AMMONIUM EFFECTS ON CHEMORECEPTION AND PHYSIOLOGY OF THE RUSTY CRAYFISH, ORCONECTES RUSTICUS

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ABSTRACT

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Sensory information is stimuli dispersed through the environment in multiple modalities. Signal detection of stimuli can be impaired when background noise is present. Background noise can include anthropogenic pollution, e.g. ammonium. Ammonium can disrupt communication in three ways: masking, sensory impairment, or physiological impairment. To test for these three hypotheses, crayfish pairs were sized-matched to 30% size difference and exposed to one of two exposures of ammonium: low exposure was 0.9 mg/L NH$_4^+$ and a high exposure of 9.0 mg/L NH$_4^+$ for eight days. A fighting test was completed the first day of the exposure and the eighth day of the exposure. Crayfish fighting pairs exposed to the low ammonium levels fought for a longer period of time, hypothesized to be a result from sensory impairment. Fighting pairs exposed to high ammonium levels fought for a shorter period of time compared to the control, which is predicted to be from physiological impairment. Changing the daily activity patterns of crayfish populations by manipulating the time spent fighting has disproportional impact on the ecosystem. Crayfish spend a deviated amount of time shredding detritus and mating, which affects nutrient cycling and predator-prey interactions in the ecosystem.
“To him who in the love of Nature holds Communion with her visible forms, she speaks a various language.” –William C. Bryant
ACKNOWLEDGMENTS

It is a pleasure to thank those who made this thesis possible. I would like to thank my advisor, Paul Moore, for guidance in academics and life; the Laboratory for Sensory Ecology for laughter and acting as a sounding board; and my family and friends for their patience and continuous encouragement.
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INTRODUCTION

Sensory cues are stimuli in the environment that an organism uses to extract information in order to respond in a behaviorally relevant fashion (Hasson 1994). An organism's behavioral repertoire can range from the negative taxis away from a harmful stimulus, including signaling in vocal playback of known predators (Andersen & Leibowitz 1978; Durant 2000; Epstein 1978), to positive taxis toward necessary resources like food (Willard & Devreotes 2006) and reproductively active conspecifics (Saxena 1978). An example of sensory information is the light cues used by copepods to mediate daily migration patterns (Hays 1996). Visual stimuli also are utilized in Fiddler crabs (*Uca pugilator*) to signal inferred dominance (How et al. 2009). In a different sensory system, field crickets use auditory signals to draw in potential mates (Wagner, Jr. & Reiser 2000). Sensory signals that evoke behavioral responses are composed of different types of stimulus energies including chemicals and vibrations.

Chemical and mechanical cues are common among aquatic animals due to the limited nature of visual signals in many habitats. Fiddler crabs (*Uca pugilator*) use mechanical signals carried in substrates to signal to reproductive conspecifics (Aicher et al. 1983). Crayfish detect vibrational signals through their antennae to sense nearby moving objects and currents (Masters et al. 1982). In addition, amphibians and cichlid use their sense of hearing to find receptive females (McGill 1960; Miranda & Wilczynski 2009) and to detect potential predators (Yan & Popper 1992). An odor cue, like an injured snail, can be used by both conspecifics and predators. Snails respond by seeking shelter whereas crayfish predators use these chemical cues as a foraging signal (Dickey & McCarthy 2007). The performance of the above behaviors involving signal detection is optimized in an ecosystem with a reduced level of noise in chemical and mechanical channels.
Background pollution in a sensory channel of an environment can affect an organism’s response to stimuli in three ways. First, sensory systems can be directly affected by chemical pollution causing an increased background noise leading to masking of a chemical. Second, the addition of chemicals in the environment can physiologically impair chemosensory systems by reducing sensory receptor functions (Sutterlin 1974). Finally, chemical pollution can also change systemic physiological processes in the organism. The first mechanism of sensory impairment involves chemical pollution that contributes to the background chemicals in the signal pathway.

Changes in background chemicals (either through biological or anthropogenic origins) can alter the signal to noise ratio of transmitted information which, in turn, would alter the ability of the receiver organism to extract relevant information. The relationship between the intensity of background stimuli and signal is termed the signal to noise ratio (Dusenbury 1992). When the background stimuli increase, the signal to noise ratio decreases which results in a loss in the detectability of a signal. If an organism using functionally related chemical compounds as sensory signals, an increase in background concentrations of chemicals can alter signal to noise ratios. When a signal is undetectable due to increased background noise, the signal is masked. If compounds of pollution are similar to the existing signal compounds, pollution has the potential to mask signals.

