THE SENSORY MECHANISMS OF CRAYFISH (ORCONECTES RUSTICUS) USED IN DETECTING PREDATORY THREATS

Jessica Clark

A Thesis

Submitted to the Graduate College of Bowling Green State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2017

Committee:

Paul Moore, Advisor

Jeffrey Miner

Daniel Wiegmann
ABSTRACT

Paul Moore, Advisor

Organisms are exposed to numerous environmental stimuli in which they must be able to distinguish threats from food sources. In order to make such distinctions, organisms rely upon sensory mechanisms, including chemoreception, vision, and mechanoreception. With the reception of chemical, visual, and/or mechanical cues prey species can determine the location, size, and movement of a nearby predator. Then, with the information gathered, prey can determine the severity of the threat and respond accordingly, whether to flee or to display a defensive stance. Various studies suggest that several aquatic species, including crayfish, rely on the integration of sensory modalities to accurately assess predatory threats. This study aimed to determine whether a hierarchy in the reliance upon sensory modalities exists in crayfish (Orconectes rusticus) and if this hierarchy is altered across different sensory environments (such as flowing and non-flowing environments). We also sought to determine the significance of sensory multimodality in crayfish. To study the relevance of each of the sensory modalities, as well as the integration of these modalities, in crayfish combinations of lesions/blocks were conducted. Two sensory mechanisms (chemical and mechanical, chemical and visual, or visual and mechanical) were lesioned/blocked at once, leaving one sensory mechanism (vision, mechanoreception, or chemoreception) functional. Each of the crayfish were then exposed to a predatory largemouth bass (Micropterus salmoides) in either a flowing or non-flowing stream where their behavior was recorded for 30 minutes. The behaviors and movements of the crayfish were then analyzed with the use of Ethovison Noldus XT. Linear mixed models were then conducted to determine the impact of the lesions, flowing environments, and the combination of
the lesions and flowing environments on the ability of crayfish to detect predatory stimulus.

Significant Least Squares Means (LSM) test were followed by Type II Wald Chisquare tests.

Results from this study support the significance of sensory multimodality in crayfish for accurately detecting and assessing predatory threats. When the sensory multimodality of crayfish was eliminated the animals were challenged to successfully assess the severity of the predator. Crayfish with only the full use of chemoreceptors or mechanoreceptors showed a greater avoidance of the predator, indicating that these individuals could detect the threat but could not accurately locate the source. Results from this study also suggest that a hierarchy in the reliance upon sensory modalities does exist in crayfish, with a bias towards chemoreception, followed by mechanoreception, and finally vision.
ACKNOWLEDGMENTS

First, I would like to thank my advisor and mentor, Dr. Paul Moore, for his guidance over the past two years. I’m thankful he went out on a limb and gave me this opportunity to start my scientific career. His advice on being a good scientist and wisdom on life has made me the scientist I am today, and for that I’m forever grateful. I’m also thankful for the lab mates and friends that I have gained as being a member of the Laboratory for Sensory Ecology. I would like to thank the members of the LSE for their endless support over the past two years, whether that be countless edits on papers, late night crayfishing trips, or days of cinderblock lifting.

Second, I would like to thank Bowling Green State University for funding my Masters research for the past two years. My committee members here at BGSU, Dr. Jeffrey Miner and Dr. Daniel Wiegmann, thank you for taking the time to give me comments on my project design, edits on my thesis, and for attending my presentations. I would also like to thank the University of Michigan Biological Station for the opportunity to conduct my research at the Stream Research Facility. A special acknowledgement to Dr. David Gates and the Gates family for their investment, in the form of a fellowship, in graduate research and housing at the UMBS.

Finally, I would like to thank my family and friends for their never-ending support for the past two years. Mom and Dad, Lynn and Ron Clark, thank you for always encouraging me to follow my dreams and having confidence in me, believing that I can achieve anything even when times are challenging. Josh Clark, my brother, thank you for the endless support and laughter. Nana and Papa, Lois and Bobby Barnett, thank you as well, for your endless love and support. I would have never made it this far without the encouragement and support from them.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS</td>
<td>6</td>
</tr>
<tr>
<td>Animals (Collection and Holding)</td>
<td>6</td>
</tr>
<tr>
<td>Monitoring Health and Safety of Fish</td>
<td>7</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>7</td>
</tr>
<tr>
<td>Restraint and Handling of Crayfish Through the Lesioning Process</td>
<td>9</td>
</tr>
<tr>
<td>Chemical Lesion/Sham</td>
<td>9</td>
</tr>
<tr>
<td>Visual Lesion/Sham</td>
<td>10</td>
</tr>
<tr>
<td>Mechanical Lesion/Sham</td>
<td>12</td>
</tr>
<tr>
<td>Experimental Arena</td>
<td>12</td>
</tr>
<tr>
<td>Starvation, Acclimation, and Protocol</td>
<td>14</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>15</td>
</tr>
<tr>
<td>Path Digitization</td>
<td>15</td>
</tr>
<tr>
<td>Behavioral Measures</td>
<td>16</td>
</tr>
<tr>
<td>Extraction of Behaviors</td>
<td>17</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>17</td>
</tr>
<tr>
<td>RESULTS</td>
<td>18</td>
</tr>
<tr>
<td>Significance of Lesions</td>
<td>18</td>
</tr>
<tr>
<td>Time in Zones</td>
<td>18</td>
</tr>
<tr>
<td>Movement in Zones</td>
<td>18</td>
</tr>
<tr>
<td>Significance of Flow</td>
<td>20</td>
</tr>
</tbody>
</table>
Time in Zones................................................................. 20

Movement in Zones.......................................................... 20

Significance of the Interaction Between Lesion and Flow .................. 20

Time in Zones................................................................. 20

Movement in Zones.......................................................... 22

DISCUSSION ............................................................................. 23

REFERENCES .......................................................................... 29

APPENDIX A: FIGURES .......................................................... 36

APPENDIX B: TABLES ............................................................ 41

APPENDIX C: IACUC APPROVAL ............................................. 44
INTRODUCTION

Predators impact ecosystems through consumptive and non-consumptive effects. Consumptive effects (CEs), defined as the direct physical interactions between predators and prey, impact prey populations by reducing the density of populations through the act of consumption (Davenport & Chalcraft, 2013; Turner & Peacor, 2012; Weissburg et al., 2014). Examples of consumptive effects include *Helicoverpa armigera* larvae feeding on Australian cotton crops (Rendon et al., 2015) to a lion attacking and consuming a gazelle. Non-consumptive effects (NCEs) are defined as the indirect interactions between predators and prey (Davenport & Chalcraft, 2013; Elvidge & Brown, 2015; Weissburg et al., 2014). These indirect interactions refer to the factors of intimidation prey demonstrate while in areas of previous and potential predatory threats. For example, several species of *Daphnia* increase their body size, reducing their chances of predation by smaller fish, with the addition of spines, neckteeth, and/or helmets in response to kairomones released by predators (Dzialwoski et al., 2003; Preisser et al., 2005). Multiple studies have shown that NCEs can have greater impacts on the dynamics of prey populations as compared to CEs (Preisser at al., 2007; Rendon et al., 2015; Sih et al., 2010; Turner and Peacor, 2012; Weissburg et al., 2014).

NCEs occur when the threat of predation alters the behavior and/or lifecycles of prey species (Lima & Dill 1990). These short- and long-term behavioral and morphological changes caused by NCEs include reduced foraging, avoidance of certain areas within a habitat, and altered growth rates (Davenport and Chalcraft, 2013; Larsen, 2012; Preisser at al., 2007; Weissburg et al., 2014). For example, after grey wolves were reintroduced to Yellowstone National Park, the population of elk reduced foraging on aspens, which resulted in an increase in the growth of aspen trees and other native plants (Ripple and Beschta, 2006). In the presence of
Mojave Desert sidewinders (*Crotalus cerastes*) two species of kangaroo rats (*Dipodomys deserti* and *D. merriami*) avoided certain areas of the experimental habitat. Areas with the highest risk of snake interactions were actively avoided by the kangaroo rats (such as under bushes), resulting in a reduction of foraging (Bouskila, 1995). Previous studies, including those mentioned, have shown the impact NCEs have on food webs within ecosystems and on the behavioral decisions of prey species.

