Effects of Atrazine on Virile Crayfish, *Orconectes virilis*, Agonistic behavior

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Abstract

Atrazine(ATR) a common herbicide, was for a period of time the most common herbicide in surface waters of the U.S. Midwest. ATR has been found to have various negative effects on aquatic organisms. Crayfish specifically, a keystone species in aquatic ecosystems, appear to be affected negatively, with previous research noting the effects on crayfish olfaction and chemoreceptors. We hypothesized that short term exposure to ATR would affect the aggressive behavior of Virilis crayfish, *Orconectes virilis*, when fighting other crayfish. This was accomplished by using two identical simulated streams, with one side for naive crayfish, and the other side with introduced ATR for the exposed crayfish. After 24 hours these crayfish were removed from the streams and pitted against one another in a crayfish arena. Five (5) minute bouts where recorded and score to determine a numerical winner. We found that there is no significant difference in agonistic behavior between the control and exposed groups of crayfish. These results suggest that Virilis crayfish exposed to acute amounts of ATR are resilient, or given long enough to recover, display the same behavior as those not exposed to any ATR. suggesting acute ATR exposure is relatively benign with regard to virilis crayfish health.

Introduction:

Biota in aquatic ecosystems can be severely degraded by byproducts of large scale commercial agriculture through contaminated runoff that enters streams and rivers (Belanger et al, 2017). Pesticides have been found to have significant impacts on water quality and the associated flora and fauna (Tong and Chen, 2002). In this study we examined the downstream impact of Atrazine (ATR), a common herbicide used to control broadleaf plants and grassy weeds, on the behavior of female virilis crayfish.

In 2016, ATR was the most commonly detected herbicide surface waters of the in Midwestern United States (Belanger et al, 2016). With severe weather events and groundwater percolation, it has been found that ATR easily reaches streams and rivers (Belanger et al, 2017). More concerning, however, is that the half-life of ATR in surface water can vary from 8 days to several years (Comber 1999, EPA 2007). ATR changes food and social odor response in rainbow trout, *Oncorhynchus mykiss,* Atlantic salmon, *Salmo salar,* and goldfish, *Carassius auratus* (Moore and Waring 1998; Nieves-Puigdoller et al. 2007; Saglio and Trijasse 1998; Tierney et al. 2007). It has also been shown that ATR, delivered to streams through runoff, can cause adverse effects in chemoreceptors and olfaction in Virilis Crayfish, which alters feeding behavior (Belanger et al, 2015). Crayfish, a keystone species, are a vital link between terrestrial and aquatic food chains. Crayfish are a prey source for terrestrial predators as well as aquatic predators (Lodge et al. 1994). Crayfish are sensitive to changes in water quality and chemistry, meaning they are a good bioindicator of water quality (Belanger et al, 2015). It has been shown that ATR ranges from slightly to highly toxic to freshwater invertebrates, with significant reductions in invertebrate numbers occurring between, 62 to 92 ppb, a range which has been commonly documented in the field (EPA 2003; Belanger et al, 2015). ATR effects the chemoreceptors of Crayfish which alters many of their behaviors which rely upon this chemical reception ability. This, in turn, affects the crayfishes chances of survival (Belanger et al, 2015).