Masking is a sensory phenomenon that can prevent aquatic organisms from detecting chemical signals. Masking can result from background chemicals causing an increase in the firing of sensory cells such that a change in firing rate to a new stimulus is lost among the background activity (Harris & Dallos 1979). The humic substances of unicellular green algae chemically mask toxic compounds (Erbes & Webler 1997). The signals that salmon use to home to their breeding grounds have been susceptible to masking from background levels of metals in
aquatic systems (Hasler 1954). In addition to masking, background metals and other chemicals
that are of anthropogenic origin can cause injury to sensory systems.

The physiological impairment of chemosensory mechanisms could result from damage to
receptors or receptor neurons. One of the first steps in signal detection is the binding of
molecules to receptors in the cell membrane. Background chemicals can prevent the chemical
signal from binding to the receptor in two ways. An agonistic chemical will bind to the receptor
and cause a reaction that will compete with the signal detection in the nervous system. The
second type of binding is an antagonistic chemical that binds to the receptor preventing other
chemicals to be bound but does not cause activation of the nerve pulses that decrease the signal
to noise ratio (Negus 2006). In addition, background chemicals may cause irreversible damage to
chemoreceptor cells resulting in receptor death and permanent prevention of signal detection.
Receptor damage to the membrane or cell organelles can be reversible, preventing signal
recognition temporarily (Sutterlin 1974). Whether binding or damage occur, both cases result in
a period of time that the organism loses sensory capabilities. In addition to sensory impairment,
there may be metabolic or physiological implications that would reduce an organism's ability to
appropriately respond to sensory cues.

The third effect on organisms exposed to chemical pollution is changes in whole animal
physiology. For example, the pollutant dichlorodiphenyltrichloroethane (DDT) affected the
reproductive physiology of many organisms, including changing the secondary sex coloration of
the reed frog (Noriega & Hayes 2000) and testis deformation in domestic roosters (Blomqvist et
al. 2006). Some fishes have shown physiological changes as an adaptation to ammonium spikes
in rivers by air breathing, actively excreting of ammonium, and lessening of ammonium
production (Ip et al. 2004). Crustaceans have been shown to be physiologically affected by
ammonium as well including toxicity resulting in rapid death (Arthur et al. 1987; Romano & Zeng 2010; Young-Lai et al. 1991). An increase of free ammonium in the habitat of the freshwater crayfish, *Pacifastacus leniusculus*, leads to an increased accumulation of nitrogen in the hemolymph of the body (Harris et al. 2001). The physiological changes of the chemosensory system of crayfish are not the only measurable consequence in the species as a result of an increase in environmental ammonium.

As a keystone species, crayfish are central to the carbon cycle. Crayfish release carbon by shredding detritus for other organisms like insects (Sharma et al. 1984). Plants are consumed by crayfish (Olsson et al. 2008). Top predators like bass and mink consume crayfish continuing the cycle (Kellogg & Dorn 2012; Sidorovich et al. 2010). Crayfish can be used for studying ammonia because presence of crayfish influences the ecosystem.

Crayfish are the model organisms to use in an ammonium study because of the species’ life history and habitat. The natural habitat of *Orconectes rusticus* fluctuates in ammonium levels from 1.9 x10\(^{-5}\) M NH\(_4^+\) to 5.3 x10\(^{-5}\) M NH\(_4^+\) following peak fertilizing times (Ohio Environmental Protection Agency 2010). In addition, *O. rusticus* has the sensory capabilities to detect ammonium (Corotto et al. 2007). Finally, *O. rusticus* is accessible and has the capacity to withstand high ammonium toxicity (14.7 mg/L NH\(_4^+\)) in the environment (Arthur et al. 1987; Harris et al. 2001). All three sensory consequences that result from increased levels of ammonium as a background chemical have the potential to impact chemosensory mediated behavior in the crayfish.