Most often the response of prey upon the detection of predatory signals is avoidance, which has been called a “fear” response (Laundré et al., 2010). From such concepts, arose the “landscape of fear” theory (Laundré et al., 2010). Several studies have shown that prey alter their use of the landscape according to a perceived threat of predation (Elvidge and Brown, 2015; Fine et al., 2011; Laundré et al., 2010; Weissburg et al., 2014). In areas of greater potential lethality, prey become more vigilant, alter their foraging and mating behaviors, and avoid certain areas even at the cost of resource acquisition. Whereas, in low probability areas of interactions with predators prey are less vigilant, therefore, forage more often and for greater durations. Laundré et al. (2010) demonstrated how mule deer (*Odocoileus hemionus*) alter their use of a landscape according to predation risks of pumas (*Puma concolor*). Most often the deer were documented to avoid the forest edges, where pumas are successful hunters, and preferred open areas, where pumas are not as efficient at hunting (Laundré et al., 2010). When exposed to visual and chemical cues of predatory largemouth bass (*Micropterus salmoides*), even with no physical interaction, juvenile channel catfish (*Ictalurus punctatus*) reduced foraging habits (Fine et al., 2011). This foraging reduction resulted in slower growth rates compared to control groups with no exposure to predatory threats (Fine et al., 2011). This landscape of fear can be dependent upon the sensory landscape an individual inhabits.
Jurcak and Moore (2014) defined sensory landscape as the temporal and spatial distribution of stimuli throughout an individual’s environment. The sensory landscape for organisms is created by the presence of others, such as prey, conspecifics, and predators, and the transmission of sensory signals through the environment (Jurcak & Moore, 2014). Each unique predator creates a set of sensory stimuli, whether those stimuli are visual, chemical, auditory, or mechanical, detectable by prey. For example, avian predators cast shadows from above and/or create disturbances in the treetops with the beating of their wings, alerting prey of their presence (Lohrey et al., 2009). Also, ambush predators, such as snakes, secrete chemical cues (kairomones) into the environment which species of prey, including lizards, can detect their presence (Cabido et al., 2004). However, the sensory landscape can be dependent on the characteristics of the landscape itself.

The mixing, dispersal, and quality of stimuli throughout an area is reliant upon the habitat in which it occurs. For example, highly turbid environments will limit the transmission of light, and therefore, visual cues (whereas turbidity does not likely affect chemical cues) (Lunt and Smee, 2015; Weiss et al., 2012). Chemical cues are carried by bulk flow and remain in aquatic systems longer compared to those of terrestrial systems. The wind speed of terrestrial systems and the hydrodynamic forces of aquatic systems, including flow velocity and turbulence (Hazlett et al., 2006), can affect the structure of odor plumes, which alters the distribution of chemical cues and how organisms perceive the available information (Turner and Peacor, 2012; Weissburg, 2012). Over contrasting substrates, odor plumes distribute at different rates and concentrations, affecting the distribution of information (Wolf et al., 2009). Obstructions in the landscape, such as trees and rocks, can disrupt the flow of signals through the environment, including blocking visual cues and/or accumulating chemical cues in certain areas. Conversely,
sound travels at a higher speed in aquatic environments as opposed to terrestrial ones. Mechanical/tactile cues are limited to short distances (Weissburg et al., 2014). The sensory landscape, as described above, contains the stimuli from which prey extract information about the threat of predation.

Aquatic organisms rely upon multiple sensory mechanisms to detect potential predators. Most often chemoreception, mechanoreception, and vision are utilized to assess such predatory threats (Smith & Dunham, 1990; Weiss et al., 2012). Chemical cues provide prey species with the locality of the threat and the degree of threat (higher concentrations of chemical cues can indicate a predator, or multiple predators, are nearby) (Weiss et al., 2012). The reception of mechanical cues indicates the movement of the predators in close proximity (Weiss et al., 2012). Visual cues provide information of the characteristics, such as body size/shape, of predators within a limited distance (Smith & Belk, 2001). The integration of such sensory mechanisms allows organisms to assess predatory threats with accuracy and thus respond in a proper way (Partan & Marler, 2005). Studying the function of sensory modalities can further distinguish the significance of modality integration, an individual’s reliance upon such modalities, the information gathered from each, and how individuals use the information gathered to make decisions to limit the likelihood of consumption. This study focused on the importance of sensory integration and whether a hierarchy of reliance upon sensory mechanisms exists in crayfish.

By limiting the functional sensory modalities of crayfish (Orconectes rusticus) to one, we hoped to determine the degree of multimodality that occurs during predatory threats, which in turn allows us to determine how each sense influences the NCEs caused by predatory largemouth bass (Micropterus salmoides). Orconectes rusticus inhabit both flowing (rivers and streams) and
non–flowing (lakes and ponds) environments (Bergman et al., 2006) and have been shown to rely on vision, chemoreception, and mecahnoreception to detect nearby prey and potential predators (Callaghan et al., 2012). This multimodality and ubiquitous presence in aquatic habitats made *Orconectes rusticus* appropriate experimental organisms for this question. *Micropterus salmoides* are known predators of *Orconectes rusticus* and their presence has been shown to alter the behaviors of crayfish (Keller & Moore, 2000). Through sensory lesions/blocks the multimodality of *Orconectes rusticus* was limited to one functional sensory mechanism, either chemoreception, mecahnoreception, or vision alone. *Orconectes rusticus* were then exposed to two environmental conditions, flowing and non–flowing streams, to further distinguish the role of sensory modalities and the impact of environmental conditions.
METHODS

Animals (Collection and Holding)

Rusty crayfish, *Orconectes rusticus*, were collected from Maple Bay in Burt Lake, MI (lat. 45°28’N, long. 84°40’W) throughout the summer of 2016 by use of hand nets. Both adult males and females (post orbital carapace length: mean ± SEM = 3.06 ± 0.033 cm) were used. All individuals were in form II (non-reproductive form), free from injuries with all appendages (chelae and walking legs), including sensory appendages (antennules and antennae), intact. Crayfish were housed at the University of Michigan Biological Station Stream Research Facility in streams made of cinder blocks lined with plastic sheeting (304.8 cm x 81.3 cm x 40.6 cm; L:W:H). Unfiltered water from the nearby Maple River was pumped into the holding streams, which provided crayfish with river water and detritus. Within the streams, crayfish were housed individually in Tupperware containers (18.1 cm x 16.2 cm x 7.3 cm; L:W:H) where they were physically and visually isolated from other individuals. Crayfish were isolated a minimum of one week before use in trials. This isolation ensured a limited diet of natural detritus and no interactions with other individuals that could have resulted in dominant and subordinate statuses of individuals.

Thirty adult male and female (12.7 cm - 25.4 cm standard length) largemouth bass, *Micropterus salmoides*, were provided by Harrietta Hills Trout Farm in Harrietta, MI. The bass were equally divided and housed in two flow through metal horse troughs (237.5 cm x 86.4 cm x 60.1 cm: L:W:H). Unfiltered water from the nearby Maple River filled the holding troughs. A sand substrate, approximately 2 cm deep, covered the bottoms of the troughs to reduce the risk of physical injury to the fish. Several PVC Pipes were provided as shelters for the bass. Window
screening covered the tops of the troughs to prevent the loss of fish to predatory animals. The fish were fed a diet of Purina Aquamax Sport Fish/Grower 600 pellets every day.