Virilis crayfish, *Orconectes virilis,* is a freshwater crustacean native to Michigan (Rubenstein & Hazlett, 1974). Also known as the Northern Crayfish or Lake Crayfish, they can often be found in lakes and streams. They feed on detritus or macrophytes and they build shelters from which to hide from predators. Foraging sites are often the location of intense agonistic intraspecific and sometimes interspecific interactions (Bergman & Moore, 2003). These fights tend to be asymmetrical, with one contestant usually favored (Parker, 1974; Maynard Smith and Parker, 1976). Some intrinsic asymmetries that have been noted include (Bergman & Moore, 2003): body size, in our case carapace size (Bovbjerg, 1953, 1970; Rubenstein and Hazlett, 1974; Berrill and Arsenault, 1984; Pavey and Fielder, 1996), chelae size (Garvey and Stein, 1993; Rutherford et al., 1995) and sex (Stein, 1976; Peeke et al., 1995, 1998). Extrinsic asymmetries include: prior residence (Peeke et al., 1995, 1998), differing fight strategies (Guiasu and Dunham, 1997) and previous aggressive encounters (Rubenstein and Hazlett, 1974; Daws et al., 2002; Bergman et al., 2003) All of these factors will have an effect on the outcome of agonistic interactions. The goal of this study is eliminate as many of these potentially confounding factors as possible and determine the effect ATR has on female Virilis crayfish agonistic behavior. Although there is a great deal of knowledge on the effects of ATR on rusty crayfish, *Orconectes rusticus* (Belanger et al, 2017), a closely related species of crayfish that is invasive in Michigan, there is one study conducted specifically on the agonistic interactions of Virilis crayfish (Rubenstein, 1974). In this study we plan to expose Virilis Crayfish to ATR under Dynamic flow conditions. After 24 hours of exposure they will be removed and prepared for a fight against a symmetrical opponent exposed to different naïve conditions. The Crayfish's behaviors will be scored and a winner will be determined. The goal of this experiment is to determine how short term ATR exposure effects Virilis crayfish agonistic behavior.

We hypothesized that exposure to ATR would affect the agonistic behavior of Virilis crayfish, We predict that Crayfish exposed to atrazine will lose in fights more often against naïve (unexposed) crayfish. This prediction is based upon previous research that shows that pesticides such as ATR effect the chemoreceptors of crayfish causing abnormalities in their foraging behavior as well as their responses to external stimuli (Belanger et al, 2015) so they would be less likely to fight back, resulting in less intense aggressive interactions.

Methods:

Female Virilis crayfish were collected from Burt Lake (Cheboygan Co, MI) at the Maple Bay state forest campground. They were stored in an aerated container overnight with water from the lake. The following day, the crayfish were transported to the University of Michigan Biological Station (UMBS) stream lab. The carapace of each individual crayfish was measured and they were assigned a number. Once they were assigned a number they were placed into individual plastic containers with corresponding numbers and left in an isolation tank, filled with water from the East branch of the Maple River. The plastic containers were weighed down to the bottom of the tank by a rock in order to keep the crayfish out of direct sunlight.

The experimental design (figure 1) consisted of two simulated streams built from cinder blocks and plastic liner. The right and left streams held .108m^3 and .114m^3 respectively. The discharge for the streams was 0.078L/s and 0.072L/s with respect to the naive and exposed (right and left). Feeding the water was a headtank that was connected to water source, coming from the east branch of the maple river, through a variety of PVC fittings. The water drained through the end of the simulated stream into the drainage system which empties into the East branch of the Maple River. Furthermore, a piece of plexiglass was placed into the middle of both of the stream dividing the stream into half while still allowing streamflow, this was done to increase the number of crayfish that could be exposed during a single trial from 2 to 4. Four small tether devices where created using three small tiles zip-tied together and attached to a fishing line that was tied off to a small sheet of velcro. The crayfish were paired up based upon similarity in carapace size, before fighting the crayfish's right chelae was also recorded. They were dried off and a corresponding piece of velcro was stuck to their carapace. They were then attached to the tether and left in the simulated stream for 24 hours.

After 24 hours the crayfish were removed from the stream apparatus and brought into the crayfish arena, a dark plastic box with dimensions of 40.5 x 20.5 x 15 cm. One crayfish was marked with white out in order to distinguish it from it's opponent. The Crayfish arena was a tank separated in the middle by a removable wall of plastic. The tank was filled with 6 liters of water, coming from the East branch of the Maple River. The crayfish were placed into the separate rooms of the tank created by the removable wall. Once the camera was recording, the middle wall was removed and the crayfish were allowed to fight for 5 minutes. No attempt was made at scoring the live fight. After the bout was over the control crayfish were returned to their containers in the isolation tank, and the exposed crayfish were frozen**.** The footage was reviewed and the crayfish were individually scored using Table 1 (from Bergman & Moore, 2003).

