DEFINING THE REACTION SPACE OF PREDATOR-PREY INTERACTIONS

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A Dissertation

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ABSTRACT

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This dissertation contributed to the call for a greater comprehension of sensory ecology within predator-prey interactions, particularly in the non-consumptive effects (NCEs) of predators. I investigated how stimulus modality, predator movement, environmental transmission, prey sensory ecology, pollution, and the interaction of these factors modify prey behavioral responses to predators. Specifically, I experimentally tested three research questions: 1) how the reaction space of predators with different hunting modes in different flow environments altered prey behavior, 2) how modulating signal intensity and prey detection thresholds altered the reaction space, and 3) how the exposure to anthropogenic chemicals altered the reaction space of prey.

First, I placed prey (crayfish) in two different environments (flow and no flow) in one of three predator treatments (active predator [bass], sit-and-wait predator [catfish], no predator) and monitored the behavior of the crayfish in a resource patchy environment. Predator hunting mode changed prey behavior, but only in flowing water that would enhance the transmission of predator cues. The most significant interaction between predator treatment and flow environment was found with the active predator in flowing habitats, but this same interaction did not alter NCEs from a sit-and-wait predator. Second, I exposed virile and rusty crayfish to low, medium, or high concentration of odor from largemouth bass and to controls without bass odor and monitored crayfish. The results showed that the behavior of virile crayfish was significantly altered across concentrations more than rusty crayfish, indicating that the virile crayfish may have larger reaction space. Finally, I exposed virile and rusty crayfish to a pesticide (carbaryl)
then placed the crayfish in a two-choice flume containing predator odor and clean river water to monitor their behavior. I found that the exposure to a carbaryl did not affect the anti-predator behavior of either species.

The findings show that each factor of the reaction space is important in understanding and altering NCEs of predators. Additionally, NCEs may be hidden unless the interaction of factors is taken into consideration. Investigating the sensory environment of predator-prey interactions is crucial for better understanding the mechanisms driving the NCEs of predators and their consequences.
This dissertation is dedicated to my family, John, Maria, and Natalia Jurcak, and Douglas Detter.

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CHAPTER I. GENERAL INTRODUCTION

Predator-prey research has focused on the capture, handling, and consumption of prey, known as consumptive effects (CE) (Lima 1998, Schmitz et al. 2004, Peckarsky et al. 2008). Consumption of prey by predators can have significant effects on prey population dynamics and affect entire ecosystem (Lima 1998). In marine ecosystems, the predation of sea otters on sea urchins limited the overgrazing of kelp (Estes and Duggins 1995). In Yellowstone National Park, the reintroduction of wolves led to increased predation on elk, in turn, limiting the grazing of elk on aspen (Philip and Smith 1997, Ripple et al. 2001). Since the introduction of wolves, aspen growth was found to be significantly higher in areas with high wolf presence (Ripple et al. 2001). However, the non-consumptive effects of predators (NCEs) arguably have a larger impact on prey and the structuring of populations and communities than CEs (Brown et al. 1999, Turner and Peacor 2012).

NCEs can elicit changes in prey behavior, morphology, and physiology due to the presence of predators in the environment (Brönmark and Miner 1992, Preisser et al. 2007, Peckarsky et al. 2008, Brönmark and Hansson 2012). The length of protective spines on common rotifers depend on the size of the predator (Zhang et al. 2017). Male black field crickets experience greater metabolic rates in the presence of cues from predatory water skinks (Lagos and Herberstein 2017). Narwhal movement patterns became more transiting and spent more time closer to shore when arctic killer whales were present (Breed et al. 2017). Research on NCEs have led to the creation of the “landscape of fear” concept in ecology (Laundré et al. 2001, Altendorf et al. 2001).
The landscape of fear theory states that the sensory environment in the presence of predators is composed of aversive signals which alter prey behavior (Laundrè et al. 2001, Hernández and Laundrè 2005, Tolon et al. 2009). In particular, “fear” of predation or an increase in the calculated probability of a risk drives the spatial distribution, behavioral changes, and physiological alterations exhibited by prey (Laundrè et al. 2001, Ripple and Beschta 2004, Matassa and Trussell 2011). Since the introduction of this theory, a number of studies have found empirical support for this theory (Laundré et al. 2001, Ripple and Beschta 2004, Preisser et al. 2005, Matassa and Trussell 2011, Kuijper et al. 2013). However, most of studies have focused on the response of prey from the presence of predators. These same studies have provided less insight as to how the stimuli and movement of predators can influence NCEs. For there to be NCEs, prey need to detect the threat of predation which elicit these changes.

