22	64	14	44	16	31	16	4	6	3	10
Hover	68	29	80	33	64	27	45	17	17	23	15	34
Patrol	13	23	13	20	6	3	15	29	11	37	39	18
Chase	42	32	9	22	8	9	47	30	12	33	38	44
Spawn	0	19	36	2	0	0	0	0	0	0	0	0

These are the total reproductive behaviors from the males in the various tank sections that were observed every other day for two weeks.

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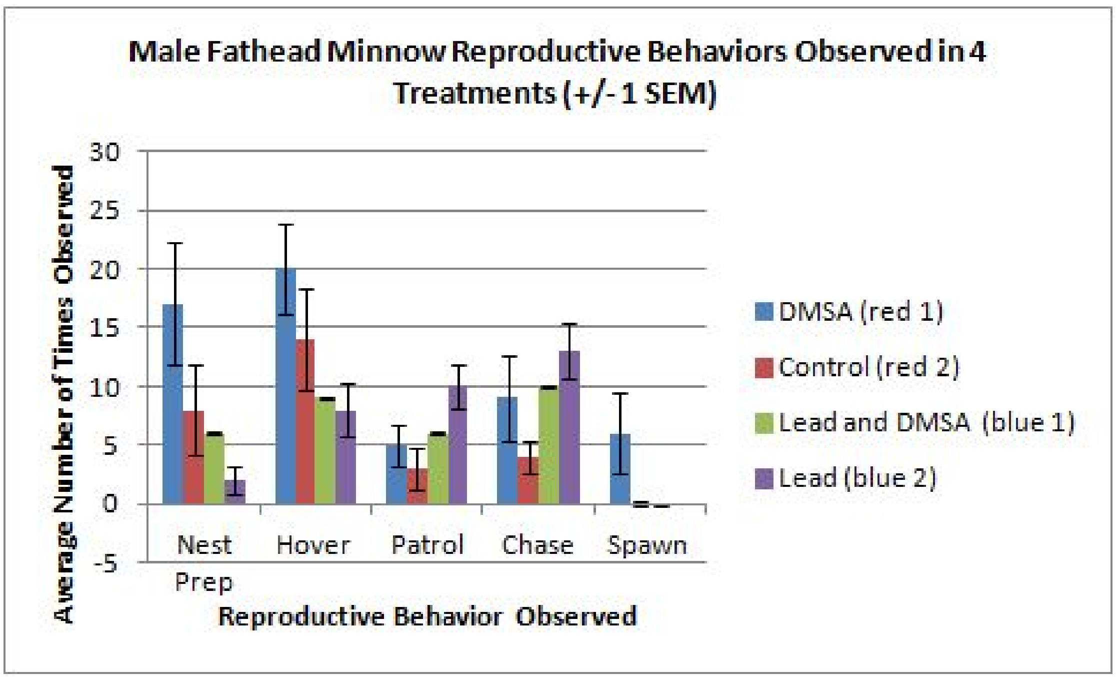
	DMSA (red 1)	Control (red 2)	Lead and DMSA (blue 1)	Lead (blue 2)
Nest Prep	17	8	6	2
Hover	20	14	9	8
Patrol	5	3	6	10
Chase	9	4	10	13
Spawn	6	0	0	0

These are the calculated averages for male reproductive behaviors in all four tanks.



This graph depicts the average male reproductive behaviors in all four tanks over two weeks. Error bars were used to show the significance of the data.

Standard error of the mean was used to determine the significance of the data, as seen in the error bars on the graph above. I can infer that the data I have collected is significant. For example, the control tanks displayed more hovers and spawns than the lead-exposed tanks, which primarily showed patrols and chases. However, the DMSA tanks had increased observations of nest preparations, hovers, and spawns and less observations of patrols and chases. This shows that the DMSA tanks showed dominance over all the other tanks in regards to noticeable reproductive behaviors. These observations therefore implicate significant data, despite overlapping error bars in the graph.

Discussion

Throughout the course of the experiment, the control tanks (red 1 and red 2) were more active in nest preparation, hovering, and spawning than the experimental tanks (blue 1 and blue 2). However, fewer chases and patrols on average were performed in the control tanks. This could be due to the fact that the fish in these tanks took more interest in reproducing, and thus spent more time in these activities that involved the preparing for and laying of eggs. The impact was more noticeable in blue 2, as the DMSA could have flushed most of the lead out of the minnows' bodies and thus healed more of the lead's effects on reproductive and mental capabilities. However, in the experimental tanks, the lead may have affected the neurological processes of the minnows and thus made them less willing to reproduce. This is shown through our data, in which the lead exposed tanks (blue 1 and blue 2) performed more patrols and chases on average, while nest preparations, hovers, and spawns were very low compared to the control tanks. Since the lead had a bigger impact on reproductive and mental processes, the inclination of the males in these tanks to reproduce were inhibited, and thus, fewer reproductive behaviors were performed. I can partly conclude that my observations support my hypothesis, since the effects of lead were detrimental to some of the reproductive behaviors of male fathead minnows, while adding DMSA to previously exposed minnows allowed for improvements to be made in the number of reproductive behaviors that were performed.

This experiment had various limitations and errors that possibly impacted the results. One limitation was that the student groups did not observe every tank and section each day, due to time constraints. Data was only shared within small groups and not with the entire class, meaning that not all behaviors were noted everyday, and different classifications of behaviors and characteristics could have occurred between individuals. Observations were also not performed everyday. Because of this, some tanks could have been more active on days when observations were not occurring, and were therefore not taken note of. An error during this experiment was the secureness of the section dividers within the tanks. Some of these dividers were not securely fit, which allowed some fish to squeeze into other sections of the tank. Because of this, some fish that were observed might have came from another section and performed differently, which could lead to some skewed results. One occurrence of this incident actually made one section's results null. Another potential error of this experiment was the discovery that our water that was being used in all of the tanks had a high pH level. This could have affected our results. If more time was allowed for complete observations and the tanks were made more secure, then this experiment could be improved and yield better results.

The observance of fathead minnows exposed to lead and DMSA has allowed us to see how these agents could impact humans. The detrimental effects of lead on reproduction was observed through the lead-exposed tanks, where the reproductive behaviors of the fathead minnows were affected over time. These observations show that lead interrupts the natural inclination to reproduce that is common in all life on Earth, including humans. Additionally, the improvements to reproductive behaviors through DMSA treatment was also observed. The inhabitants of the DMSA-exposed tank were noted to have gradually improve their performance of reproductive behaviors over time. This demonstrates the rehabilitative abilities of DMSA and how they can relate to the treatment of lead poisoning in humans. Overall, through this experiment, a greater understanding of the importance of lead contamination prevention has been obtained.

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