Table 1. Behaviors used to score the fights with corresponding numerical values (from Bergman & Moore, 2003).

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

Each individual's points were scored and the crayfish with the most points after the five minute bout was deemed the winner. For the last four fights a five gallon bucket was used, filled with three liters of water, due to complications with others research.

The ATR exposure trails were nearly identical besides the introduction of the atrazine. On the left side of the artificial stream we used a 10L solution of 0.2% atrazine and introduced at a rate of 4.32ml/s, using a piece of surgical tubing attached to weight to hold the tube down to the bottom of the ATR solution bucket. The Atrazine was introduced at the top of the stream in order for proper mixing to occur, for equal exposure to both crayfish located on either side of the plexiglass divider. These crayfish where exposed for 24 hours and then removed from the stream and prepped for a fight with a naive counterpart of equal size. The exact same recording, fight protocol, and scoring system was applied.

Figures were created to show the differences in total scores and win/loss from the naive vs exposed. The Control bouts (naive vs naive) were also carefully scored and total and average points scored by each crayfish were used as a frame of reference for determining if atrazine effects virilis crayfish agonistic behavior to a statistically significant degree using a wilcoxon test.

Figure 1.B The Crayfish fight arena.

Figure 1

Results:

Table 2. Depicts the results of a Wilcoxon rank sum test with continuity correction (n=22). Comparing fight scores of naïve and exposed crayfish.

Wilcoxon Test	
W	**p-value**
61.5	**.9736**

Figure 2. Depicts the Average points scored per crayfish per bout under (Bergman & Moore, 2003) Table 1(n=22), ±2.29(SE). Control=Naive, Exposed=ATR treatment.

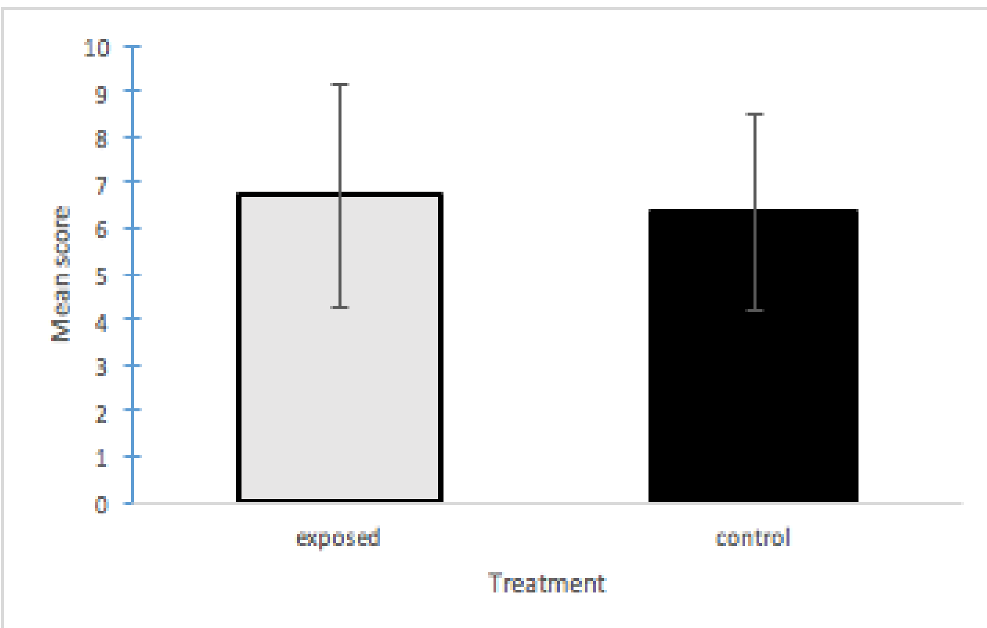

Table 3. Depicts the Average, Standard Deviation, and Variance of the Sample of female Virilis Crayfish used in the study (n=22).