The recognition of a more inclusive perspective regarding the role of sensory processes has led to a call for a broader application of sensory ecology to the analysis of NCEs (Sih et al. 2010, Weissburg et al. 2014). To create a more complete picture of NCEs, future research needs to consider the entire process from stimulus production by the predator, activity and movement of predators (such as hunting mode; Weissburg et al. 2014), transmission and movement of stimuli through an environment (Weissburg et al. 2014), and the sensory ecology of the prey (sensitivity, modality, thresholds). When the sensory environment from predators is intense enough to elicit responses in the prey, this environment can be termed the reaction space. I formally define reaction space as a spatially and temporally dynamic area where stimuli from predators alter the behavior, physiology, and/or morphology of prey. There are four factors which comprise the construction of the reaction space: stimulus modality, predator movement, environmental transmission, and prey sensory ecology.
Prey detect predatory threats by perceiving cues emanating from the predator. These cues come from different types of stimuli, including, visual, mechanical, and chemical stimuli. Fiddler crabs use the angular position and angular size of an organism relative to the horizon to distinguish predators, such as sea birds (Layne et al. 1997). Caterpillars used substrate-borne vibrations to detect the presence of predatory threat as well as distinguish the type of predator (Castellanos and Barbosa 2006). Spider mites exhibited a strong decrease in activity when presented with the chemical cues from killed conspecifics (Gyuris et al. 2017). Stimuli from predators include information such as predator location, predator identity, and predator diet. The distribution of predatory stimuli can be dictated by the movement and hunting mode of predators.

There are multiple predator hunting modes which differ in how the predators moves through the environment searching for prey. Active predators continuously move throughout the environment to search and follow prey (Preisser et al. 2007). Sit-and-wait predators remain stationary in a location for long periods of time before capturing prey while sit-and-pursue predators wait until prey comes into their pursuit distance until prey comes within capturing distance (Preisser et al. 2007). As more active predators move through habitats, the origin of predatory cues also move. Highly mobile predators can create a broader distribution of predatory stimulus from which prey obtain information on potential threats. The exact structure of the prey reaction is a result of the interaction of the movement of the stimulus source (predator) and the transmission mechanics of the environment.

Changes in the mechanics of environmental transmission of predatory signals also alters the influence of NCEs. In aquatic environments with intermediate flow velocities, dogwhelks responded to green crab cues more often than in low or high flow velocities (Large et al. 2011).
As turbulence increases, the ability of bivalves to detect predators, such as blue crabs and whelks, decreases (Ferner et al. 2009, Smee et al. 2008, 2010). Concurrently with this decrease in predator detection comes an increase in the hunting success of whelks (Ferner and Weissburg 2005, Ferner et al. 2009). These studies show that the transmission and overlap of stimuli by environmental factors (chemical signals in slow or fast flow) create the landscape from which prey extract information about the potential threats from predators (Moore and Crimaldi 2004). Finally, the sensory ecology (sensitivity, modality, and thresholds) of prey can affect the NCEs of predators. Not all landscapes are perceived equally by specific prey organisms. Tadpoles significantly decreased their activity when presented with cues from dragonflies that recently consumed prey compared to the presence of the predators alone (van Buskirk and Arioli 2002). Larval central newts were unable to distinguish predatory tiger salamanders from non-predatory grey tree frogs by visual stimuli and reduced their activity after receiving visual stimuli from both species. However, larval newts were able to distinguish between predator and non-predator when presented with chemical stimuli (Mathis and Vincent 2000). The prey response to predatory stimuli depends on the threshold of differences within stimuli (i.e. chemical) or between stimuli (i.e. visual vs. chemical).

