Effects of Atrazine on Virilis Crayfish (*Orconectes virilis*) Agonistic Behavior

Tatiana Cuevas

University of Michigan Biological Station
EEB 381 General Ecology
August 15, 2018
Brendan O'Neill

Abstract

Atrazine is an herbicide that has been intensively used in the Great Lakes basin for about 25 years and has a long half-life in cold, low productive aquatic systems (Schottler and Eisenreich 1997). Atrazine has been increasingly found in drinking water, and has been proven to impact aquatic flora and fauna (Guasch, 1997). One of the organisms possibly affected by this chemical is crayfish. Crayfish are considered to be a keystone specie to Michigan aquatic ecosystems due to their feeding habits and the food source they provide to predators. Previous studies suggest that crayfish species have been negatively affected by the addition of atrazine to waterways because it inhibits their olfactory and chemoreceptor processes. For this study we subjected female Orconectes virilis crayfish to short term exposure of atrazine within a artificial dynamic stream system. We believe that the short term atrazine exposure will affect the agonistic behavior by being out performed by their naïve counterparts. After a 24 hour period, the crayfish were placed in 5 minute bouts and scored on both passive and aggressive actions. From our results, we did not find any significant differences in the agonistic behavior of control and exposed groups of crayfish. Although we could not see the impact of acute exposure of atrazine on agonistic behavior, prior studies have longer exposure has negatively impacted behavior and reproduction. This in combination with our study may suggest that acute exposure to atrazine is less harmful to stream ecosystems.

Effects of Atrazine on Virilis Crayfish (Orconectes virilis) Agonistic Behavior

Tatiana Cuevas
EEB 381
Summer 2018
Brendan O'Neill

Abstract

Atrazine is an herbicide that has been intensively used in the Great Lakes basin for about 25 years and has a long half-life in cold, low productive aquatic systems (Schottler and Eisenreich 1997). Atrazine has been increasingly found in drinking water, and has been proven to impact aquatic flora and fauna (Guasch, 1997). One of the organisms possibly affected by this chemical is crayfish. Crayfish are considered to be a keystone specie to Michigan aquatic ecosystems due to their feeding habits and the food source they provide to predators. Previous studies suggest that crayfish species have been negatively affected by the addition of atrazine to waterways because it inhibits their olfactory and chemoreceptor processes. For this study we subjected female *Orconectes virilis* crayfish to short term exposure of atrazine within a artificial dynamic stream system. We believe that the short term atrazine exposure will affect the agonistic behavior by being out performed by their naïve counterparts. After a 24 hour period, the crayfish were placed in 5 minute bouts and scored on both passive and aggressive actions. From our results, we did not find any significant differences in the agonistic behavior of control and exposed groups of crayfish. Although we could not see the impact of acute exposure of atrazine on agonistic behavior, prior studies have longer exposure has negatively impacted behavior and reproduction. This in combination with our study may suggest that acute exposure to atrazine is less harmful to stream ecosystems.

Introduction

The absence of one organism in an environment can drastically change the community composition of an ecosystem or prevent it from existing all together. Species that have this power over an ecosystem are referred to as a keystone specie. In Northern Michigan, the species that fills this niche is *Orconectes virilis*. Crayfish affect freshwater ecosystems by removing plants from the aquatic system that may increase turbidity, regulating fish populations by consuming fish eggs, and being prey for both aquatic and terrestrial predators (Lodge,1987). In addition to this, crayfish are sensitive to changes in water quality and chemistry, meaning they are a good bioindicator of water quality (Belanger et al, 2015). In this study, we will examine the sensitivity of this keystone specie to the addition of an herbicide.

