The Effects of Atrazine on the Agonistic Behavior of Virile Crayfish, *Orconectes virilis*

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Abstract

Atrazine is the most commonly used herbicide throughout the United States and its overuse creates problems for aquatic ecosystems. Crayfish are a keystone species in these aquatic ecosystems and the absence of keystone species can alter an entire ecosystem. Therefore, keystone species like crayfish should be protected from substances leaking into their environment that can harm them, such as atrazine. This study looks at the effects of atrazine on the agonistic behavior of *O. virilis* crayfish. To examine this, crayfish of similar chelae and carapace sizes were introduced to an artificial stream under specific conditions for 24 hours and then the crayfish engaged in a fight. One crayfish would be exposed to atrazine at 200 micrograms per liter for 24 hours and one crayfish would be exposed to normal stream-like conditions. Our results showed no statistical evidence to support our hypothesis that *O. virilis* exposed to atrazine would be less aggressive compared to crayfish unexposed to atrazine. Regardless of our evidence, there are multiple studies that show the negative effects of atrazine on aquatic organisms and thus, atrazine should not be used as heavily as it has been in the United States.

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Introduction

Atrazine is an herbicide commonly used for crop protection. The intent of many monocultural growers is to produce a maximum crop yield, but the herbicides used to control the growth and herbivore consumption of the crops can leak into aquatic environments. When atrazine is introduced to an aquatic ecosystem it can negatively affect the reproductive organs of aquatic flora and fauna and it can disrupt their endocrine system (Fernandez-Domene, 2018). In addition, atrazine has a long half-life, which can cause the atrazine to be carried to reservoirs far away from the initial source of pollution (Fernandez-Domene, 2018). This paper looks at the effects of this atrazine on aquatic ecosystems, specifically crayfish behavior.

Crayfish are a keystone species and atrazine affecting keystone species can alter an entire community structure (Graymore, Stagnitti, and Allison, 2001). This alteration can occur by decreasing the function of reproductive organs in these organisms or by eliminating primary producers in the system that will indirectly affect the survival of the keystone species. Atrazine interferes with the photosynthetic mechanisms of aquatic flora and with decreased growth in plants, the higher organisms on the trophic pyramid in an aquatic ecosystem may decrease, leaving less food for the keystone species (Graymore, Stagnitti, and Allison, 2001).

Crayfish have been used in many atrazine studies to examine the effects of atrazine on keystone species. Crayfish rely heavily on chemoreception, perception of ecological information using chemical stimuli, with agonistic behaviors, such as avoiding predation or physical competing with other crayfish. Chemicals introduced to the environment of the crayfish can reduce sensory receptor functions when they physiologically impair chemosensory systems and thus cause the crayfish to be more easily predated on or defeated by another crayfish in a competitive situation (Edwards, Klotz, and Moore, 2017). The agonistic behavior of the invasive rusty crayfish, *Orconectes rusticus*, has been widely studied throughout the United States since they have been introduced to the area of native crayfish. However, the crayfish species native to Southeastern Michigan, *Orconectes virilis*, has not been as widely studied and therefore, little is known about the influence of herbicides on this species (Hale et al., 2016).

Our study examined the effects of atrazine on agonistic behavior in *O. virilis* under stream-like conditions. We hypothesized that *O. virilis* in high concentrations of atrazine would decrease their ability to defend themselves in the presence of naïve, or unexposed, *O. virilis*. We predict that naïve crayfish should outcompete the crayfish at high concentrations of atrazine. We also predicted that the intensity of a fight between an exposed crayfish and another exposed crayfish would decrease compared to other trials and the scores for each crayfish would be lower than the naïve bouts.

Methods

To determine the effects of atrazine on virile crayfish, we created an artificial stream at the UMBS Stream Lab to conduct our tests in. The stream was separated into two sections and both sections were 120 x 39.5 x 23 centimeters (Figure 1). Additionally, each side of the stream introduced water from the Maple River at similar flow rates. The flow rate of the left section of the stream was constant at 4.70 liters per minute and the right section was constant at 4.32 liters per minute throughout our experiments. Each section of the stream held different conditions, the left section of the stream held naïve conditions, conditions without atrazine, and the right side introduced atrazine to the stream to test exposed conditions. In addition, each section of the stream was split into two with plexiglass barrier to expose four crayfish at a time to their specific conditions.

Once the stream was set, female *O. virilis* crayfish were collected from a boat launch dock on the western side of Burt Lake, called the Maple Bay State Forest Campground, during July of 2018. To eliminate any initial hierarchy, the crayfish were isolated in separate containers to stay in a holding tank until they were needed. The chelae and carapace lengths of the crayfish were recorded to determine which crayfish should be paired to fight in the trials according carapace and chelae sizes (Figure 2). Eleven total trials were performed with the crayfish, the first two trials included our controls that were place in naïve conditions, the next seven trials had the right section of the stream exposed an atrazine mixture to test naïve-conditioned crayfish against exposed, and the last two trials introduced atrazine into both sections of the stream to conduct a trial with only atrazine exposed crayfish. Each trial was set up so that the crayfish were tethered in their corresponding sections for 24-hour periods.