Once each of these four factors (stimulus modality, predator movement, environmental transmission, and prey sensory ecology) are taken into consideration, the concept of the reaction space naturally arises. Predicting the intensity and extent of NCEs is dependent on understanding the size and construction of the reaction space of the prey. Temporally, prey with a large reaction space will respond earlier to a predator than prey with a small reaction space. Thus, prey may be prone to more NCEs from a predator. Conversely, prey with a small reaction space may be less prone to NCEs. However, a small reaction spaces requires the prey to detect predatory stimuli at
closer proximities than prey with a larger reaction space, which could lead to greater chances of predator consumptive effects. An environment where the predatory stimuli can be dispersed over a large area, will increase the potential for predator detection leading to more NCEs than an environment where the predatory stimuli is concentrated to a small area. Variations in the size of reaction spaces within and between species and environments will influence the magnitude and frequency of NCEs.

Pollution can have large effects on nontarget organisms, directly causing mortality but also having sublethal effects on their physiology and behavior (ref). Specifically, pollution may modify NCEs by interfering with prey ability to detect and react to predation risk (Lurling and Scheffer 2007). For example, chinook salmon exposed to diazinon showed reduced anti-predator responses when presented with chemical alarm signals (Scholz et al. 2000). The alteration a reduction in the size of the reaction space, potentially leading to a greater risk in predation. A shift in the dynamics of predator-prey interactions in favor of predators could alter ecosystem trophic interactions.

The focus of my dissertation research was to understand the factors that create and alter reaction space. I investigated how stimulus modality, predator movement, environmental transmission, prey sensory ecology, and the interaction of these factors modify prey behavioral responses to predators. Exploring these features allowed for the comprehension of how each part influences the creation of the reaction space and how this alters NCEs. I aimed to answer the call for knowledge proposed by Sih et al. 2010 and Weissburg et al. 2014 for a greater comprehension of the sensory ecology of predator-prey interactions. I also aimed to answer the request of Bronmark and Hannson 2012 to understand how anthropogenic chemicals can alter the chemical ecology of aquatic systems, particularly in predator-prey interactions. The three
chapters that follow will define and elucidate the reaction space by determining 1) how the reaction space of predators of different hunting modes in different flow environments alter prey behavior, 2) how modulating signal intensity and prey detection thresholds alter the reaction space. 3) how the exposure to anthropogenic chemicals alters the reaction space of prey.
CHAPTER II. SENSORY SIGNALS AND THE REACTION SPACE IN PREDATOR-PREY INTERACTIONS