The signal chemical in the urine cues of crayfish is an important cue in agonistic behavior. Dominant crayfish are known to urinate more than the subordinate opponents during fights (Bergman et al. 2005). Pre-exposure to urine signals without fighting affects agonistic
battles. For example, if a crayfish is exposed to dominant urine signal before fighting, the crayfish will display less intensity in fighting; while if pre-exposed to subordinate odors, the crayfish will fight with a higher intensity level (Bergman & Moore 2005). However, if two crayfish meet each other in two concurrent agonistic battles, urine cues often correlate with decreased time and intensity of the second fight (Zulandt-Schneider et al. 2001). Similarly when two fighting lobsters are rid of urine cues by catherization, the fight will have a significantly higher intensity and increased durations (Karavanich & Atema 1998). Therefore, the urine cues and the resulting behavior could be affected by an increase in the levels of the ammonium in the environment.

Given that crayfish utilize urine signals in agonistic battles, an increase in the amount of ammonium an agonistic pair of crayfish is exposed to would potentially result in masking in the chemosensory channel. In nature, the presence of ammonium in urine is hypothesized to be a communication signal for fighting ability and receptiveness to reproduction in the crayfish, *Orconectes rusticus*. The crayfish detects chemicals, like the ammonium in urine, in the environment by antennual whipping, decreasing the boundary layer and allowing for more chemical diffusion to the chemosensory receptors (Mellon 1997). An increase in ammonium in the environment may result in more noise and a higher threshold for detectability. Masking of urine cues is hypothesized to be the most likely complication that could result from the environmental increase in ammonium. I hypothesized that an elevated ammonium exposure prior and during an agonistic fight would result in escalated fighting behavior and an increased bout duration of the fight as a result of masked urine cues.
METHODS

Animals

Male, form I crayfish, *Orconectes rusticus*, were collected from the Portage River outside of Bowling Green, Ohio. Crayfish were placed in isolated containers in a flow-through holding tank with a constant temperature (23 °C) at least one week before the experiment. The animals were retained in a light:dark cycle (12:12 h) and one rabbit pellet was provided three times a week. Crayfish had a mean carapace size of 2.8 ± 0.1 cm, chelae length of 2.4 ± 0.1 cm, and weight of 9.3 ± 0.6 g (mean ± SEM). Sixty crayfish were paired throughout the experiment by size-matching with one crayfish at least thirty percent larger in carapace and chelae length than the opponent to predict a dominance hierarchy (Pavey & Fielder 1996). To identify individuals of the fighting pair, crayfish were marked with White-Out®. Animals were grouped into small (carapace: 2.4 ± 0.1 cm, chelae: 1.9 ± 0.1 cm, weight: 5.7 ± 0.4 g; mean ± SEM) and large (carapace: 3.3 ± 0.1 cm, chelae: 3.0 ± 0.1 cm, weight: 13.4 ± 0.8 g; mean ± SEM) sizes. Animals were returned to the river after a week of recovery following the experiment.

Experimental Habitats

Crayfish were held in the same experimental habitats for both treatment exposures and fight tests. The aquaria were ten-gallon tanks split into two equal sections with an opaque divider in the middle which prevented visual signaling, mechanical signaling, and chemical signaling by diffusion.

Treatment Exposures

A. Control; exposed to artificial pond water; N=10

B. Low concentration of ammonium; 0.9 mg/L NH₄⁺ in artificial pond water (vol/vol); N=10
C. High concentration of ammonium; 9.0 mg/L NH$_4^+$ in artificial pond water (vol/vol);

N=10

The low concentration of 0.9 mg/L NH$_4^+$ was determined by the average ammonium run-off as reported for the Portage River (Ohio Environmental Protection Agency 2010). The high concentration of 9.0 mg/L NH$_4^+$ was chosen by taking a logarithm of 10 to test for a higher threshold of chemosensory abilities.

**Behavioral Tests**

Two behavioral tests were performed; one at the beginning of the experiment (day one) and once after a week of exposure elapsed (day eight). Ammonium or control treatment was introduced into the aquarium by pipette in accordance with the volume in the tank and an acclimation period of ten minutes preceded each test. Behavioral tests began after the removal of the divider and lasted fifteen minutes. An agonistic bout, or a single aggressive encounter, began when a crayfish approached the opponent and was considered complete when the two crayfish are at least one body length apart for ten seconds (Fero 2007). In this study, the first bout was recorded and analyzed. Consecutive tests did not consist of a separate acclimation period because the behavioral tests were in the exposure habitats. After behavioral tests, crayfish were removed from the habitat and isolated while the habitat was cleaned with distilled water, the divider replaced, and tank was refilled with the ammonium or control treatment. (Fluorometric analysis of ammonium levels was completed over a two-day period to estimate ammonium loss (See Table 1)). Crayfish were then returned into the habitat. Tests were video-taped with a Panasonic HDC-HS700K 3MOS Hybrid Full HD 1920 x 1080 60p Camcorder.
Data Analysis