In recent years the use of herbicides and pesticides have threatened freshwater ecosystems due to agricultural runoff. For instance, atrazine is used in the agricultural industry to manage the growth of broadleaf weeds and to increase yields of crops like corn, sorghum, and sugarcane (Belanger, 2017). Atrazine was first introduced into the herbicide program in 1967 (Ryan 1970). From then on, it has been found to leave fields during severe weather events and to enter groundwater through percolation, atrazine can easily reach streams and rivers which are the tributaries to larger bodies of water (Belanger et al, 2015). In 2001, it was the most common herbicide found in drinking water in the United States (Gilliom et al, 2007). Despite this, The EPA has stated that atrazine is a potential endocrine disruptor, but does not pose health concerns to humans (Atrazine, 2007). This, however, can not be said about aquatic life.

Atrazine, delivered to streams through runoff, can affect the chemoreceptors and olfactory processes of *O. Virilis* crayfish, which, in turn, changes feeding behavior (Belanger et al, 2015). This alteration can lead to reduced survivability for this keystone specie. In addition to this, past studies have found that the reproductive processes of crayfish and other aquatic organisms have been negatively affected (Guasch et al., 1997). In this study we will focus on agonistic behavior between crayfish that usually occurs at sites of competition for resources. These fights tend to be one sided, where one crayfish usually out competes the other (Parker, 1974). Intrinsic asymmetrical characteristics that impact the outcome of these fights include: body size, also known as carapace (Bovbjerg, 1953, 1970), chelae size (Garvey and Stein, 1993) and sex (Stein, 1976). Other factors that impact fights are extrinsic asymmetries, and these include: prior residence (Peeke et al., 1995), differing fight strategies (Guiasu and Dunham, 1997) and other aggressive encounters (Rubenstein and Hazlett, 1974). Each of these factors when combined will change the outcome of crayfish fights.

Atrazine is known to have a very high aquatic half-life, meaning it takes up to 7 years to completely disappear from most freshwater basins including groundwater and river systems (Comber, 1999). This study was created to observe the impacts of atrazine on the behavior of *O. Virilis* crayfish after a 24 hour exposure at 200 micrograms per liter concentration within a dynamic system. We hypothesize that *O. Virilis* crayfish subjected to the atrazine will not perform as well as naïve *O. Virilis* crayfish which were not subjected to these concentrations. Our determination of a good performance is based on more offensive tactics than passive. In this study, we attempted to limit as many intrinsic and extrinsic asymmetrical factors as possible to truly focus on the behavioral changes of *O. Virilis* crayfish after short term exposure to atrazine.

Methods

Using crayfish nets, female *O. Virilis* crayfish were collected from Burt Lake at the Maple Bay State Forest Campsite in Cheboygan, MI. The carapace of the individual crayfish was measured, and each was assigned a number. After numbering the *O. Virilis* crayfish, they were placed into individual plastic containers and marked them with the corresponding number for the crayfish. Once in the containers, they were placed into an isolation tank stream system filled with water from the East Branch of the Maple River. To keep the crayfish out of direct sunlight, the containers were weighed down in the tank and held there by a stone.

Fig 1. Picture of the artificial streams created at UMBS Stream Lab

We created two parallel artificial streams; one held 0.108m3, and the other held 0.114m3. As for stream discharge rates for both the right and left, they were 4.32L/m and 4.70 L/m respectively. We placed the naïve crayfish on the right side of the stream and exposed crayfish on the left side. The water was fed into this system by a headtank that was connected to the water source from the Maple River by PVC pipes. Additionally, a piece of plexiglass was placed in the middle of both of the streams so that they were divided, but not enough to restrict the stream flow on either the side of the glass. The reason for this was to maximize the amount of tests we could run at one time. Focusing on the crayfish, four tether devices were created using three tiles connected together by zip-ties. A fishing line was also tied to the tiles, and attached to the end of that is a small piece of velcro. We allowed for a 5 inch distance from the tiles to the velcro. The crayfish were paired by similarity in carapace size, and the crayfish's right chelae was recorded. Before being put into the tank, another piece of velcro was placed on the carapace of the crayfish and attached to the tether.

