Effect of Chemical Stressors on Crayfish Chemical Cue Response

Brianna Henry*, R. Berry, E. Fazio, K. Kovacs, and A. Keth
Clarion University of Pennsylvania, Clarion, PA 16214

Abstract:
Atrazine, a triazine herbicide used to kill broadleaf weeds in agricultural and roadway applications, is one of the most widely used pesticides in the United States. While this herbicide is meant for landscape use, rain and other storm events often carry the chemical from terrestrial to aquatic systems, where it has been observed to cause several negative effects. A number of studies have related Atrazine exposure to reduced olfactory reception in fish and suggest that Atrazine acts as an endocrine disruptor in fish and amphibian species that come into contact with this chemical at ecologically relevant levels. As aquatic species sense most of their information about environmental conditions through olfaction, this is particularly important for crayfish, who only replace their olfactory cells during molting. In this study we examine the impacts of the herbicide Atrazine on crayfish responses to food cue and pursue possible explanations for behavioral changes based on observation of olfactory structure surface morphology utilizing scanning electron microscopy. Because Atrazine has been observed to be an endocrine and olfactory disruptor in other species, its non-lethal effects in crayfish could potentially disrupt the food web dynamics of aquatic systems. We hypothesize that exposure to ecologically relevant concentrations of Atrazine will result in irregular behavior in crayfish presented with chemical cues indicating the presence of a food source. Behavioral changes in response to these chemical cues may indicate a need to further consider the non-lethal effects of Atrazine, in addition to other understudied pollutants.

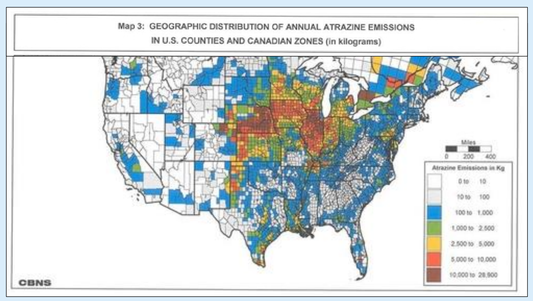

Figure 1. Atrazine use intensity is highest in farmlands surrounding the Mississippi River drainage, making it highly applicable to aquatic systems.

Crayfish as Olfactory-Sensitive Bioindicators

Crayfish are:
- Moderately sensitive to pollution
- Fairly long-lived macroinvertebrates
  - Life-span of about 3 years
- Food for many aquatic organisms
- Aquatic decomposers

Crayfish only replace olfactory cells when molting.
- May only molt 5-6 times during lifetime
- Rely on these cells for detection of chemical cues
  - "Smelling" the water
- Olfactory damages can be long-lasting
  - Impact chances of survival and finding mate, predator, or food source

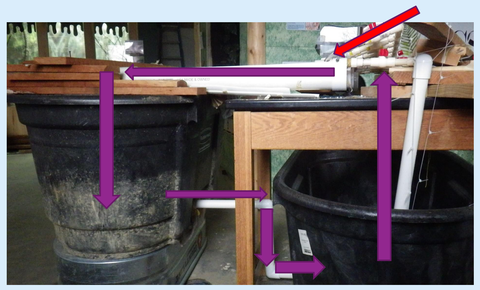

Figure 2. Artificial stream testing system; purple arrows indicate water flow and red indicated cue introduction.

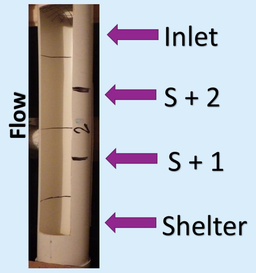

Figure 3. Labeled positions in testing set-up for analysis.

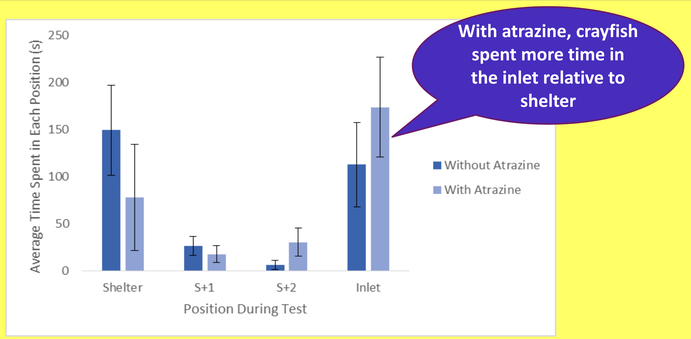

Figure 4. A comparison of average time spent in each section of the single cue testing trough among atrazine exposure treated and untreated tests. Results are pooled food and alarm cue response, from 2014 tests with p = 0.0165.