Monitoring Health and Safety of Fish

The procedures with largemouth bass were approved by research protocols 856543-2 (BGSU) and PRO00004591 (Michigan). The bass were monitored for signs of distress and disease throughout their time in the holding troughs and during trials. If/when signs of stress, such as strange swimming patterns or gasping at the surface of the water, were displayed by an individual, the individual was removed immediately from their housing trough or experimental arena. Once removed, the fish were transported in a cooler equipped with an aerator and placed into a quarantine tank located inside the Stream Research building. While quarantined, the stress levels of the fish were monitored. Fish that displayed any signs of disease were also removed from the holding streams and placed into quarantine tanks. The quarantine tanks allowed for the administration of antibiotics and close monitoring, along with minimizing the spread of disease. Fish that displayed any signs of distress or disease were not used in the trials.

Experimental Design

A 2 x 4 fully factorial experimental design was conducted to examine the dependence of sensory mechanisms utilized by crayfish to detect predators in different flow environments with flow as one treatment and sensory mechanisms as the second treatment. Within the flow treatment, two conditions existed (flowing and non–flowing). Within the sensory mechanism treatment, a total of four treatment conditions, three lesions/blocks and one control, were created. For each treatment within the sensory mechanisms condition, one sensory channel remained active, while two sensory channels were either blocked or lesioned. For example, in the chemical
active treatment, mechanoreceptors and vision were blocked while chemoreception was sham lesioned, leaving this sense active. In a similar way, the mechanical active treatment had a sham block performed on the mechanoreceptors with vision being blocked and chemoreceptors being lesioned. In the visual active treatment, vision had a sham lesion, while mechanoreceptors were blocked and chemoreceptors were lesioned. In the control condition, shams were performed on all three sensory mechanisms. Details for the sham and lesion techniques follow this section.

While the mechanical and visual mechanisms were technically blocked rather than lesioned, we will use the term lesion for all three treatments for ease of communication. The experimental design is outlined below.

Flow environment:

- Control; N = 10
- Chemical Active; N = 10
- Vision Active; N = 10
- Mechanical Active; N = 10

Non-flow environment:

- Control; N = 10
- Chemical Active; N = 10
- Vision Active; N = 10
- Mechanical Active; N = 10

Each of the four conditions were replicated 10 times in both environments, and crayfish were only used once within a trial. A total of 80 crayfish were used.
Restraint and Handling of Crayfish Through the Lesioning Process

Once selected for a treatment, individuals were physically restrained, ventral side down while facing downward, and placed on a bath of well water (5 ° Celsius) for at least 2 minutes. The restraints limited the movements of the individuals throughout the process, while the cold water helped to further slow their movements. During this process the restraint boards stood at a slight angle (approximately 70°), ensuring that water did not spill from the antennule reservoirs (micro-pipette tips). River water was applied to the gills and along the body of the crayfish every 10 to 15 minutes. The health of the crayfish was monitored throughout the process. The total duration of the lesioning process, and the amount of time crayfish were restrained, was 2 hours and 10 minutes. Once the crayfish were exposed to the appropriate combination of lesions and/or shams the individuals were placed in their holding containers to await trial the next morning.

Chemical Lesion/Sham

Chemical Lesion: All four antennules (lateral and medial filaments) of the crayfish were placed in a reservoir filled with 50 ppt saltwater for 2 hours (Kraus-Epley et al., 2015). To ensure the antennules were exposed to the saltwater throughout the 2 hours, additional saltwater was added to the reservoir as needed. The saltwater within the reservoirs was also replaced after each watering of the crayfish, every 10 to 15 minutes. After 2 hours of exposure, the saltwater was replaced with deionized water for 10 minutes. This change in osmotic pressure lysed the dendrites of chemoreceptors while the mechanoreceptors remained intact (Belgane et al., 1997; Kraus-Epley et al., 2015). This technique has been used for lesioning chemoreceptors of crayfish in several past studies and has proven to provide an effective chemosensory lesion (Belgane et al., 1997; Kraus-Epley et al., 2015).
Chemical Sham: Both pairs of antennules were placed in a micro-pipette tip filled with river water, from the crayfish holding stream, for 2 hours and 10 minutes (the same duration as treated animals). To ensure the antennules were exposed to the river water for the entire time the reservoir was filled with water every 10 to 15 minutes, or as needed.

**Visual Lesion/Sham**

Visual Lesion: To block the reception of any visual cue/signal, thin black flexible goggles were placed over the eyes of the individuals. While restrained and antennules in reservoirs, a small wooden peg (4.7 ± 0.2 mm long and 2 mm round) was secured to each side of the carapace, just behind the eyes, with Loctite brand epoxy. The epoxy was allowed to dry completely. Correct size goggles, large or small, were then selected based on the size of each individual. Black goggles were made by mixing the standard 1:1 ratio of Black Sylgard® 170 Silicone Elastomer (Dow Corning Corporation, Midland, MI). Four grams of Part A and 4 grams of Part B were mixed together. A thin layer (0.8 ± 0.08 mm) of the mixture was then poured into a glass Pyrex petri dish cover (9.3 cm in diameter and 1.1 cm deep). Seven to ten metal ball bearings (7 mm in diameter or 5 mm in diameter) were then strategically placed in the dish. The dish was placed in an oven at 125° Celsius for 30 minutes, or until the mold had cured. Once the mixture had hardened, the dish was removed from the oven and allowed to cool. A small metal spatula was then used to carefully remove the Sylgard® mold from the dish. The ball bearings were then removed from the mold, leaving a circular indentation. Teardrop shaped metal cutters were used to shape the goggles, ensuring that the larger/rounder end of the shape was around the circular indentation in the molds. The small goggles were created from the molds with 5 mm indentations, which were shaped with a 1.85 cm x 1.3 cm (L:W) teardrop shaped metal cutter (Figure 1a,b). The 2.5 cm x 1.85 cm (L:W) metal cutter was used to make the larger goggles
from the molds with the 7 mm indentations (Figure 2a, b). Once the correct size of goggles was selected, the goggles were cutout and trimmed to best fit for each animal. Goggles were held in the correct position on the animal, eye in eye slot and goggle not in the way of the antennae, and the location of the hole for the peg was marked (using a sharpie). A 3 mm hole was then punched, using a cork borer, in the goggle. One at a time, the goggles were positioned correctly on the pegs, being sure the eyes were blocked, and secured in place with Loctite superglue. The superglue was allowed to dry.

Visual Sham: Using the same application methods as black goggles above, clear goggles were applied to the control individuals. The clear goggles were made following the same protocol as the black goggles, however the standard 10:1 ratio (10 grams of the base to 1 gram of the curing agent) of Sylgard® 184 Silicone Elastomer was used. When the clear goggles were applied correctly the vision of the crayfish was not hindered in any way and therefore remained active.

To ensure that the goggles had no impact on the vision of the crayfish preliminary trials were conducted. In the lab, 10 control animals (crayfish without goggles) and 10 experimental animals (crayfish with clear goggles) were exposed to a scare stimulus (a row of 3 ping pong balls coming towards them; Rapin & Moore, 2016). The distance between the scare stimulus and the initiation of a tailflip (a common retreat response) was recorded for each individual. Animals in both treatment conditions exhibited similar response distances and therefore we concluded that the goggles had no impact on their vision.
Goggled animals were also exposed to slightly increasing flow velocities (from 5 cm/s to 15 cm/s) in the lab. The findings from this exposure ensured that the goggles would not flap up during the following trials and, therefore, the animals remained blinded throughout.