Introduction

Animals extract information from the environmental to make important decisions regarding foraging, predator avoidance, and mating (Dill 1987; Keller and Moore 1999). This information can be considered within the sensory landscape of the habitat, which is the spatial and temporal distribution of various stimuli of an organism’s environment (Wilson and Weissburg 2013; Jurcak and Moore, 2014). Given the spatial and temporal complexity of the sensory landscapes, organisms experience multiple stimuli simultaneously and need to extract the relevant information, which can be considered stimulus patterns that may signal the degree of predatory threats, the location of food patches, or the potential receptivity of mates (Hazlett 1999; Bytheway et al. 2013). The environmental stimuli used in these decisions include both attractive stimuli for the focal organism (i.e. mating signals; Resink et al. 1989; Rodríguez et al. 2012; Wilgers and Hebets, 2012) and aversive stimuli (i.e. predatory signals, alarm signals; Chivers et al. 2001; Mirza and Chivers 2001; Gall and Brodie Jr. 2009). The assessment of the threat of a predation risk compared to the reward of close resources has been shown to be based on the distribution, intensity, and types of sensory stimuli within the dynamic sensory landscapes within natural habitats (Dill 1987; Corcoran et al. 2013; Jurcak and Moore 2014). In addition to attractive and aversive, environmental stimuli are composed of cues, where selection is solely on the receiver to detect the stimuli, and signals, where selection acts upon both the sender and the receiver to detect the stimuli (Smith and Harper 2003). Thus, sensory landscapes contain both signals (e.g., mating and alarm signals) and cues (e.g., food patches and predatory cues).
Prey detect predatory threats by perceiving cues emanating from the predator and by extracting critical information such as predator location and predator identity (Weissburg et al. 2014). Given the aversive nature of predatory cues, prey often respond to perceived threats by altering their behavior (changing foraging decisions), physiology (increasing production of stress hormones), or their morphology (altering body structure for defense) (Boonstra et al. 1998; Relyea 2000; Matassa and Trussel, 2011). Changes in prey traits, such as foraging behavior, can have large effects on multiple trophic levels (Turner 1997; Turner and Montgomery 2003; Sih et al. 2010; Weissburg et al. 2014). Predator foraging, or Consumptive effects CEs include predators catching, consuming, and removing prey from the environment, whereas non-consumptive effects NCEs include all of the influences predators have on prey traits by their presence and movement through an environment (Lima 1998; Schmitz et al. 2004). NCEs may exert a stronger effect on ecosystem function than consumptive effects because predators have the potential to simultaneously alter the traits of many prey (Preisser et al. 2005; Preisser et al. 2007; Turner and Peacor 2012). In many NCEs studies, prey respond behaviorally predatory cues to reduce their risk of being detected. Changes in prey anti-predator behavior are variable within the environment, creating a landscape of fear (Laundré et al. 2001; Ripple and Beschta 2004; Preisser et al. 2005; Matassa and Trussell 2011; Kuijper et al. 2013). The landscape of fear predicts that the presence of predators creates a sensory landscape dominated by predatory cues that alters prey movement, restricts or relocates foraging, and increases vigilance during foraging (Preisser et al. 2005; Preisser et al. 2007; Matassa and Trussell 2011). While these studies demonstrated significant alterations to prey behavior based on predator presence and the generation of predatory cues, there is little to no information as to the sensory ecology underlying NCEs. That is, the role of neurobiology, physiology, and anatomy of the senses that
is used to extract ecologically relevant information for organisms. For example, previous studies have not quantified intensity of predatory cues and the sensory thresholds of prey in order to establish the size of these landscapes of fear. In addition, this missing role of sensory ecology of the prey has led to a call for a broader application of sensory ecology to the analysis of NCEs (Sih et al. 2010; Weissburg et al. 2014).

Previous sensory ecology research has shown that prey can accurately determine risk levels based on the intensity and types of sensory cues being released by predators (Turner 1997; Turner and Montgomery 2003). Based on these findings, Turner and Montgomery (2003) suggested that the changes in traits of prey (NCEs) are dependent of the sphere of influence of the predator. The size and structure on the sphere of influence are driven by the predator density, predator movement, the medium through which the predator stimuli are transmitted, and prey sensory modality (Turner and Montgomery 2003). Here, the medium and habitat can determine the spatial and temporal distribution of stimuli as the absorption and refraction of light is different in air vs water (Denny 1993). In addition, the sensory modality (audition, vision, and olfaction) has specific response thresholds that determine the potential detection distance to the predator. Further, the type of sensory stimulus (e.g., sound, chemical, light), stimulus production from the predator (e.g., movement, metabolic processes), and the environmental conditions within a medium (e.g., clear vs turbid water) also play a role in determining the intensity, size, and location of the sphere of influence or landscape of fear.

