Developing a Snail Model for Behavior Toxicity Evaluations

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Abstract

As humans expand further and further, we constantly are increasing the impact we have on the environment, especially factoring in how often people pollute and contaminate nature. We often don't understand the full effect we have on our environments and the organisms living there. One example of something we don't understand is heavy metal contamination and how it affects the organisms in contaminated areas. This is a difficult task since there can be tremendous amounts of variations in behaviors of even the same species. This study will help us set a baseline for how two snail species react to food cues. We will expose these snails to food cues and measure the time it takes them to find food and their general activity levels. This will let us have a standard to later compare contaminated snails to in the future and further gauge the effects of heavy metals on snails.

Keywords: Behavior, Food Cues, Snails, Lead, Tar Creek

Introduction

Mining in northeastern Oklahoma started around 1891. Some surface mining occurred before the American Civil War, but mining commercially started when the Peoria Mining Land Company moved into present-day Ottawa County. Many new mines started popping up in the area. One of these operations began on Harry Crawfish's allotment, the future site of Picher, Oklahoma, in 1913 (Everett 2007).

Picher became the largest supplier of lead and zinc in the Tri-State Lead and Zinc District, and one of the top suppliers in the world. Picher produced half of all the lead and zinc used in World War II and put out $20 billion in ore from 1917 and 1947. Picher reached a peak of fourteen thousand men working inside the mines during the boom years (Matthews & Wood 2007).

By the time mining ceased at Picher in 1967, there were over 1,400 abandoned mine shafts and 70 million tons of lead- and zinc-laced chat piles. With no pumps constantly clearing it out, water filled mine shafts and was left to sit and stagnate. The chat piles were left towering over the town and are still there today (Matthews & Wood 2007).

Picher occupied land was originally owned by Quapaw who had leased and then sold it to the mining companies. Since the Quapaw tribe still owned much of the surrounding land and the underground was riddled with mine shafts, new industry never found its way into Picher. As mining started slowing down, the population steadily declined to 1,640 residents by the year 2000. This was a dramatic change since there were 11,187 people employed in the area in 1924, the highest employment year for the Picher mining site (Matthews & Wood 2007). According to the 2010 census, Picher's population had reached 20 residents.

Picher became part of the Tar Creek Superfund site in September of 1983 and still is rated the worst Superfund site in the United States (Matthews & Wood 2007). In 1993, the Indian Health Services (IHS) office in Ottawa County and the Agency for Toxic Substances and Disease Registry (ATSDR) began to evaluate blood lead level tests taken from children at the Ottawa

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County IHS office. They found that 35% of the subjects had elevated blood lead levels (BLLs). The ATSDR investigated nine of the homes of children with elevated blood lead levels and found elevated lead levels in the soil around the nearby homes, the dust being blown off of the chat piles, and the lead paint used inside the homes (U.S. Agency for Toxic Substances and Disease Registry 2004).

The cause of the elevated lead levels of the dust and soil on ASTDR's report was the 70 million tons of mining waste left in huge piles called chat or tailing piles. This mining waste contains dangerous heavy metals such as lead, zinc, and cadmium. These chat piles could contaminate any water around them and seep contaminants into the ground around them. Dust could carry contaminants on the wind to nearby areas and towns (Kansas Geological Service 2005).

In 1973, 76,800 acre-feet of contaminated mine water started to leak from the ground into nearby streams and creeks. The water had turned orange and become contaminated with lead, zinc, and cadmium during the nine years it had been in the mine shafts (Matthews & Wood 2007). The hazardous water now in these waters can collect in the sediment and be washed up onto the banks, further spreading the contamination. Tar Creek is one of these water sources.

Tar Creek eventually empties out into Grand Lake, a 46,500 surface acre lake in northeast Oklahoma. Grand Lake was created in 1940 with the completion of the Pensacola Dam. It is regarded as one of the best bass fishing spots in the region and boasts 1,300 miles of shoreline. Every year it is visited by thousands of fishermen, sailors, skiers, and migratory birds. It is home to a collection of wildlife, including many species of snails (Grand River Dam Authority 2014).

Recent work demonstrates that Grand Lake has elevated levels of lead, zinc, and cadmium at levels threshold for probable hazard to wildlife in 1.5%, 40.3%, and 10.5% of Grand Lake sample sites (Morrison, unpublished data, 2014). However, the two species in this study, an amphipod (Hyalella azteca) and a pond snail (Helisoma trivolvis), experienced an averaged 10% and 2% death rate respectively in Grand Lake conditions. This survival rate is unexpected considering the high levels of heavy metal concentrations.

Often, scientists will use the accumulation of contaminants like heavy metals in organisms to measure the bioavailability of chemicals to the organisms which helps determine the severity of the ecosystems contamination. It is known that snail shells can accumulate heavy metals, like lead, in their shells (F. Pyatt et al. 1997, Beeby and Richmond 1989), and thus snails have often been used as a biomarker for contaminants. Nica et al. (2012) studied the ability of heavy metals to accumulate in a terrestrial environment using the Roman snail (Helix pomatia) and found that species to have the potential to help judge heavy metal pollution levels. Another study looked at a snail species, Lymnaea stagnalis, in an aquatic environment and how well its soft tissues and shell accumulated metals (F. Pyatt et al. 1997).