**Mechanical Lesion/Sham**

Mechanical Lesion: While restrained, Gorilla® Super Glue was applied, using the brush applicator, along the entirety of the antennae, making sure to cover each of the aesthetasc hairs (Kraus - Epley and Moore, 2002). When applied correctly, the super glue blocked the mechanoreceptors along the antennae inhibiting the collection of mechanical cues. Once the glue had dried the mechanoreceptors were then considered lesioned. This lesioning technique has been used for blocking mechanoreceptors of crayfish in several past studies and has been shown to be an effective mechanical and not chemoreceptor lesion (Kraus - Epley and Moore, 2002).

Mechanical Sham: Gorilla® Super Glue, similar to the amount applied to experimental crayfish, was applied to the base of the carapace of the individuals. This was to ensure that the odor of the glue had no effect on the behavior of the crayfish. The antennae were then washed with a 1 mL pipette filled with river water. This was to replicate the stimulation of antennae in which experimental animals were exposed to in the gluing process. The super glue was allowed to dry. The mechanoreceptors were not altered in any way and therefore remained active.

**Experimental Arena**

Four experimental arenas (118 cm x 81 cm x 41 cm L:W:H) were constructed using cinder blocks lined with 4 mil plastic sheeting. To create a flow through arena, water from the Maple River entered from a head tank and flowed through the arena at a rate of 4 cm per second and exited through an 81 cm x 21 cm (L:H) piece of plywood with 8 mm holes at the opposite
end of the stream. The water level remained at a depth of 20 cm. Nylon stockings placed over the supply pipe helped reduce the quantity of macroinvertebrates and fine organic matter in the water. The bottom of the arenas were covered with approximately 2 cm of sand. Streams were divided into two areas, upstream for the bass, 40 cm x 81 cm x 41 cm (L:W:H), and downstream for the crayfish, 81 cm x 81 cm x 41 cm (L:W:H). The two areas were separated by egg crating, which allowed the individuals to visually and chemically sense each other but avoided physical contact. During acclimation, a non-porous divider, 81 cm x 25.4 cm (L:H) plywood, was placed beside the egg crating to prevent mixing of stimuli between the bass and crayfish sections (Figure 3). To ensure that both animals received constant flow during acclimation, a secondary outflow (for the bass) and a secondary inflow (for the crayfish) were constructed. This allowed water to enter in the upstream (bass) area and exit right before the barrier. On the opposite side of the barrier (downstream/crayfish area), water entered from secondary hoses from the head tank and then exited in the outflow of the arena. Once the acclimation period ended, these secondary inflow and outflow were deactivated and the non-porous divider removed, leaving only the egg crating divider between the animals. The no-flow arenas (Figure 3) were identical in size, shape and made of the same materials as the flow streams, however there were no inputs or outputs of water, the hoses from the head tank were removed and the holes in the outflow were plugged. For acclimation periods, the same non-porous dividers used in the flowing streams were placed beside the egg crating, to prevent any exchange of information between the crayfish and bass. After the 15 minute acclimation period, the non-porous dividers were removed and the trials began. The downstream/crayfish area of the experimental arenas contained a shelter along the right wall, 30 cm from the egg crating divider, and a food source along the left wall, opposite the shelter. The shelters were made of PVC pipes cut in half (8.3 cm
x 7.6 cm; L:D) attached to a Plexiglas base. The available food source consisted of a small plastic cap (2.5 cm in diameter and 1 cm deep) filled with fish gelatin. The fish gelatin was made by mixing 28 grams of Knox’s unflavored gelatin, 46 grams of homogenized canned sardines, and 600 ml of boiling water (Wolf et al., 2004). This gelatin mixture was then poured into individual small plastic caps and allowed to set in a refrigerator for 12 hours. Caps of food were only used for one trial each. Using four low light security cameras (Model #PRO-615) mounted on a wooden structure above the arenas, the behaviors of the crayfish were recorded from above. The cameras were connected to a SWANN DVR4-3250 model DVR housed within the Stream Research building where the trials were recorded for further analysis. Black tarps (9.1 m x 9.1 m) covered the wooden structure above the arenas to ensure consistent lighting throughout each of the trials. Four 25W A19 red transparent lightbulbs were arranged on the structure to provide lighting.

*Starvation, Acclimation, and Protocol*

Each of the crayfish were housed in Tupperware for a minimum of 7 days before use in trials. During the 7 days, the Tupperware limited the amount of natural detritus available to the animals. This limited diet was implemented to increase motivation for foraging during trials. Due to their role in the experimental trials, limited to simply providing the presence of a predator, the bass were not starved.

For each of the trials, one crayfish and one bass were selected at random. Crayfish were used for only one trial each while bass, due to a limited quantity, were used for multiple trials. The handling time for each animal was as limited as possible. Using a fine net, bass were individually collected from the holding trough and placed in a cooler equipped with an aerator.
and filled with water from the river. From the cooler the bass were transported to the upstream area of the experimental arena. The selected crayfish, once lesioned and marked with whiteout, were safely transferred, in Tupperware, and released into the downstream area of the arena. With the non-porous divider in place, both of the individuals were allowed to acclimate simultaneously for 15 minutes. This acclimation time allowed both the bass and the crayfish to adjust to their new surroundings before being exposed to one another.

The trials took place between the hours of 08:00 and 12:00. For each trial a fresh cap of fish gelatin was placed in the arena. Each of the trials ran for 30 minutes. Once the trials were complete, the food cap was collected and weighed and the fish were placed in the cooler and carried to their holding troughs. Due to the lesioning of sensory mechanisms and to comply with our DNR permit, the crayfish were frozen. The streams were allowed to flush for a minimum of one hour between trials.

Data Analysis

Path Digitization

To quantify any effect that sensory lesions may have had on predator mediated behaviors, one point on the carapace of the crayfish was used to generate an x, y position during the entire 30 minute trials. A single point on the animals was used because crayfish can move backwards and sideways while keeping their sensory apparatus pointed toward a stimulus. So, we wanted to capture movement of the animals as opposed to body orientation of the animals. The movements made by each individual were analyzed using Ethovision Noldus XT (Leesburg, Virginia, USA), a motion tracking software. Using the x, y data points, the position of the crayfish was recorded every second and the x, y points were analyzed to determine movement patterns within the arena.
Previous work has shown that this level of spatial resolution provides an excellent assessment of movement (Moore & Grills, 1999; Moore et al., 2015; Wolf & Moore, 2004). In addition, crayfish tracks were compiled using these data and we extracted the following behavioral measures.

**Behavioral Measures**

From the x, y data points we could determine the time spent in each of the zones of the arena. The downstream/crayfish area of the experimental arenas were equally divided into three zones. The fish zone was defined as the area closest to the upstream/fish area of the arena. The middle one-third, which consisted of the shelter and food cap, was defined as the resource zone. The section furthest from the fish and containing no resources was defined as the empty zone. The animal was considered walking when its moving speed was greater than 0.5 cm/s and considered stationary when the walking speed was lower than 0.5 cm/s. Using these definitions, the total time spent walking and stationary in each of the zones were recorded. The walking speed in each section was then calculated for each animal. When an animal was more than 5 cm, or 1 average body length, from the wall it was considered to be in the open/away from the wall. An animal was considered to be near the wall when it was within 5 cm from the wall. Climbing behavior was defined to have occurred when all walking legs of an individual were off the substrate and on at least one wall of the arena. When an animal climbed over the shelter but did not climb up onto a wall the behavior was not considered as climbing. The near food behavior was defined to have occurred when the crayfish was within one body length from any angle of the food cap. When the crayfish was near the food source for more than 3 seconds, moving the maxillipeds and/or scraping the substratum with its chelae, the behavior was defined as foraging. Before and after each trial the cap of fish gelatin was weighed to determine the amount of food
consumed by the crayfish. Shelter behavior was defined to have occurred when more than half of the crayfish body was within the shelter. Near shelter behavior was defined as the crayfish being located within one body length of the shelter and not in the shelter. The amount of time the mentioned behaviors were displayed was recorded and further analyzed. Many of the behaviors did not display significant differences between lesions and/or flow; therefore, we only reported the behaviors which showed significant differences between the treatments.