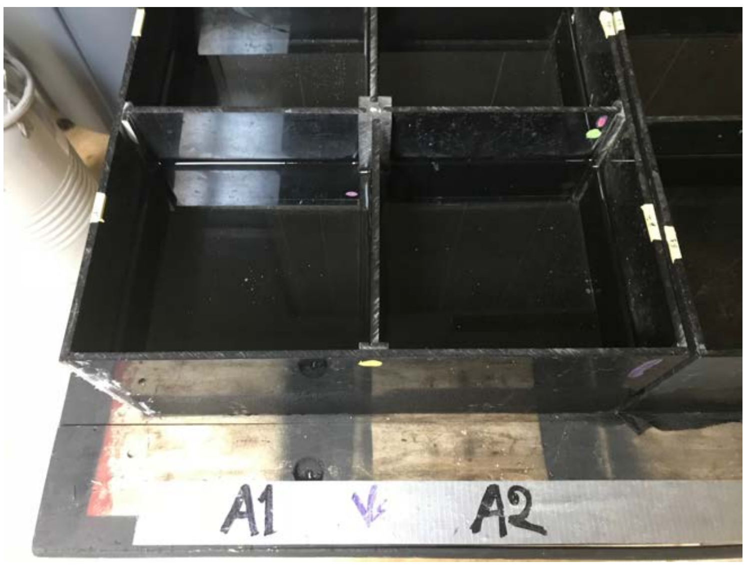

Fig 2. Picture of the arena used to host crayfish fights

The atrazine solution that we used was 10 liters with a 0.2% concentration of atrazine. This solution was stored in a three-gallon bucket placed on the wall dividing the two streams. The atrazine was distributed to the left stream from the bucket using a small tube at the rate of 4.32 mL/m. This was done until all of the 10 liter solution had been delivered to the system. 24 hours after the initial exposure, the crayfish were collected and put into an arena (40.5 x 20.5 x 15 cm, Fig 2.). The arenas were filled with 3 liters of water, exposed crayfish were marked with a white line, and crayfish were placed on each side of the divider. Once the divider was lifted, we timed and recorded the bouts for 5 minutes. After the fight, the crayfish were scored on their performance. Points were given or taken away based on passive or aggressive actions (Bergmann and Moore, 2003, Table 1). We performed 2 control fights of Naïve vs Naïve and 2 control fights with Exposed vs Exposed. Additionally, we had 7 fights of Naïve vs Exposed. The data was then analyzed using R studio using a Wilcoxon and t-test.

Table 1. Scoring rubric for crayfish fights (Bergmann and 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

Results

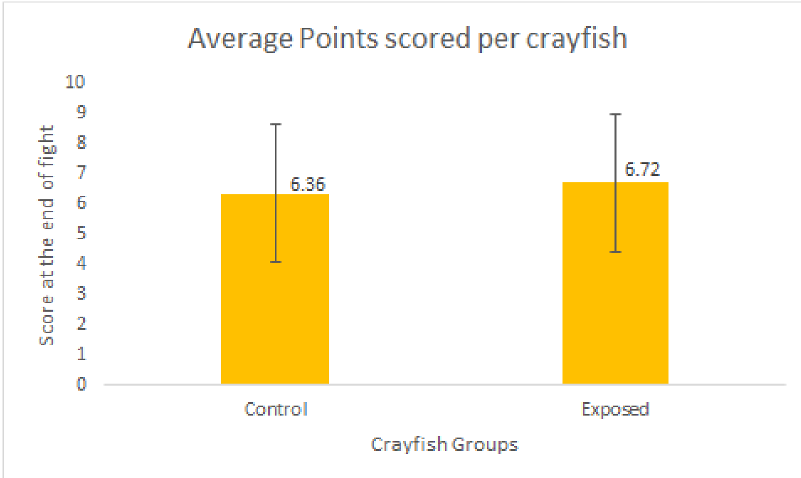

Fig 3. Average points scored for Virilis crayfish. Control is the Naïve crayfish and the Exposed are the crayfish subjected to the atrazine. n=22, +-2.29(SE)