The toxicity of a contaminant in water depends on many factors. Wren and Stephenson (1991) reviewed previous literature to discern if the acidification of the water effected the toxicity and bioaccumulation of lead in freshwater invertebrates. Datta and Das (2003), also wrote an article discussing many different factors that could potentially change the toxicity of a metal. One factor was hardness with lower lead toxicity levels in water with higher hardness. Another factor was acidity since higher acidity caused higher

bioavailability of metals. The presence of soil sediments also decreases lead toxicity.

This study could help further future research in animal behavior studies but also in the field of toxicology. It could help show people the effects of human interference and call attention to Superfund site projects. Another beneficial point is that with the direction of this research, we could potentially use these snails as a future indicators for contamination, by looking at their behavior or heavy metal accumulation. Some light will also be shed on the unfamiliar subject of snail behavior. Mainly, this study will set the standard for snail behavior that we will compare later trials with, giving more insight into how heavy metals affect these organisms.

Methods

For this experiment, we focused on measuring the time taken to find food and the general activity of the snails. For this experiment, we used Helisoma trivolvis and Physa acuta. Both species are found in freshwater lakes and ponds across the United States, where they can be vulnerable to exposure to heavy metal contamination (Mahler et al. 2006).

The two species were kept in separate tanks under similar conditions. The tanks were aerated and the bottoms were lined with gravel, with water changes done when necessary. The snails were fed as often as necessary until they were fasted for the trials.

Food consumption was restricted either 24 or 72 hours prior to starting trials. We filled each 15 cm petri dish with 150 mL of dechlorinated water and placed these over the quadrant templates. The templates were set up with four concentric circles labeled A, B, C, and D, with A being the outermost quadrant. Each quadrant was then split into 12 regions, except for quadrant D which would be covered in food that would have obstructed the regional lines. We weighed out 100 mg or 300 mg of romaine lettuce for each petri dish. One snail was placed in each dish and left to acclimate for 5 minutes. After acclimation, we moved each snail to quadrant A1 in their respective dishes. Location at time 0 was marked at A1 for their starting location. We placed 100 mg or 300 mg of romaine in the center of each petri dish after the 5 minute acclimation period had ended. We recorded the snails' locations in the petri dishes every minute for 15 minutes. After the trial, each snail was measured with calipers to record their width and length. The snails were then placed in a separate tank to avoid reuse of organisms. Any snail that yielded less than 5 movements between quadrants was thrown out.

Results

Often with behavioral assays, exact answers are hard to discern since there can be many factors on an organism's behavior. The same can be said, if not more so, about snail behavioral studies. Thankfully, we were able to notice a few trends throughout the study.

We were able to compare the two species against each other, as well as groups fed 100 mg of food against groups fed 300 mg of food. Also, we found some significant differences between the snails that were fasted for 24 hours and the snails fasted for 72 hours. We even tried snails with different regular diets, looking at broccoli-fed snails and romaine-fed snails but were not able to find any significant differences in their time to food and activity levels. One of the trends noticed can be seen in Figure 2. It seemed as if Physa that were given 100 mg of food were overall more active than the Physa given 300 mg.

When we looked over the information presented in Figure 4, we saw another trend. The Helisoma that were

fasted for 24 and 72 hours and given 100 mg of food had much lower activity levels than the Helisoma that were given 300 mg from both fasting groups.

We also noticed one very drastic difference. As shown in Figure 5, there is a stark contrast between the 24-hour-fasted Helisoma and the 72-hour-fasted Helisoma when given 100 mg of food. The 24-hour Helisoma took much less time to find the food. This is strange when compared to the 300 mg trials where the 24 hour and 72 hour Helisoma s' times were much closer together.

Discussion

It should be noted that this experiment setup could have been (and probably will be) greatly improved to reduce the risk of the snails finding the food by chance. This could be accomplished by using a Y-maze, a tube setup, or even simply a larger tank. In future experiments, everything should be done to minimize the effects of random chance.

Due to a shortage in snails, many of our sample sizes were different. Also, the sample sizes were even more disturbed when we took out any snail that had showed than 5 movements. We tried to account for this disturbance by using the proportion of snails that found quadrants C or D in Figure 1 instead of the number of individuals. Another discrepancy is that the Helisoma shows trials run with snails fasted for 24, 72, or 0 hours in Figure 4 that shows Helisoma's activity levels, while Figure 2 that shows Physa's activity levels only marks snails that were fasted for 72 hours. This was because the Physa part of the experiment was simply supplemental to our Helisoma data. This should also be fixed in the future.

As shown in Figure 2, there is a difference in activity levels for Physa given different food amounts. This could be explained by Figure 1, which shows that a larger proportion of 300 mg Physa found the food than 100 mg Physa. Since more 300 mg Physa found the food, they would almost certainly not leave the food after finding it, possibly making them seem to have lower activity levels.