The exposed sections were set up with atrazine so that a three-gallon bucket held the mixture as a tube supplied it to the relative section at a constant flow of 4.32 milliliters per second. The mixture of atrazine was made by diluting 0.5 liters of 4% atrazine with 9.5 liters of water to create 10 liters of a 0.2% atrazine mixture.

After the crayfish were exposed to their conditions for 24 hours, the paired crayfish were put into an artificial arena (40.5 x 20.5 x 15 centimeters) to fight for five minutes (Figure 3). Each five-minute bout for the crayfish was scored by a system of points based off a previous study's table scoring intensity levels when they were working with the agonistic behaviors of crayfish (Table 1).

Once each trial was scored, the mean scores for each naïve and exposed crayfish were calculated and a Wilcoxon test was performed to determine if there was a significant difference between the average scores. A paired t-test was also performed to determine if there was a significant difference between each individual fight's score.

Figure 1 Artificial stream at the UMBS Stream Lab, Cheboygan, MI with three-gallon bucket filled with atrazine mixture.

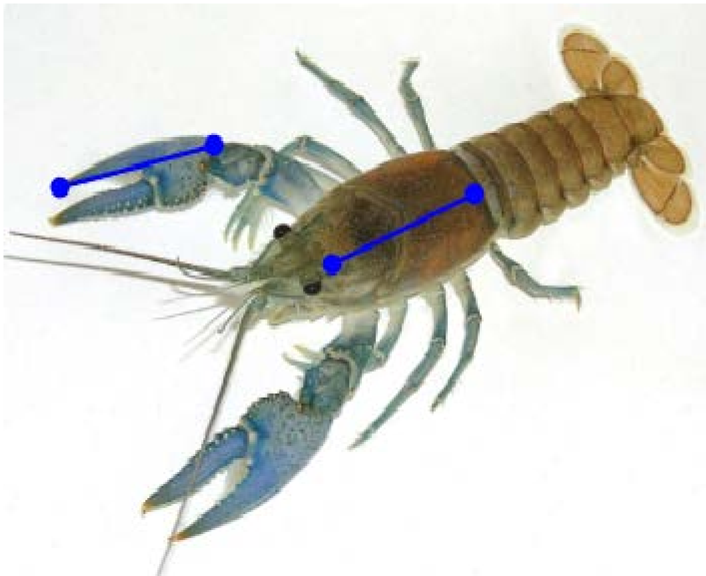

Figure 2 Chelae (left blue line) and Carapace (right blue line) Measurement Areas

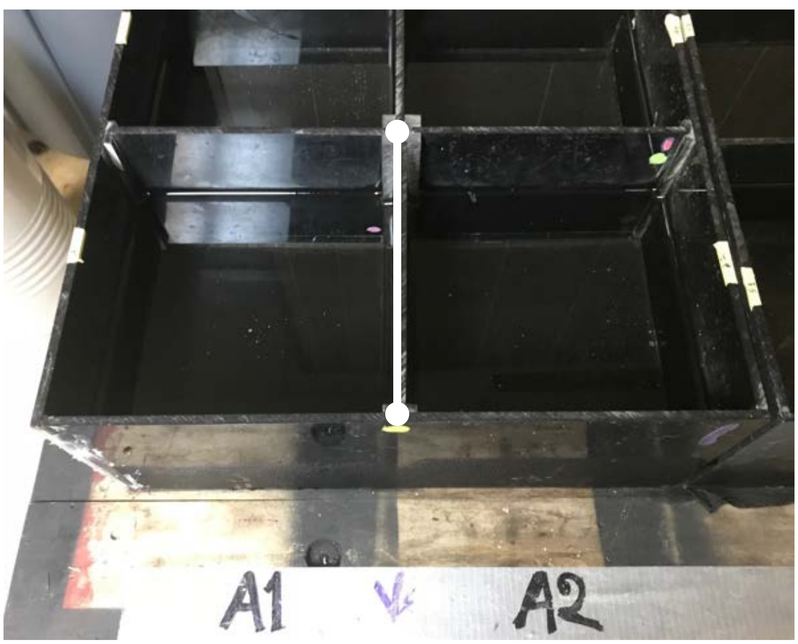

Figure 3 Artificial arena for the crayfish to fight. White bolded line represents the barrier that was lifted for the crayfish to interact when the bout began.

Table 1 Bergman & Moore (2003) Table used for Scoring Crayfish Bouts

Intensity level	Description
−2	Tailflip away from opponent or fast retreat
−1	Slowly back away from opponent
0	Ignore opponent with no response or threat display
1	Approach without a threat display
2	Approach with threat display using meral spread and/or antennal whip
3	Initial claw use by boxing, pushing, or touching with closed claws
4	Active claw use by grabbing opponent with open claws
5	Unrestrained fighting by grasping and pulling opponent's claws or appendages

Results

The average lengths of the carapace and chelae were determined to observe the standard deviation and variance of the two (Table 2). The mean scores for each crayfish were determined (Figure 4) and put into a Wilcoxon test that showed there was no significant difference between the mean scores of naïve crayfish versus exposed crayfish ($W = 61.5$, $p < 0.97$). A paired t-test was also performed to determine if the mean scores for each fight were significantly different ($t = 1.15$, $df = 6$, $p < 0.29$).