The first bout of each test was viewed and behaviors were assigned and timed by an ethogram (Table 2). With these behaviors, six dependent variables were measured: bout duration, maximum intensity level, time at intensity level 1, time at intensity level 2, time at intensity level 3, and slope of bout duration. Bout duration (N=60) was defined as the time from the initiation of the bout to the point where one crayfish retreated. Maximum intensity level (N=60) was the highest level of aggression that any single crayfish exhibits. Time at intensity level 1 (N=58) was the total amount of time that the maximum intensity level that either crayfish exhibited was intensity level one. Time at intensity level 2 (N=56) was the total amount of time that the maximum intensity level that any single crayfish was displaying was intensity level two or approaching the opponent with a meral spread. Time at intensity level 3 (N=25) was the total amount of time that the maximum intensity level of any single crayfish was intensity level three or when the one opponent boxes and grasps the other opponent open-clawed during the entire bout. Slope of bout duration was the difference in the length of the bout from day one to day eight. Slope was calculated by subtracting the bout duration at day eight from the bout duration at day one and dividing by the number of days.
Table 2. Agonistic ethogram used to determine levels of fight intensity as adapted from Moore 2007.

<table>
<thead>
<tr>
<th>Assigned Number</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2</td>
<td>Tail flip away from the opponent</td>
</tr>
<tr>
<td>-1</td>
<td>Back away slowly from the opponent</td>
</tr>
<tr>
<td>0</td>
<td>No threat and no forward or backward movement</td>
</tr>
<tr>
<td>1</td>
<td>Approach opponent</td>
</tr>
<tr>
<td>2</td>
<td>Approach opponent with a meral spread</td>
</tr>
<tr>
<td>3</td>
<td>Boxes and pushes opponent open-clawed</td>
</tr>
<tr>
<td>4</td>
<td>Grasps opponent with claws and dances</td>
</tr>
<tr>
<td>5</td>
<td>Unrestrained fighting and tearing of appendages</td>
</tr>
</tbody>
</table>

A two way, repeat-measures fully factorial MANOVA with time and concentration as factors followed by a post-hoc analysis (Fisher-LSD) was used to analyze the significant differences in maximum intensity, bout duration, time at one, time at two, and time at three to test for general differences between treatments and weeks. The slope of duration was evaluated by a one-way ANOVA with concentration as a factor. A one-way ANOVA was used because the calculation for slope includes both behavioral tests over time. Since significant results were found, a post-hoc analysis using a Fisher-LSD test was performed. Duration was analyzed by a two way, repeated-measures ANOVA with time and concentration as factors to test specifically for the designed hypotheses of masking, sensory impairment, and physiological impairment. A significant difference was found and a Fisher-LSD post-hoc was conducted.
RESULTS

Number of Behaviors

Not all behaviors were found in each bout. Crayfish in the control treatment on day one spent time at intensity level 1 (N=10), at intensity level 2 (N=9), at intensity level 3 (N=4), and at intensity level 4 (N=1). Crayfish in the control treatment on day eight spent time at intensity level 1 (N=10), at intensity level 2 (N=10), and at intensity level 3 (N=6). Crayfish in the low treatment on day one spent time at intensity level 1 (N=9), at intensity level 2 (N=9), and at intensity level 3 (N=3). Crayfish in the low treatment on day eight spent time at intensity level 1 (N=10), at intensity level 2 (N=8), at intensity level 3 (N=4). Crayfish in the high treatment on day one spent time at intensity level 1 (N=10), at intensity level 2 (N=10), at intensity level 3 (N=4), and at intensity level 4 (N=1). Crayfish in the high treatment on day eight spent time at intensity level 1 (N=9), at intensity level 2 (N=10), and at intensity level 3 (N=2).

General MANOVA

The comparisons of the overall two-way, repeat-measures fully factorial MANOVA were significant between day one and day eight ($F_{5,23,0.05} = 9.064, p < 0.001$). The comparisons between treatments were not significant ($F_{10,46,0.05} = 1.658, p > 0.05$). The comparisons between treatments and weeks ($F_{10,46,0.05} = 1.264, p > 0.05$) were not significant.