**Extraction of Behaviors**

The x, y data points, provided by Ethovision XT Noldus, were then ran through an in-house macro, which was created using excel. This macro provided the walking speed, time walking, time stationary, and the total time in each of the three zones for all of the trials. The data was then compiled into one table to be analyzed in R Studio.

**Statistical Analysis**

In order to assess the effect of sensory lesions, flowing environments, and the interaction of sensory lesions and flowing environments on the detection of predators in crayfish, the behaviors stated above were recorded and analyzed from the trial videos. Using the lme4 and lmerTest packages in R statistical software v 3.3.1 (R Core Team, 2016; Bates et al., 2015), linear mixed models were used to determine the stated effects. Mixed models were chosen because stream arenas were considered a random effect within the model. The models were developed using sensory lesion (control, chemical active, visual active, and mechanical active) and environment (flow or no flow) as the fixed effects and the stream arenas (1, 2, 3, or 4) as a random effect. Post-hoc tests for the models were conducted using the diffslsmeans package in R studio.
RESULTS

Significance of Lesions

Time in Zones

Of the four lesion groups, mechanical active and chemical active animals showed a greater avoidance to the predatory threat. Chemical active animals spent significantly less time in the fish zone of the arenas as compared to control animals (Table 1: LSM: $t = -2.66; p < 0.05$). Mechanical active animals spent significantly less time in the fish zone than both control and visual active animals (Table 1: mechanical vs. control LSM: $t = 3.88; p < 0.05$; mechanical vs. visual LSM: $t = -2.23; p < 0.05$). As for the resource zone, mechanical active animals spent significantly more time than control (Table 1: LSM: $t = -5.49; p < 0.05$), visual active (Table 1: LSM: $t = 2.85; p < 0.05$) and chemical active (Table 1: LSM: $t = -2.94; p < 0.05$) animals. Chemical active and visual active animals spent significantly more time in the resource zone than the control animals (Table 1: chemical vs. control LSM: $t = 2.55; p < 0.05$; visual vs. control LSM: $t = -2.65; p < 0.05$). Although the lesion groups spent different amounts of time in the resource zone there were no differences in the time spent near the food or the shelter (Type II Wald Chisquare Test: $p > 0.05$). Each of the four lesion groups spent similar amounts of time in the empty zone of the arenas (Table 1: Type II Wald Chisquare Test: $p > 0.05$).

Movement in Zones

The movement of the crayfish throughout the arenas was also influenced by the differences in the lesion combinations. In the fish zone, both chemical active and mechanical active animals walked significantly faster than control and visual active animals (Table 2: chemical vs. control LSM: $t = 2.38; p < 0.05$; chemical vs. visual LSM: $t = 2.63; p < 0.05$;
mechanical vs. control LSM: \( t = -4.15; p < 0.05 \); mechanical vs. visual LSM: \( t = 4.40; p < 0.05 \).

There were significant differences between the four lesion groups in the amount of time crayfish spent stationary in the fish zone (Table 3: Type II Wald Chi-square Test: \( p < 0.05 \)). Chemical active and mechanical active animals remained stationary in the fish zone for shorter periods of time than control animals (Table 3: chemical vs. control LSM: \( t = -2.06; p < 0.05 \); mechanical vs. control LSM: \( t = 3.41; p < 0.05 \)). Visual active animals spent significantly more time stationary than mechanical active animals in the fish zone (Table 3: LSM: \( t = -2.47; p < 0.05 \)).

Significant differences were observed between the amount of time crayfish spent stationary in the resource zone of the arenas (Type II Wald Chi-square Test: \( p < 0.05 \)); however, there were no significant differences in the walking speeds between the lesion groups in the resource zone (Type II Wald Chi-square Test: \( p > 0.05 \)). Mechanical active and visual active animals remained stationary for longer periods of time as compared to control animals in the resource zone of the arenas (Table 3: mechanical vs. control LSM: \( t = -3.83; p < 0.05 \); visual vs. control LSM: \( t = -2.31; p < 0.05 \)). Mechanical active animals also spent significantly more time stopped in the resource zone than chemical active animals (Table 3: LSM: \( t = -2.38; p < 0.05 \)).

In the empty zone, mechanical active animals had significantly faster walking speeds than control, chemical active, and visual active animals (Table 2: mechanical vs. control LSM: \( t = -3.28; p < 0.05 \); mechanical vs. chemical LSM: \( t = -1.97; p < 0.05 \); mechanical vs. visual LSM: \( t = 3.64; p < 0.05 \)). However, there were no significant differences in the amount of time crayfish spent stationary in the empty zone between the lesion groups (Type II Wald Chi-square Test: \( p > 0.05 \)).
Significance of Flow

Time in Zones

There were no significant differences between the two environmental conditions in the times crayfish spent in the fish, resource, and empty zones of the arenas (Table 1: Type II Wald Chisquare Test: $p > 0.05$).

Movement in Zones

Crayfish in the fish zone of the flowing arenas had significantly faster walking speeds than crayfish in the non–flowing arenas (Table 2: LSM: $t = 2.02$: $p < 0.05$). Crayfish spent significantly less time stationary in the fish zone of the flowing arenas as compared to crayfish in the fish zone of the non–flowing arenas (Table 3: LSM: $t = -2.31$: $p < 0.05$).

There were no significant differences in the walking speed and the time crayfish remained stationary in the resource zones of both the flowing and non–flowing arenas (Table 2: Type II Wald Chisquare Test: $p > 0.05$).

Crayfish had significantly faster walking speeds in the empty zone of the flowing arenas than crayfish in the non–flowing arenas (Table 2: LSM: $t = 2.00$: $p < 0.05$); however, there were no significant differences in the time crayfish spent stationary between the different flowing environments (Type II Wald Chisquare Test: $p < 0.05$).

Significance of the Interaction Between Lesion and Flow

Time in Zones

Crayfish spent significantly different amounts of time in the resource zone of the arenas (Type II Wald Chisquare Test: $p < 0.05$); however, the combined effect of both the lesions and
the environments did not influence the time crayfish spent in the fish zone and empty zone of the arenas (Figure 4a,b: Type II Wald Chisquare Test: $p > 0.05$). Chemical active, visual active, and mechanical active animals in the flowing arenas, as well as mechanical active animals in non–flowing arenas, spent significantly more time in the resource zone than control animals in both the flowing and non–flowing arenas (Figure 4a,b: Table 1: chemical flow vs. control flow LSM: $t = 2.00$: $p < 0.05$; visual flow vs. control flow LSM: $t = -2.64$: $p < 0.05$; mechanical flow vs. control flow LSM: $t = -2.71$: $p < 0.05$; mechanical no flow vs. control flow LSM: $t = -4.12$: $p < 0.05$; chemical flow vs. control no flow LSM: $t = 2.93$: $p < 0.05$; visual flow vs. control no flow LSM: $t = 3.57$: $p < 0.05$; mechanical flow vs. control no flow LSM: $t = 3.64$: $p < 0.05$; mechanical no flow vs. control no flow LSM: $t = -5.05$: $p < 0.05$). Visual active and mechanical active animals in the flowing arenas remained in the resource zone of the arenas for significantly longer periods of time than chemical active and visual active animals in the non–flowing arenas (Figure 4a,b: Table 1: visual flow vs. chemical no flow LSM: $t = 1.96$: $p < 0.05$; visual flow vs. visual no flow LSM: $t = 2.48$: $p < 0.05$; mechanical flow vs. chemical no flow LSM: $t = 2.03$: $p < 0.05$; mechanical flow vs. visual no flow LSM: $t = 2.55$: $p < 0.05$). Mechanical active animals in the non–flowing arenas spent significantly more time in the resource zone than visual active animals in the non–flowing arenas, as well as chemical active animals from both the flowing and non–flowing arenas (Figure 4a,b: Table 1: mechanical no flow vs. visual no flow LSM: $t = 3.95$: $p < 0.05$; mechanical no flow vs. chemical flow LSM: $t = -2.12$: $p < 0.05$; mechanical no flow vs. chemical no flow LSM: $t = -3.44$: $p < 0.05$). Although there were significant differences in the times spent in the resource zone, there were no significant differences in the time crayfish spent near the shelter or food cap (Type II Wald Chisquare Test: $p > 0.05$).
Movement in Zones