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**

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

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

The variance between chelae size was 12.42 while the variance in carapace length was less at 8.73 (Table 3). Additionally, there was a large SE of +-2.29 (Fig. 3) with n=22. This shows that there were a total of 11 fights. According to our Wilcoxon test there was no significant difference in the performance of the Naïve or Exposed crayfish (w=61.5, p=.9736). The results of the crayfish bouts show that the the average fight score for the naïve was 6.36, and the average for the exposed crayfish was 6.72 (Fig 3).

Discussion

Our hypothesis posited that the short term exposure to atrazine would impact the behavior that female *O. Virilis* crayfish will display when faced with a situation where aggression is needed, such as competing for space or resources. Specifically for this experiment, we believed that female *O. Virilis* crayfish exposed to atrazine would score less points than the naïve crayfish. From the research, our p-value of 0.9736 suggested that our results were not statistically significant in relation to our initial hypothesis. Because of this, it is difficult to make any assumptions about the effects of atrazine on crayfish behavior. This acute exposure to atrazine did not seem to impact the chemoreceptors of *O. Virilis* by their seemingly unchanged behavior and similar studies were seen in the preliminary research and showed that the chemoreceptors were affected, but they did not study female *O. Virilis* specifically (Tierney, 2007, 2008).

Despite all of our attempts to make this study as controlled as possible, there were a couple factors out of our control. For instance, when putting the crayfish in the isolation stream, some of them died or lost a claw. When this happened, we rearranged the pairings in an attempt to keep crayfish of similar sizes together. Sadly, this was difficult to achieve, so the crayfish were usually paired with ones that had the carapace of chelae a millimeter or two larger or smaller. Also, even if we were able to find crayfish of the same carapace length, the chalae size was often different. This is seen in the high variance of 12.42 for chalae length (Table 3).

Also, the short term atrazine exposure was shorter than we initially expected. The 10 liters of atrazine being deposited at a rate of 4.32 mL/m took only 38 minutes for the atrazine to enter the system. This time was also cut in half when both streams were fed atrazine from the same bucket. This would make the exposure time only 19 minutes for each of the crayfish. Since we did not score these crayfish until 24 hours after the initial exposure, with this short exposure period, the crayfish might have had enough chemoreceptor recovery time and would not show the effects of atrazine. The recovery process of crayfish exposed to atrazine in terms of their chemoreceptor abilities was discussed in a previous study (Belanger et al. 2015). To further this research, we are interested in understanding the impacts, if any, that atrazine would have on the reproductive processes of female *O. Virilis* crayfish. As mentioned before, reproductive changes have been seen in some organisms at low concentrations, and we would like to see if it impacts crayfish in the same capacity.

In addition to the manipulation of the stream inputs, we encountered complications with the stream system. For instance, we were unable to get the outflow rates of the streams the same in both of the streams, but we created them to be as close as possible. Also, we were unable to change the sediment on the bottom of the stream after every treatment group. There is evidence of atrazine sinking to the bottom of river systems and becoming imbedded into the sediments (Mengjie et al., 2017). With this in mind, there is a possibility that the atrazine accumulated in the sediment could have impacted the data collected for the exposed crayfish. We decided to use a dynamic stream system because it is most representative of areas of environmental concern such as rivers and small streams, but a positive addition to this research would be to see the impact that static conditions have on crayfish agonistic behavior. In future research, we will test different species and sexes of crayfish in effort to get a more representative accumulation of data. From this, we hope to bring attention to the potential impacts of atrazine in waterways, not only to crayfish, but to other aquatic flora and fauna as well.

Citations

Atrazine: Chemical Summary. Toxicity and Exposure Assessment for Children's Health (PDF) (Report). U.S. Environmental Protection Agency. 2007-04-24. Archived from the original (PDF) on 2012-03-16.