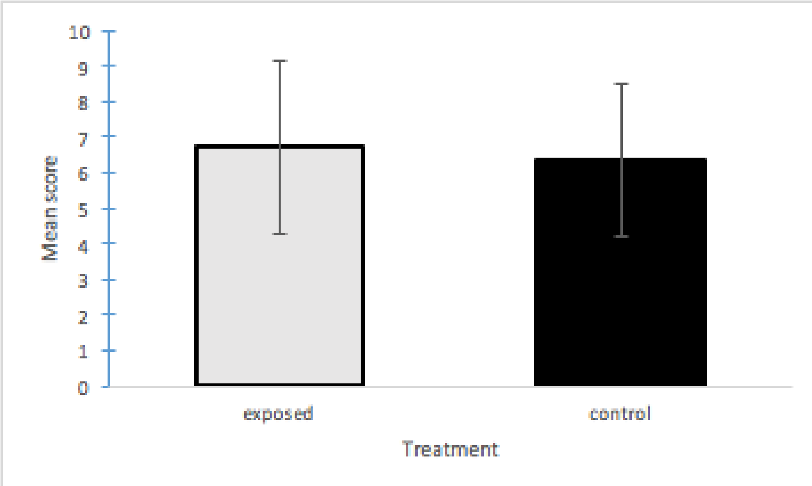

Figure 4 Mean Scores for Naïve (Control) and Exposed Crayfish; n = 22; ± 2.29 (SE).

Table 2 Carapace and Chelae Average, Standard Deviation, and Variance of Female *O. virilis*

O. Virilis Crayfish Parameters					
Carapace			Chelae		
Average (mm)	SD	Variance	Average (mm)	SD	Variance
33.59	2.95	8.73	30.26	3.52	12.42

Discussion

Many monocultural growers tend to disrupt aquatic ecosystems by agricultural runoff leaking into the environments of the aquatic organisms. This study tried to show the negative effects that certain herbicides used in agriculture have on the aquatic organisms living in nearby streams, specifically crayfish. However, this study did not end up showing the results we expected. Therefore, we cannot make concrete assumptions of the effects of atrazine on crayfish agonistic behavior.

The fact that we had a minimal amount of time to run our experiment and thus a minimal time to expose the crayfish may explain why we did not have any significant results with atrazine exposure on *O. virlis*. One study looked at the exposure of crayfish with atrazine over a longer period, 96 hours, and saw that the herbicide caused *O. rusticus* chemoreceptors to be impaired (Belanger et al., 2017). Therefore, crayfish exposed under longer intervals can show the results we expected. Another factor contributing to the short-term exposure of atrazine was the fact that our atrazine flowed out of the bucket at a constant rate of 4.32 milliliters per second. Over a 24-hour period, this would mean that the atrazine was completely depleted from the bucket within the first 38 minutes of introducing the atrazine. This implies that the crayfish were exposed to atrazine for an extremely short amount of time and then they were recovering from any effects caused by the minimal exposure of atrazine.

Time was an extremely crucial factor in the efficiency and accuracy of our trial. For example, we did not have enough time to capture similar sized crayfish so that we could pair them evenly based on their carapace and chelae lengths. The variance in chelae length was 12.42 millimeters. The chelae length was sometimes greater than four millimeters between the exposed crayfish and its naïve opponent. The weapons of crayfish, such as claws and legs, are an important in the fighting strategies of crayfish and therefore, having slightly different sizes in chelae length could explain why our data did not show a significant difference between the average scores of the fights or the crayfish (Schroeder and Huber, 2001). Another issue involved the atrazine for one 24-hour exposure not being completely emptied from the bucket, but because of our strict time limit, we decided to go forward with the bout anyway. This would have exposed the crayfish to very little or no atrazine and then had the crayfish fight its opponent.

In future studies, there should be a greater deal of crayfish caught in order to conduct more replicate trials. Our data may not have been significant because of the limited amount of data we collected as a result of our small number of crayfish and replicates. Collecting additional crayfish would also prepare for crayfish deaths and limb detachments throughout the process of the experiment. We encountered at least four crayfish deaths and two instances where our crayfish in the 24-hour exposure ended up losing their claws and thus, we could not use these crayfish in a fight and it set our trials back a few days.

Even though our study contained multiple flaws that caused our results to be insignificant, this study is important because it tried to understand the negative impacts of atrazine exposure to aquatic organisms and keystone species. The contamination of aquatic ecosystems can alter the type of species in an environment and if they are keystone species, the entire ecosystem may collapse (Liu and Sumpter, 2017). Therefore, studies such as this one can provide support for the need to restrict herbicide use to help our aquatic ecosystems.

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