Crayfish spent significantly different amounts of time stationary in the fish zone of the arenas (Figure 3a,b; Type II Wald Chisquare Test: $p < 0.05$); however, the interaction of lesions and environments did not significantly influence the walking speeds of the crayfish in each of the zones of the arenas (Figure 2a,b; Type II Wald Chisquare Test: $p > 0.05$). Visual active and mechanical active animals remained stationary for significantly shorter periods of time in the fish zone of flowing arenas than control animals in the fish zone of non–flowing arenas (Figure 3a,b: Table 3: visual flow vs. control no flow LSM: $t = -2.26$: $p < 0.05$; mechanical flow vs. control no flow LSM: $t = -2.58$: $p < 0.05$). Chemical active animals in the flowing arenas and mechanical active animals in the non–flowing arenas spent significantly less time standing still in the fish zone of the arenas than control animals in both the flowing and non–flowing arenas (Figure 3a,b: Table 3: chemical flow vs. control flow LSM: $t = -2.01$: $p < 0.05$; mechanical no flow vs. control flow LSM: $t = 2.24$: $p < 0.05$; chemical flow vs. control no flow LSM: $t = -3.81$: $p < 0.05$; mechanical no flow vs. control no flow LSM: $t = 4.04$: $p < 0.05$). Both chemical active and visual active animals spent significantly more time stationary in the fish zone of the non–flowing arenas as compared to chemical active animals in the flowing arenas and mechanical active animals in the non–flowing arenas (Figure 3a,b: Table 3: chemical no flow vs. chemical flow LSM: $t = -2.90$: $p < 0.05$; visual no flow vs. chemical flow LSM: $t = -2.94$: $p < 0.05$; chemical no flow vs. mechanical no flow LSM: $t = 3.13$: $p < 0.05$; visual no flow vs. mechanical no flow LSM: $t = -3.17$: $p < 0.05$).
DISCUSSION

The results from this study clearly demonstrate three findings supporting the importance of sensory integration for crayfish to correctly assess potential predatory threats in their environment. First, eliminating the sensory multimodality of crayfish challenged the animals to accurately assess the present predatory threat. Crayfish with the full use of only chemoreceptors (chemical active) or mechanoreceptors (mechanical active) frequently walked around the arenas and had, on average, faster walking speeds (Table 2). Conversely, visual active animals remained stationary for longer periods of time throughout the trials (Table 3). Second, mechanical active and chemical active animals showed a significantly greater avoidance of the predatory threat. When limiting the functional senses of crayfish to only chemoreceptors or only mechanoreceptors individuals maintained a farther distance between themselves and the bass (Table 1). Third, vision has a limited role in the ability of crayfish to detect and respond to predators. The crayfish that were limited to only the reception of visual cues (visual active) remained closer to the bass (Table 1).

Limiting crayfish, a multimodal organism, to only one functional sensory modality tested the individual’s ability to accurately distinguish stimuli in their environment. Crayfish with the ability to receive only chemical cues likely expended more energy walking around the arenas, as they were mobile for longer periods of time and at faster speeds (Table 2). This behavior could be indicative of a search strategy for refuge or an escape (Kats & Dill, 1998). Mechanical active animals displayed similar behaviors, with only slightly faster walking speeds, as chemical active animals. The behaviors displayed by chemical active and mechanical active animals suggest that the individuals detected the threat but could not accurately locate the source. Visual active animals displayed behaviors, such as sitting next to the threat (Table 3), which would indicate
the individuals might not be able to identify the severity of the threat. Those individuals who lack the ability to assess predatory threats are less likely to avoid predation. The differences in behavior for each of the lesion treatments (chemical active, mechanical active, and visual active) in this study indicate a hierarchy of dependence in the modalities, with the most dependence on chemoreceptors, secondarily on mechanoreceptors, and finally on vision.

Our findings show a noticeable sensory preference toward chemoreceptors in detecting predatory threats. The reception of chemical cues allows aquatic organisms to communicate, interact, and orient within their environment (Weiss et al., 2012). Crayfish not only rely upon chemoreception to detect predatory threats, but also in daily activities, such as foraging (Moore & Grills, 1999), identification of social status/conspecifics (Callaghan et al., 2012; Crook et al., 2004), and mating (Acquistapace et al., 2002). Callaghan et al. (2012) found that the reception of chemical cues plays a significant role in the formation of hierarchies in *Orconectes rusticus*. Crayfish also rely upon chemoreception to detect food sources; however, Kraus-Epley et al. (2015) found that crayfish rely on mechanoreception, in the absence of chemoreception, to detect food odors.

The findings from this study suggest that crayfish do not depend highly on visual cues to detect threats. However, previous studies have suggested that crayfish respond to visual cues when combined with other cues, such as chemical cues (Acquistapace et al., 2002; Crook et al., 2004). Crayfish can be found in habitats ranging from clear rivers to murky and lentic ponds. Clear, steady rivers offer the optimal reception of visual cues. However, murky ponds, or other turbid environments, restrict the accuracy of visual cues while not hindering the reception of chemical and mechanical cues (Lunt & Smee, 2015; Weiss et al., 2012). Crayfish are also nocturnal animals; thus, they must be able to navigate through landscapes which have limited
light availability. In the absence of light, the reception of visual cues is hindered (Delgado-Morales et al., 2004; Elvidge & Brown, 2012; Weiss et al., 2012), supporting limited reliance on vision in crayfish. Although there is a clear hierarchy in the reliance on modalities in crayfish, each modality has a role in the reception of signals in crayfish.

Each sensory mechanism provides crayfish with beneficial information on stimuli, such as nearby threats, within their environment. Chemoreceptors detect chemical cues, such as those produced by predators, in which prey can assess potential threats by determining the presence, diet, size, and proximity of predators (Smith & Belk, 2001; Turner & Peacor, 2012; Weissburg et al., 2014). The reception of visual cues of predators provide prey with information on the size and approach rate of the threat (Smith & Belk, 2001; Weissburg et al., 2014). Prey species can determine the movement of predators through the environment by relying on mechanoreceptors to detect the disturbances created in the water, air and substrates (Warkentin, 2005; Weiss et al., 2012; Weissburg et al., 2014). However, each modality has its own set of limitations, such as distance (mechanoreceptors), light variations (vision), and environmental mixing (chemoreceptors). Therefore, to gather the most accurate information on incoming threats integration of these modalities is important.

In predator – prey interactions, sensory cues are used to determine the degree of threat from predation and, as a result of the measurement of this threat, prey can alter their behavior in different ways. For example, crayfish can determine not just the diet of a bass (chemical cues), but also the swimming speed of the bass (visual cues) (Weissburg et al., 2014). Crabs (Heterozius rotundifrons) can determine a greater predatory threat through the reception of multiple signals, such as tactile disturbances and chemical odors, and adjust their behavior accordingly (Hazlett & McLAY, 2000). Sensory modalities most often provide unique
information, however, some modalities can provide redundant information (Partan & Marler, 2005; Smith & Dunham, 1990). For example, crayfish can detect the chemical cues released from an injured conspecific and the visual cues of a bass consuming that conspecific, from this information the crayfish knows the risk of predation is high. This redundancy in modalities provides insurance for the receiver of signals, especially when considering environmental noise. Environmental noise is omnipresent; however, what might be noise for one mechanism might not be noise for another mechanism (Rubi & Stephens, 2016; Troïanowksi et al., 2014).