The trend found in Figure 4 might be explained by looking at the food cues themselves. The 100 mg Helisoma of both fasting times often found the food faster than the 300 mg Helisoma. This could be because there is so much food cue in the water that the cue diffuses too quickly. This would destroy the gradient the snails might use to find the food, leaving them lost.

The most drastic difference that we were able to discern was on Figure 5. The 24-hour fasted Helisoma found the food much faster than the 72-hour fasted Helisoma, when given 100 mg of food. This goes against what many people would expect, with the hungrier snails taking longer. This could be because the 72-hour fasted Helisoma are very hungry and fatigued at this point making it harder to pinpoint the foods location. They could be able to find the 300 mg faster because the signal might be stronger.

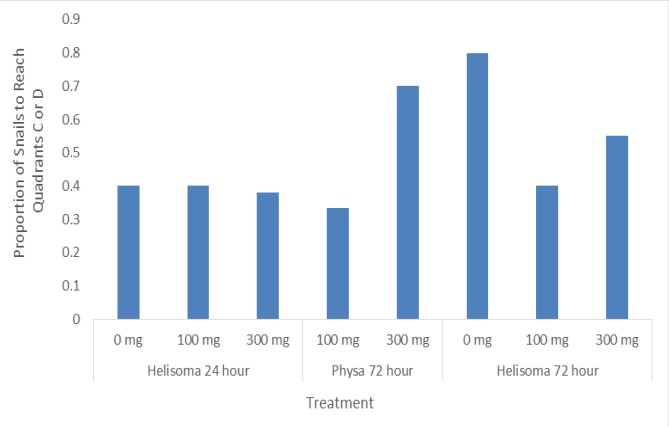

Figure 1 - This figure shows the proportion of each experimental group that reached quadrants C and D, the quandrants that were closest to the food.

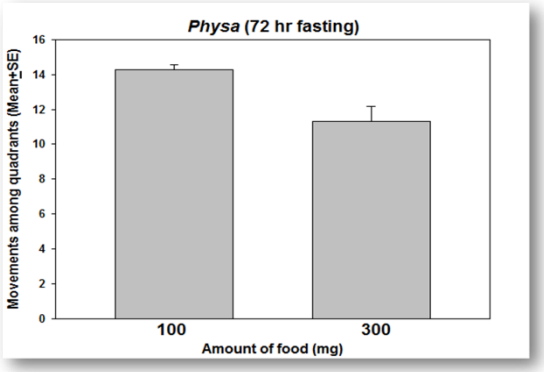

Figure 2 - This figure shows Physa movements when given different amounts of food cue

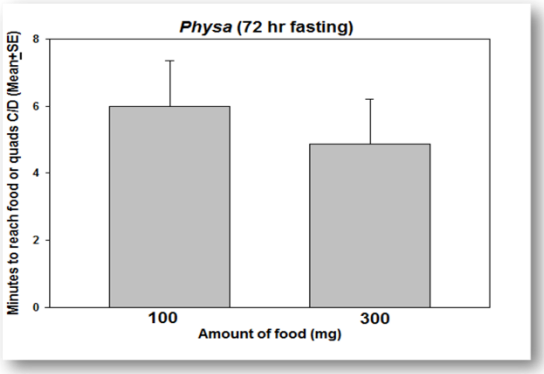

Figure 3 - This figure shows the time for Physa to reach the food using 100mg and 300 mg of food cue

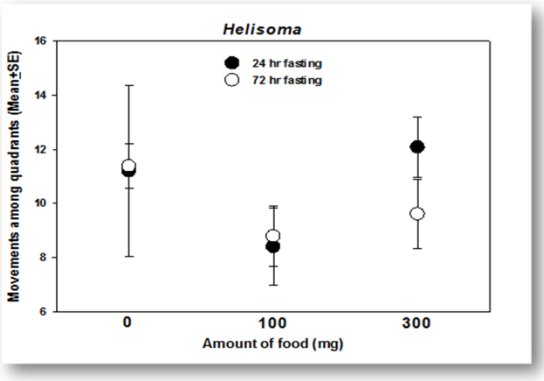

Figure 4 - This figure shows the general activity levels of Helisoma for the different levels of fod cue given

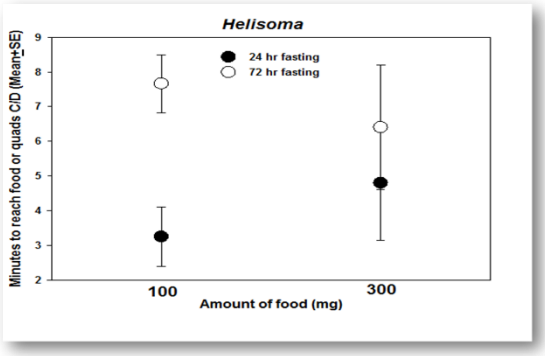

Figure 5 - This figure shows an interaction between fasting time and food amount. The 24-hour fasted snails took much less time to find food than the 72-hour fasted snails, but only at 100 mg of food.

Acknowledgements

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