Comparisons between day one and day eight

From the behavioral measures, one measure was not statistically significant from day one to day eight. Time at one was not significantly different from day one to day eight ($p > 0.05$; Figure 1). All other measures between day one and day eight were significant.

Four significant statistics resulted from the intensity level measurements. On day one, the fighting pair spent more time of the first bout at intensity level two than on day eight ($p < 0.001$;
Figure 2). Similarly, the fighting pair spent a significantly greater amount of time at intensity level three on day one than on day eight as well (p < 0.001; Figure 3). The maximum intensity level of the first bout was significantly different from day one to day eight (p < 0.001; Figure 4). Finally, the duration of the bout on day one was on average longer than the duration of the bout on day eight (p < 0.05; Figure 5).

**Slopes One-way ANOVA**

The one-way ANOVA showed significant overall differences in the slope of duration ($F_{2,65.54,0.05} = 3.886, p < 0.05$; Figure 5). There was a significant difference in Fisher-LSD test between the high treatment and low treatment ($p < 0.05$). The slope of the bout duration for the control treatment was found between the high treatment and the low treatment. There were no significant differences between the control and the low treatment ($p > 0.05$). There were no significant differences between the high treatment and the control ($p > 0.05$).

**Bout Duration MANOVA**

The overall two-way, repeat-measures fully factorial ANOVA for duration was significantly different between treatments and weeks ($F_{2,1228,0.05} = 3.52, p < 0.05$). The ANOVA was not significant between treatments ($F_{2,313,0.05} = 0.636, p > 0.05$) or weeks ($F_{1,437,0.05} = 1.25, p > 0.05$). The Fisher-LSD post-hoc test revealed significant decrease in bout duration at day one of the low treatment compared to the control at day one ($p < 0.05$), but no significant differences at day eight between the control and the low treatment ($p > 0.05$). The post-hoc revealed a significant decrease in bout duration of the high treatment at day eight compared to control at day one ($p < 0.05$).
DISCUSSION

Crayfish aggression is altered in the presence of ammonium. When exposed to low levels of ammonium, the time the crayfish pair spent fighting increased compared to the control. Fighting pairs exposed to high levels of ammonium had a decreased time spent fighting during the first bout. Deviations from time normally spent in aggressive encounters alter the time spent in other activities like shredding detritus or mating. When crayfish cannot forage normally, detritus can begin to accumulate in aquatic ecosystems disrupting energy and nutrient flow. Decreased time spent mating would alter population dynamics which can, subsequently, alter predator population levels, as well as prey densities. These changes in crayfish aggression and foraging due to ammonium can cascade through the ecosystem.

Changes in crayfish behavior, physiology, and ecology have been demonstrated for other background chemicals. In five species of crayfish (Orconectes placidus, O. virilis, Procambarus acutus, P. alleni and P. clarkii) exposed to cadmium for 96 hours, three species had a decreased tail-flip response and two species had an increased claw raise response (Wigginton et al. 2010). Metolachlor has been shown to alter crayfish chemosensory ability to forage and the ability to react to alarm signals (Wolf & Moore 2002). Metachlor also affects the fighting ability of crayfish where treated individuals were less likely to initiate a fight and also lost significantly more fights (Cook & Moore 2008). The impact of background chemicals like metachlor and cadmium on behavior and other life history characteristics can vary according to the chemical and level of exposure.

I have hypothesized that background pollution in the environment can affect organisms in three ways. One hypothesis is masking of the chemosensory system due to an increased amount of chemical background noise. The second hypothesized way is that chemical pollution will
interrupt the physiological processes in the organism. The third hypothesis is chemicals introduced into the environment will cause physiological damage to the chemosensory systems by reducing sensory receptor functions (Sutterlin 1974). Results were found that supported two of the three hypotheses.

The results from exposure to low levels of ammonium could be explained by physiological impairment of chemosensory system. The physiological impairment causes a temporary or permanent damage to the receptors over time as the animal is exposed to the chemical. If the ammonium treatments are causing chemosensory impairment, the bout duration would increase over the eight days as the flow of chemical information used during fighting would decrease (Zulandt-Schneider et al. 2001). Previous work has demonstrated that chemical signals are critical for recognition of familiar individuals and dominant status (Zulandt-Schneider et al. 2001). The results from the crayfish fights that were exposed to the low ammonium treatment support the hypothesis that ammonium would cause sensory impairment. The bout duration of the low ammonium treatment for day one was significantly shorter than the bout duration of the control group. However, at day eight, there was no significant difference between the low ammonium treatment and the control group (Figure 5).