Previous studies have shown that multiple species exhibit a ‘multimodal shift’, in that individuals switch between modalities when one is limited in order to gather the most beneficial information (Kraus-Epley et al., 2015; Troïanowksi et al., 2014). Differences in environmental conditions can cause such shifts. For example, a shift in modality has been observed in adult three – spined stickleback fishes, who most often rely on visual cues in clear waters but shift to chemical cues in turbid environments (Troïanowksi et al, 2014). Rabin et al. (2006) observed an increase on the reliance of visual cues, rather than auditory cues, in California ground squirrels (Spermophilus beecheyi) in areas with higher auditory noise. Although our results did not indicate shifts in modalities due to differences in flow environments, we did see a shift to a reliance on mechanoreceptors when chemoreceptors were absent in crayfish. Several studies recently have indicated the impact of additional environmental noise, pollutants (Callaghan et al., 2012; Lahman et al., 2015; McIntyre et al., 2008). McIntyre et al. (2008) found that environmentally relevant concentrations of copper impaired the olfaction of juvenile coho salmon (Oncorhynchus kisutch). Therefore, shifts in modalities might not just be occurring due to natural environmental noise but, human-created noise as well. Such shifts between modalities support the benefit of multimodal communication.
The sensory landscape, within an organism’s habitat, contains information on predators, prey, and conspecifics (Jurcak & Moore, 2014). Crayfish are exposed to several predatory species including ambush and active hunters as well as benthic and more pelagic predators. These unique predators, coupled with their hunting modes, create a diverse sensory landscape of fear (Laundré et al., 2010). On top of these predator signals, con- and heterospecifics create sensory signals and cues that are important for the social and competitive behaviors of different organisms. For example, crayfish can determine the sex, size, and social status of nearby conspecifics through the reception of cues and signals (Acquistapace et al., 2002; Weissburg et al., 2014). The ability to recognize individuals, such as previous opponents, from neighboring territories allows individuals to know whether to fight or flee (Crook et al., 2004). Injured conspecifics release alarm/chemical cues which alert nearby individuals of the presence of a predatory threat (Elvidge & Brown, 2012; Sih et al., 2010; Weiss et al., 2012). Prey species, or food sources in general, create sensory cues and signals as well, alerting predators of a potential meal. Individuals must then be able to determine the degree of threat from predators, the hierarchical rank of nearby conspecifics, and the resource value of the potential prey to determine whether the meal is worth the risk.

Thus, the reliance upon multiple sensory modalities allows individuals to accurately extract information from this complex sensory landscape. The information gathered from multiple modalities on predators allows prey species to alter their behavior to reduce their chances of predation. The behavioral (or physiological and morphological) responses to the information extracted from these landscapes form the NCEs that play an important role in the ecology of a habitat. Once an increased predatory threat (detected through multiple modalities) is detected most species show a reduction in foraging and mating behaviors and resort to predator
avoidance behaviors, such as seeking shelter, fleeing, and/or displaying a defensive stance (Smith & Belk, 2001). Such antipredator responses result in energetic costs which could lead to negative changes in the growth and survival rates of prey populations (Preisser et al., 2007). NCEs potentially have a greater impact on organisms which lack the ability, due to the loss of sensory modalities/integration or environmental conditions, to accurately assess predatory threats, for these individuals most often overcompensate their antipredator reactions. For this reason, organisms who can detect such predatory signals have greater chances of survival, due to accurate responses to NCEs.

In order to fully comprehend the spatially and temporally dynamic nature of NCEs on prey populations, an understanding of the sensory biology of organisms is needed (Sih et al., 2010; Weiss et al., 2012; Weissburg et al., 2014). Different habitats will have unique sensory landscapes of predatory cues. Furthermore, the interaction of the cues, their transmission through the environment, and prey’s sensitivity to those cues create the landscape of fear. As demonstrated here, changing the sensitivity (such as lesioning) or the environmental transmission of stimuli (altering background flow) alters the animal’s detection and subsequent response to the presence of a predator. Thus, NCEs and landscapes of fear need to incorporate the idea of different layers of landscape which depend upon several factors including the sensory capabilities of the prey. For example, Sherman and Moore (2001) found that *Ameiurus nebulosus* orient to odor sources more successfully in non–flowing streams as compared to flowing streams. Thus, the incorporation of sensory ecology and environmental influences on cue transmission can potentially lead to a better understanding of the impacts of NCEs.
REFERENCES


APPENDIX A: FIGURES

1A) 1B)

Figure 1. Dimensions of small goggles

Small goggles were created from the molds consisting of 5 mm indentations (Fig. 1A). Figure 1A shows the correct placement of the indentation. Small goggles measured 1.8 cm in length and 1.3 cm wide (Fig. 1B).

2A) 2B)

Figure 2. Dimensions of large goggles

Large goggles were created from the molds consisting of 7 mm indentations (Fig. 2A). Figure 2A shows the correct placement of the indentations. Large goggles measured 2.5 cm in length and 1.8 cm wide.
Figure 3. Dimensions and setup of arenas

All four arenas (both flowing and non-flowing) were a total of 121 cm long and 81 cm wide. As shown in this schematic (not to scale), during acclimation flowing arenas had secondary input and outputs, as well as a non-porous divider. These secondary outflows and dividers allowed both the bass and the crayfish to acclimate to the arena without any mixing of sensory stimuli. The divider was removed after acclimation while the egg-crating remained in place, allowing the crayfish to sense the bass without physical interaction. The non-flowing arenas were constructed the same, however, there were no input or output of water during the acclimation times and trials.
Figure 4. Total time crayfish remained in each of the zones of the arenas

A) Mean (± SEM) time (sec) for the interaction effect crayfish spent in each of the zones of the flowing arenas. B) Mean (± SEM) time (sec) for the interaction effect crayfish spent in each of the zones of the non-flowing arenas.
Figure 5. Walking speed of the crayfish in each of the zones of the arenas

A) Mean (± SEM) walking speed (cm/sec) for the interaction effect of crayfish in each zone of the flowing arenas. B) Mean (± SEM) walking speed (cm/sec) for the interaction effect of crayfish in each of the zones of the non–flowing arenas.
Figure 6. Total time crayfish remained stationary in each of the zones of the arenas

A) Mean (± SEM) time (sec) crayfish spent stationary for the interaction effect in each of the zones of the flowing arenas. B) Mean (± SEM) time (sec) crayfish spent stationary for the interaction effect in each of the zones of the non-flowing arenas.
## APPENDIX B: TABLES

<table>
<thead>
<tr>
<th>Lesion Effect</th>
<th>Control</th>
<th>Fish Zone</th>
<th>Resource Zone</th>
<th>Empty Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Active</td>
<td>818 ± 113</td>
<td>228 ± 63.8</td>
<td>467 ± 80.4</td>
<td></td>
</tr>
<tr>
<td>Visual Active</td>
<td>956 ± 106</td>
<td>530 ± 111</td>
<td>312 ± 52.9</td>
<td></td>
</tr>
<tr>
<td>Mechanical Active</td>
<td>652 ± 88.8</td>
<td>849 ± 98.6</td>
<td>297 ± 51.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flow Effect</th>
<th>Flowing Arenas</th>
<th>Fish Zone</th>
<th>Resource Zone</th>
<th>Empty Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non – Flowing Arenas</td>
<td>864 ± 72.6</td>
<td>595 ± 70.0</td>
<td>338 ± 43.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interaction Effect</th>
<th>Flowing Control</th>
<th>Fish Zone</th>
<th>Resource Zone</th>
<th>Empty Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowing Chemical Active</td>
<td>1129 ± 120</td>
<td>302 ± 118</td>
<td>365 ± 93.2</td>
<td></td>
</tr>
<tr>
<td>Flowing Visual Active</td>
<td>706 ± 124</td>
<td>616 ± 94.3</td>
<td>476 ± 86.5</td>
<td></td>
</tr>
<tr>
<td>Flowing Mechanical Active</td>
<td>801 ± 183</td>
<td>728 ± 184</td>
<td>269 ± 77.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interaction Effect</th>
<th>Flowing Mechanical Active</th>
<th>Fish Zone</th>
<th>Resource Zone</th>
<th>Empty Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non – Flowing Control</td>
<td>1233 ± 175</td>
<td>410 ± 158</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non – Flowing Chemical Active</td>
<td>931 ± 190</td>
<td>458 ± 140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non – Flowing Visual Active</td>
<td>1111 ± 93.4</td>
<td>355 ± 73.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non – Flowing Mechanical Active</td>
<td>484 ± 102</td>
<td>350 ± 66.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Average time crayfish remained in each of the zones of the arenas