Carapace			Chelae		
Mean(mm)	SD	Variance	Mean(mm)	SD	Variance
33.59	2.95	8.73	30.26	3.52	12.42

It should be noted that the Variance between chelae size for the Crayfish used in this study was 12.42mm. Also note the large SE found in Figure 2. Based upon the results of our wilcoxon test (w=61.5, p=.9736) it can be said there is no significant difference in the performance of the control and exposed Crayfish.

Discussion:

We formulated the hypothesis that short term exposure to ATR would reduce the agressive behavior of female Virilis Crayfish. Specifically, we predicted that Crayfish exposed to ATR would score less points, and lose more bouts than their naive counterparts. However we did not see these results, instead we saw no relationship between ATR exposure and aggressive behavior.

This prediction and hypothesis were based on research by (Belanger et al, 2015) that suggested that Atrazine Exposure affects the chemoreceptors, causing abnormal foraging behavior and response to a stimulus. In the same study evidence was also found to suggested that short term ATR exposure may act as an olfactory sensory neuron receptor antagonist, or even temporarily denature G-proteins coupled receptors (Belanger et al, 2015).

We did not see any statistically significant results with relation to the aggressive behavior of virilis crayfish. This makes it hard to make any such assumption about the affects of ATR on female Virilis crayfish. The Crayfish matched up in fights were measured and opponents were chosen based upon similarities in carapace and chelae size. Many of our crayfish, however, died in the isolation tank and the fighting pairs had to be changed to Crayfish that were not exactly the same size, although they were as close in size as they possible. This asymmetry would immediately bias the fight. It’s known that Chalae size is a deterministic character in terms of fighting success (Garvey and Stein, 1993; Rutherford et al., 1995). We also found the even when Carapace length was the exact same size, often the chelae size still differed.

Various studies have found that Acute ATR exposure does affect the chemoreceptors of crayfish (Belanger et al. 2015; Moore and Lower 2001; Tierney et al. 2007, 2008). However, there is no previous literature on the effects of short term ATR exposure on female Virilis Crayfish Agonistic behavior.

Many potential factors could have had a confounding effect on the results observed. For instance, the 10L solution of .2% ATR solution was spent when we arrived twenty-four hours later. Using the introduction rate of 4.32ml/s it would have only take the ATR bucket approximately thirty-eight minutes to draining, meaning the Crayfish were only exposed to the ATR for a little more than thirty-eight minutes of the entire twenty-four hour exposure. It should also be noted that for the final trial, two pieces of surgical tubing were used to introduce ATR to both simulated streams. This means all four crayfish were exposed to ATR for a little over nineteen minutes. If we wanted to expose the Crayfish to a constant source of ATR, at an introduction rate of 4.32ml/s, we would need a solution of 373L of .2% ATR, for the purposes of this study such a large volume of ATR is simply not feasible. However, one could use a higher concentration of ATR and a lower introduction rate to decrease the volume of ATR solution needed. This actual exposure time could be a very important factor with regard to our results; following the acute ATR introduction the crayfish could have had enough time to recover from the ATR exposure.

More research should be conducted on Virilis crayfish ability to recover from acute ATR exposure. Finding a threshold that crayfish could withstand would have very important management implications. Allowing managers and policy makers to limit the amount of ATR to a level that is not ecologically devastating to streams and the organisms within them such as virilis crayfish.

It should also be noted that the variance between the control and exposed treatments appeared to visually differ. With the control treatment appearing to have more variance than the exposed treatment, this could also be a potential idea for further research. It could be useful to run the same experiment with many more replicates in order to increase ones confidence in any statistical tests that might expand our understanding of the effects of ATR on virilis crayfish.

Regardless of how much ATR is deemed safe in surface waters, we know it is affecting the aquatic organisms that reside in those streams. Although none of our data depicted any explicit relationships or undiscovered trends, it is important to note that our experiment was far from ecologically sound. With various malfunctions along the way, a proper ecologically accurate model of our experiment would be far more expensive, labor intensive and time consuming. Regardless, the knowledge that could be gained from such an experiment would be invaluable, potentially laying the foundations for future research regarding the susceptibility of crayfish to certain concentrations and durations of ATR Exposure, as well as the ability to recover and the threshold at which irreversible damage is done.

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