The results found in the high ammonium treatment support the hypothesis of physiological changes. Ammonium inhibits the chemical exchanges of $\text{Na}^+$ and $\text{NH}_4^+$ in the gills and causes a significant decrease in the osmotic concentrations of sodium in the hemolymph (Harris et al. 2001). If the ammonium treatments resulted in physiological changes to the body tissues, we would expect that crayfish show similar behavioral changes as those crayfish exposed to high copper levels. In that study, crayfish became lethargic (Sherba et al. 2000). If energy and activity levels decreased, bout duration would significantly decrease over the eight days and
would be significantly shorter in duration than the control. The decrease in bout duration can result from physiological damages caused by ammonium exposure. The high ammonium treatment affected the crayfish by decreasing aggression, supporting the physiological impairment hypothesis (Figure 5).

Neither results from the high ammonium treatment or the low ammonium treatment support the hypothesis of masking. If the results of a treatment supported the hypothesis, masking would have resulted from the increased number of ammonium molecules binding to chemosensory receptors and an immediate impaired ability to sense the urine signals of the opponent crayfish. The extended length of the bout duration would result from impaired chemosensory use in the agonistic battle which has been shown to increase fighting times (Bergman et al. 2005). Therefore, the bout duration of treatment would be significantly longer than the bout duration of the control on both day one and day eight. In the low treatment, the bout duration of the low treatment on day eight is increased from the control on day eight, but is not longer than the control on day one. The high treatment shows that the crayfish pair does not have an increase in bout duration than the control on day one or on day eight.

Effects of ammonium on crayfish aggression can vary by the concentration of ammonium that is present in the environment. If high concentrations of ammonium are present in the environment, the physiology of *O. rusticus* will be affected and the aggressive battles will decrease in duration. If low concentrations of ammonium are in the environment, the chemosensory system of *O. rusticus* will be impacted and aggressive battles would increase in duration.

Increased concentrations of ammonium affect other organisms directly by altering social behavior and life history. Species, such as the Rainbow Trout (*Salmo gairdneri stonei*) (0.53
mg/L NH₄⁺), Walleye (*Stizostedion vitreum*) (0.66 mg/L NH₄⁺), Channel Catfish (*Ictalurus punctatus*) (0.86 mg/L NH₄⁺), White Suckers (*Catostomus commersoni*) (15.3 mg/L NH₄⁺), Cladoceran (*Simocephalus vetrulus*) (1.71 mg/L NH₄⁺), Fathead Minnows (*Pimephales promelas*) (2.17 mg/L NH₄⁺), snails (*Physa gyrina* and *Helisoma trivolvis*) (1.95 mg/L NH₄⁺ and 2.37 mg/L NH₄⁺), and amphipods (*Crangonyx pseudogracilis*) (3.12 mg/L NH₄⁺), have shown levels of toxicity to ammonium resulting in rapid death (Arthur et al. 1987). Fingernail clams (*Sphaerium novaezelandiae*) have a decrease in burrowing ability for the first hour of recovery after a 96-hour exposure to ammonium (Hickey & Vickers 1994). Shrimp begin to clump together with conspecifics and become lethargic at ammonium levels of 0.4 g/m³ (Hickey & Vickers 1994). Ammonium impacts not only crayfish but other organisms, such as vertebrates, through direct effects like behavior alteration and indirect effects that cascade through the ecosystem.

Sensory impairment from low concentrations of ammonium can impact mating behavior in crayfish. Urine cues from female crayfish are used to communicate receptivity in mating and when female urine cues are blocked, male courtship behavior is prevented (Berry & Breithaupt 2010). Decrease in crayfish population free habitat to crayfish competitors. These habitats could be filled by other, potentially invasive, macroinvertebrates, specifically snails (Lodge et al. 1994). Less time spent matting would alter crayfish population dynamics which can, subsequently, alter the food sources of predator populations such as game fish and mink (Kellogg & Dorn 201; Sidorovich et al. 2010). Low concentrations of ammonium would manipulate ecosystem structure by altering niches and decreasing crayfish populations.