In aquatic environments with intermediate flow velocities, dogwhelks responded to green crab cues more often than in low or high flow velocities indicating the important role of environmental transmission (Large et al. 2011). Anti-predator response of tadpoles depend are strongly influenced by predator type and diet (Laurila et al. 1997). Tadpoles had a stronger anti-
 predator response when exposed to a predator that had consumed tadpoles than predators that had consumed other invertebrates (Laurila et al. 1997). Despite this advance in understanding, an important missing element of the sphere of influence is the sensory biology or ecology of prey (threshold of detection of different sensory modalities, paired or spatially distributed sensory receptor organs, and the neurobiology underlying the extraction of information from signals and cues). The sensory modality used by prey and sensitivity of that specific modality to predatory cues are what drive the behavioral, morphological, and physiological changes that are at the core of NCEs. Thus, the individual elements of the sphere of influence, along with the extension of stimulus type, environmental transmission, and sensory ecology of prey, function together to create the influence that predators have on prey. We have termed this extension of the sphere of influence as the reaction space. We formally define reaction space as a spatially and temporally dynamic area where stimuli from predators are intense enough for prey to detect and elicit an aversive response by altering the behavior, physiology, or morphology of the prey.

There are four main elements that comprise the reaction space: stimulus modality (vision, electoreception, etc.), predator movement that creates the spatial distribution of cues (active hunters, sit-and-wait ambush predators), environmental transmission (refraction, reflection, attenuation), and prey sensory ecology (sensory thresholds and neural networks that extract information). The first element is stimulus modality. For example, fiddler crabs use visual cues to detect predators, such as birds, and respond by retreating to their burrow entrances (Hemmi 2005). Light cues can travel faster and are attenuated less strongly in open field terrestrial environments than sound or chemical signals, thus visual detection distances tend to be greater. The second element, movement of predators, determines the spatial dimensions involved in the distribution of aversive signals. The continuous movement of active predators will create a larger
spatial extent of signals, but these signals may be more ephemeral in nature. Conversely, sit-and-wait predators may produce a more consistent, but locally constrained stimulus environment (Preisser et al. 2007). The third element of the reaction space is the environmental transmission of the predatory stimuli. The distribution of chemical stimuli in both air and water is primarily determined by turbulent wind or water movement which creates a fluctuating cue in space and time (Moore et al. 1994; Moore and Crimaldi 2004; Weissburg 2000). As turbulence increases in aquatic systems, the ability of bivalves to detect predators, such as blue crabs andwhelks, decreases (Ferner et al. 2009; Smee et al. 2008; 2010). Finally, the last element of the reaction space is the sensory ecology (sensitivity, modality, thresholds) of the prey. Not all landscapes are perceived equally by prey organisms. Rusty crayfish, *Orconectes rusticus*, reduced their response to food odors when alarm odors from conspecifics and two heterospecific species were present (*Orconectes virilis* and *Orconenctes propinquus*). However, *Orconectes propinquus* responded to alarm odors of *Orconectes rusticus* as well as conspecifics with significantly less of a response to *Orconectes virilis* (Hazlett 2000). All of these elements individually, as well as the interaction of these elements with each other comprise the reaction space that dictates predator-prey non-consumptive effects.

The purpose of this study was to examine how the interaction between stimulus transmission and predator movement during hunting alters the reaction space. We generated various conditions likely to alter the reaction space of a prey by pairing two different stimulus environments (flow or no flow) with three predator treatments (active, sit-and-wait, no predator). Under these conditions, we monitored prey behavioral decisions in the presence of differing resources (food and shelter). For our experiment, the prey were *Orconectes virilis* (crayfish) which are found in both flow and no flow environments (Hazlett et al. 2006; Moore et al. 2015).
Crayfish are multimodal, relying on visual, mechanical, and chemical cues for detecting potential predators (Callaghan et al. 2012). Crayfish heavily rely on their chemical senses for gaining information used for making behavioral decisions (Hazlett 1999; Keller and Moore 1999). Thus, the difference in the transport of chemical information of predators in the two environments may alter the ability of crayfish to detect a potential predatory threat, likely altering the reaction space to the predator. The contrast in flow environment may increase or decrease the extent of NCEs from chemical information. The predators in this study were an active hunter, *Micropterus salmoides* (Largemouth bass), and a sit-and-wait predator *Ictalurus punctatus* (Channel catfish), both of which are crayfish predators (Garvey et al. 1994; Adams 2007). Both predators (Channel Catfish and Largemouth bass) and prey (Virile crayfish) are native to Michigan and coexist within the lakes and rivers of the Burt Lake watershed (Seelbach and Wiley 1997; Zorn et al. 2002; Wehrly et al. 2003).