Mean (± SEM) time (sec) crayfish spent in the fish, resource, and empty zones of both the flowing and non – flowing arenas. The data is grouped by each of the statistical effects. To determine significant differences for each of the statistical effects Type II Wald Chisquare Tests were conducted in ‘R’. Significant Type II Wald Chisquare Tests were followed by Least Square Means tests in ‘R’.
<table>
<thead>
<tr>
<th>Lesion Effect</th>
<th>Control</th>
<th>Resource Zone</th>
<th>Empty Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Active</td>
<td>1.10 ± 0.17</td>
<td>1.16 ± 0.23</td>
<td>1.33 ± 0.25</td>
</tr>
<tr>
<td>Visual Active</td>
<td>0.56 ± 0.08</td>
<td>0.90 ± 0.19</td>
<td>0.82 ± 0.13</td>
</tr>
<tr>
<td>Mechanical Active</td>
<td>1.47 ± 0.24</td>
<td>1.17 ± 0.27</td>
<td>1.92 ± 0.32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flow Effect</th>
<th>Flowing Arenas</th>
<th>Non – Flowing Arenas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowing Control</td>
<td>0.66 ± 0.03</td>
<td>1.18 ± 0.18</td>
</tr>
<tr>
<td>Flowing Chemical Active</td>
<td>1.47 ± 0.23</td>
<td>1.39 ± 0.36</td>
</tr>
<tr>
<td>Flowing Visual Active</td>
<td>0.57 ± 0.10</td>
<td>0.66 ± 0.20</td>
</tr>
<tr>
<td>Flowing Mechanical Active</td>
<td>1.63 ± 0.41</td>
<td>1.39 ± 0.43</td>
</tr>
<tr>
<td>Non – Flowing Control</td>
<td>0.56 ± 0.12</td>
<td>1.04 ± 0.18</td>
</tr>
<tr>
<td>Non – Flowing Chemical Active</td>
<td>0.74 ± 0.20</td>
<td>0.93 ± 0.28</td>
</tr>
<tr>
<td>Non – Flowing Visual Active</td>
<td>0.55 ± 0.12</td>
<td>1.14 ± 0.31</td>
</tr>
<tr>
<td>Non – Flowing Mechanical Active</td>
<td>1.30 ± 0.27</td>
<td>0.95 ± 0.31</td>
</tr>
</tbody>
</table>

Table 2. Average walking speed of the crayfish for each of the zones of the arenas

Mean (± SEM) walking speed (cm / sec) of crayfish in the fish, resource, and empty zones of both the flowing and non–flowing arenas. The data is grouped by each of the statistical effects.

To determine significant differences for each of the statistical effects Type II Wald Chi-square Tests were conducted in ‘R’. Significant Type II Wald Chi-square Tests were followed by Least Square Means tests in ‘R’.
<table>
<thead>
<tr>
<th>Lesion Effect</th>
<th>Control</th>
<th>Resource Zone</th>
<th>Empty Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Active</td>
<td>418 ± 118</td>
<td>289 ± 70.8</td>
<td>192 ± 57.5</td>
</tr>
<tr>
<td>Visual Active</td>
<td>557 ± 82.6</td>
<td>394 ± 105</td>
<td>153 ± 34.5</td>
</tr>
<tr>
<td>Mechanical Active</td>
<td>252 ± 82.2</td>
<td>563 ± 105</td>
<td>74.8 ± 24.5</td>
</tr>
<tr>
<td>Flow Effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowing Arenas</td>
<td>374 ± 56.2</td>
<td>374 ± 66.7</td>
<td>130 ± 28.5</td>
</tr>
<tr>
<td>Non – Flowing Arenas</td>
<td>576 ± 83.4</td>
<td>314 ± 64.9</td>
<td>194 ± 46.1</td>
</tr>
<tr>
<td>Interaction Effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowing Control</td>
<td>516 ± 79.7</td>
<td>173 ± 112</td>
<td>163 ± 63.6</td>
</tr>
<tr>
<td>Flowing Chemical Active</td>
<td>165 ± 35.5</td>
<td>335 ± 108</td>
<td>188 ± 76.3</td>
</tr>
<tr>
<td>Flowing Visual Active</td>
<td>436 ± 124</td>
<td>562 ± 178</td>
<td>120 ± 41.7</td>
</tr>
<tr>
<td>Flowing Mechanical Active</td>
<td>380 ± 155</td>
<td>427 ± 111</td>
<td>50.9 ± 36.4</td>
</tr>
<tr>
<td>Non – Flowing Control</td>
<td>830 ± 186</td>
<td>87.5 ± 30.8</td>
<td>296 ± 149</td>
</tr>
<tr>
<td>Non – Flowing Chemical Active</td>
<td>671 ± 208</td>
<td>243 ± 94.7</td>
<td>197 ± 90.3</td>
</tr>
<tr>
<td>Non – Flowing Visual Active</td>
<td>679 ± 100</td>
<td>227 ± 93.8</td>
<td>186 ± 55.2</td>
</tr>
<tr>
<td>Non – Flowing Mechanical Active</td>
<td>125 ± 27.0</td>
<td>699 ± 175</td>
<td>98.7 ± 32.9</td>
</tr>
</tbody>
</table>

Table 3. Average time crayfish remained stationary in each of the zones of the arenas

Mean (± SEM) time (sec) crayfish spent stationary in the fish, resource, and empty zones of both the flowing and non – flowing arenas. The data is grouped by each of the statistical effects. To determine significant differences for each of the statistical effects Type II Wald Chi-square Tests were conducted in ‘R’. Significant Type II Wald Chi-square Tests were followed by Least Square Means tests in ‘R’.
DATE: April 5, 2016

TO: Paul Moore, Ph.D.
FROM: Bowling Green State University Institutional Animal Care and Use Committee

PROJECT TITLE: [856543-2] Predator-prey interactions between crayfish and fish: the role of sensory capabilities in defining Non-consumptive effects.

IACUC REFERENCE #: 
SUBMISSION TYPE: Revision

ACTION: APPROVED
APPROVAL DATE: April 3, 2016
EXPIRATION DATE: April 2, 2019
REVIEW TYPE: Designated Member Review

Thank you for your submission of Revision materials for the above referenced research project. The Bowling Green State University Institutional Animal Care and Use Committee has APPROVED your submission. All research must be conducted in accordance with this approved submission. Please make sure that all members of your research team read the approved version of the protocol.

Report all NON-COMPLIANCE issues regarding this project to this committee.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the Addendum Request form for this procedure.

This project requires Continuing Review/Progress Report by this office on an annual basis. Please use the Annual Renewal form for this procedure.

If you have any questions, please contact the Office of Research Compliance at 419-372-7716 or hsrb@bgsu.edu. Please include your project title and reference number in all correspondence with this committee.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within Bowling Green State University Institutional Animal Care and Use Committee's records.