Ammonium effects on the chemosensory systems of crayfish can be detrimental to the ecology of aquatic habitats. During foraging, crayfish utilize chemical cues to orient to and
locate food (Moore & Grills 1999). Crayfish cannot forage efficiently when chemosensory ability is inhibited (Kraus-Epley & Moore 2002). If crayfish cannot forage because of sensory impairment or physiological impairment, large packets of detritus would begin to accumulate in the system (Dorn & Wojdak 2004). The build-up of detritus in aquatic ecosystems disrupts energy flow and carbon cycling. Detritus build-up causes increased sediment deposit in ecosystems (Ansell 1974). Storms cause more turbulent flows and mixing of detritus and sediment in the rivers. Increased sediment is suspended into the water column by mixing and causes a decrease in water clarity (Swift et al. 2006). Changing water clarity would affect the visual ability of top predators to forage (Crowl 1989; McMahon & Holanov 1995). Insect larvae, like the crane fly, that feed on the detritus shredded by crayfish would have a limited food source (Sharma et al. 1984). Decrease in crayfish foraging and shredding could also cause an increase in plant species. Insect larvae populations that utilize the plant species as shelters like diving beetles and mosquitoes could increase in population levels with an increase in habitat size (Inoda 2011; Serandour et al. 2006). Because of these cascading trophic effects, inefficient foraging due to ammonium of crayfish would impact the organisms in the ecosystem indirectly from both bottom-up effects and top-down effects.

Deviations from time normally spent in aggressive encounters alter the time spent in other activities like shredding detritus or mating. Predator populations, like bass and mink, in the ecosystem are affected directly by a decrease in crayfish prey populations due to the decrease in time spent mating (Kellogg & Dorn 2012; Sidorovich et al. 2010). Organisms affected indirectly include insect that feed on shredded detritus and that inhabit plant species (Inoda 2011; Serandour et al. 2006; Sharma et al. 1984). Declining crayfish populations creates open habitats for organisms, such as snails to populate the ecosystem (Lodge et al. 1994). These changes in
crayfish aggression and foraging due to ammonium can cascade through the ecosystem, affecting any similar organism with chemosensory abilities.
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APPENDIX A. TABLES AND FIGURES

Table 1. Results of two-day fluorometric analysis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1 (mg/L)</th>
<th>Day 2 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.35</td>
<td>0.53</td>
</tr>
<tr>
<td>Low</td>
<td>5.28</td>
<td>2.54</td>
</tr>
<tr>
<td>High</td>
<td>13.2</td>
<td>9.52</td>
</tr>
</tbody>
</table>
Figure 1. Time at Intensity Level One. Mean (± SE) of time (s) that crayfish bouts spent in intensity level 1 for crayfish exposed to control (open square), low ammonium (closed circle), and high ammonium levels (X).
Figure 2. Time at Intensity Level Two. Mean (± SE) of time (s) that crayfish bouts spent in intensity level 2 for crayfish exposed to control (open square), low ammonium (closed circle), and high ammonium levels (X). Asterisk indicates a significant difference between time in seconds at intensity level two on day one versus day eight ($F_{5,23,0.05} = 9.064$, $p < 0.001$; Fisher LSD, $p < 0.001$).
Figure 3. Time at Intensity Level Three. Mean (± SE) of time (s) that crayfish bouts spent in intensity level 3 for crayfish exposed to control (open square), low ammonium (closed circle), and high ammonium levels (X). Asterisk indicates a significant difference between time in seconds at intensity level three on day one versus day eight ($F_{5,23,0.05} = 9.064$, $p < 0.001$; Fisher LSD, $p < 0.001$).
Figure 4. Maximum Intensity. Maximum intensity (± SE) that crayfish bouts reached for crayfish exposed to control (open square), low ammonium (closed circle), and high ammonium levels (X). Asterisk indicates a significant difference between maximum intensity level of the first bout on day one versus day eight ($F_{5,23,0.05} = 9.064, p < 0.001$; Fisher LSD, $p < 0.001$).
Figure 5. Duration of Fight. Duration (s) (± SE) of the first bout for crayfish exposed to control (open square), low ammonium (closed circle), and high ammonium levels (X). Asterisk indicates a significant difference between duration of the first bout in seconds on day one versus day eight ($F_{2,1228,0.05} = 3.52$, p < 0.05; Fisher LSD, p < 0.001).