Belanger, R. M., Evans, K. R., Abraham, N. K., & Barawi, K. M. (2017). Diminished Conspecific Odor Recognition in the Rusty Crayfish (Orconectes rusticus) Following a 96-h Exposure to Atrazine. *Bulletin of Environmental Contamination and Toxicology,*99(5), 555-560. doi:10.1007/s00128-017-2178-3

Belanger, R. M., Mooney, L. N., Nguyen, H. M., Abraham, N. K., Peters, T. J., Kana, M. A., & May, L. A. (2015). Acute Atrazine Exposure has Lasting Effects on Chemosensory Responses to Food Odors in Crayfish (Orconectes virilis). *Archives of Environmental Contamination and Toxicology,70*(2), 289-300. doi:10.1007/s00244-015-0234-8

Bovbjerg, R. V. 1970. Ecological isolation and competitive exclusion in two crayfish (Orconectes
virilis and Orconectes immunis). Ecology 51:225–236.

Bovbjerg, R. V. 1953. Dominance order in the crayfish Orconectes virilis (Hagan). Physiol. Zool.
26: 173–178.

Comber, Sean. (1999). Abiotic persistence of atrazine and simazine in water. *Pest Management Science,* 55(7), 696-702.

Maynard Smith, J., and G. A. Parker. 1976. The logic of asymmetric contests. Anim. Behav. 24: 159 –175.

Garvey, J., and R. A. Stein. 1993. Evaluating how chela size influences the invasion potential of an introduced crayfish (Orconectes rusticus). Am. Nat. 129: 172–181.

Guiasu, R. C., and D. W. Dunham. 1997. Initiation and outcome of agonistic contests in male Form I Cambarus robustus Girard, 1852 crayfish (Decapoda, Cambaridae). Crustaceana 70: 480 – 496.

Gilliom RJ et al. US Geological Survey “The Quality of Our Nation’s Waters: Pesticides in the Nation’s Streams and Ground Water”, 1992–2001 March 2006, Revised February 15, 2007

Guasch, H., Munoz, I., Roses, N., & Sabater, S. (1997). Changes in atrazine toxicity throughout succession of stream periphyton communities. Journal of Applied Phycology, 9(2), 137–146. https://doi.org/10.1023/A:1007970211549

Lodge, D., and Lorman J., 1987. Reductions in submersed macrophyte biomass and species richness by crayfish *Oronectes rusticus. Can. J. Fish.Aquat.Sci.* 50, 1279-1288

Mengjie Qu, Huidong Li, Na Li, Guanglong Liu, Jianwei Zhao, Yumei Hua, Duanwei Zhu, 2017,
Distribution of atrazine and its phytoremediation by submerged macrophytes in lake sediments, Chemosphere, 168: 1515-1522,

Peeke, H. V. S., J. Sippel, and M. H. Figler. 1995. Prior residence effects in shelter defense in adult signal crayfish (Pacifastacus leniusculus) results in same- and mixed-sex dyads. Crustaceana 68: 873–881.

Rubenstein, D. I., and B. A. Hazlett. 1974. Examination of the agonistic behaviour of the crayfish Orconectes virilis by character analysis.Behaviour 50: 193–216.

Ryan, G. (1970). Resistance of Common Groundsel to Simazine and Atrazine. *Weed Science* 18(5), 614-616. Retrieved from doi: 10.1017/S0043174500034330

Stein, R. A. 1976. Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. Can. J. Zool. 54: 220 –227.

Tierney KB, Singh CR, Ross PS, Kennedy CJ (2007) Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides.Aquat Toxicol 81:55–64. doi:10.1016/j.aquatox.2006.11.006

Tierney KB, Sampson JL, Ross PS, Sekela MA, Kennedy CJ (2008) Salmon olfaction is impaired by an environmentally realistic pesticide mixture. Environ Sci Technol 42:4996–5001. Doi:10.1021/es800240u