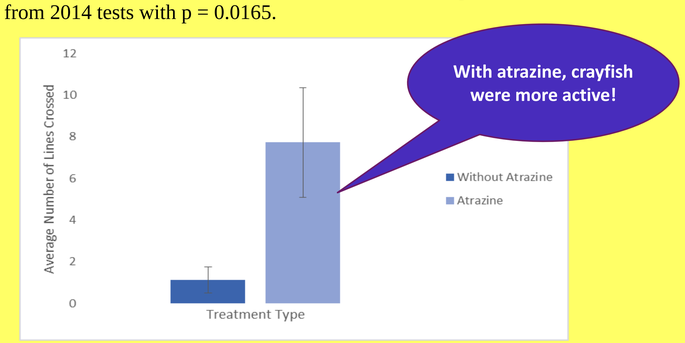

Figure 5. A comparison of the average number of lines crossed among treatment types as a measure of activity. Data pooled from both cue tests (2014). A t-test yielded p = 0.0081.

Analysis:
- Responses to each cue were assessed by time spent in various sections of the testing trough and the number of lines crossed between sections as a measure of activity (Figure 2, 3).
- Individual results were averaged and compared among treatments and cue types. Because cue responses were statistically similar, all data were pooled for an ANOVA , with pair-wise comparisons made via a Tukey's HSD. (Figure 4). A paired t-test was performed on the average number of lines crossed to compare different herbicide treatments (Figure 5).

Methods:

Acquisition and Housing
- 30 *Orconectes rusticus* (Rusty Crayfish) were wild-caught in Buffalo Creek, Newport, PA in August and housed at Clarion University until testing.
- Crayfish were starved for 6 days prior to their testing.

Testing
- Crayfish were each placed in individual meter-long troughs of 4" diameter PVC pipe in a flowing water system (Figure 2), and allowed to acclimate to the testing troughs for 24 hours.
  - Flow rate for the water was approximately 2L/min.
- After 24 hours, stimulus was introduced at approximately 0.5 L/min, and crayfish were given 3 minutes to acclimate before their behavior was videotaped for 5 minutes.
- In 2014, testing was done with food and alarm cue, both with atrazine and without atrazine present.
  - Results for these tests are shown.
- Crayfish were divided into four treatment groups for testing in 2015:
  - Ethanol (5ml/300L of water) without cue
  - Ethanol with food cue
  - 50 ppb atrazine without cue
  - 50 ppb atrazine with food cue
  - Results for 2015 trials are pending recovery of video data.

Take-Home Messages:
- Atrazine is a relevant aquatic contaminant, entering systems through run-off from agriculture or roadway applications.
- 50 ppb atrazine exposure does induce changes in crayfish chemical cue responses.
  - Increased activity
  - More time spent near cue introduction relative to shelter area
- Changes in response to chemical cues can influence behavior of aquatic organisms and decrease their survival.
  - E.g. inability to find food, mate, or sense a predator
- These results and further studies into a threshold level of atrazine contamination for affecting chemical cue responses may indicate a need for reassessing allowable surface water contamination levels.

References:
- Pecor, K.W. and B.A. Hazlett. 2008. The tradeoff between reproductive and food resources in the crayfish *Orconectes virilis*. *Marine and Freshwater Behaviour and Physiology.* 41(4): 273-280.
- Rohr, J.R. and K.A. McCoy. 2010. A qualitative meta-analysis reveals consistent effects of Atrazine on freshwater fish and amphibians. *Environmental Health Perspectives.* 118(1): 20-32.

Acknowledgements:
We would like to thank Clarion University of Pennsylvania and the Center for Conservation Studies, Inc. for their funding and use of resources. Thanks also to Dr. Keth, Dr. Boyden, Melissa Lutz, Candace White, and Emily Wilson for their support and help with testing.