We hypothesized that crayfish would spend more time in the shelter area in the presence of an active predator because the movement of the predator would create a larger distribution of predatory stimuli compared to the sit-and-wait predator. In addition, we hypothesized that the flow environment would have a greater influence on the crayfish response, such as shelter usage, to the sit-and-wait predator. The flow environment would spread the distribution of the predatory cues increasing the shelter area usage in crayfish, despite the sit-and-wait predator staying in a single location.
**Materials and Methods**

*Animal collection and holding*

Female crayfish, *Orconectes virilis*, were collected from Maple Bay in Burt Lake in Cheboygan County, MI (N 45.48°, W 84.70°). Only females were used due to the abundance of animals caught during the field season and reduce the experimental and statistical complexity by the presence of mixed sexes (statistical) and stimuli from mixed sexes (experimental). All crayfish were in the non-reproductive form (Form II). The average carapace length of all crayfish used in the experiment was 3.1 ± 0.2 cm and only crayfish with intact appendages were used in trials. Crayfish were housed in individual plastic containers (12.7 cm x 12.7 cm x 13.0 cm L:W:H) placed in either a flow through metal trough (237.5 cm x 86.4 cm x 60.1 cm L:W:H) or a flow through stream built from cinderblocks (425.5 cm x 91.4 cm x 41 L:W:H). Unfiltered river water from the Maple River was pumped into the trough and artificial stream to provide fresh oxygenated water, as well as detritus which serves as a food source. Crayfish were used only once in the trials. After trials were completed, crayfish were returned to their individual plastic containers.

Channel Catfish, *Ictalurus punctatus*, were purchased from Harrietta Hills Trout Farm in Harrietta, MI. Both male and female channel catfish used were 10.2 - 17.8 cm standard length. Catfish were collectively housed in a galvanized steel troughs (237.5 cm x 86.4 cm x 60.1 cm L:W:H) and were provided PVC tubes for shelters. Unfiltered river water from the Maple River was pumped into the trough to provide fresh oxygenated water. A mixture of sand and gravel substrate was provided to mimic natural settings and protect the fish from any potential injury from the bottom of the metal trough. A 244 cm x 91 cm sheet of window screening covered the top of the trough to protect the fish from potential predators. Twenty-five catfish were housed in
the trough. Catfish were fed Walt’s Crawlers brand worms every day and Wardley brand pond pellets every other day. Twenty-five largemouth bass, *Micropterus salmoides*, were purchased from Harrietta Hills Trout Farm in Harrietta, MI. Both male and female largemouth bass used were 12.7-25.4 cm standard length. Largemouth bass were housed in a flow through metal horse trough (237.5 cm x 86.4 cm x 60.1 cm L:W:H) under the same conditions as the channel catfish. Both catfish and bass are known predators of crayfish (Stein 1977; Adams 2007).

*Monitoring health and safety of the fish*

All procedures with the channel catfish and largemouth bass were approved by research protocols 687097-4 (BGSU) and PRO00004591 (Michigan). Fish were transferred from the housing trough to arenas in coolers filled with water from their housing trough and an aerator, to reduce stress in transportation. Catfish and bass were removed immediately from the arena or their collective housing if the fish exhibited any signs of stress during the trial. Trials where fish were removed due to stress were not analyzed and are not included in the data analysis of the results in this manuscript. The fish were put into a quarantine tank to monitor stress levels. Fish that exhibited signs of disease were removed from tanks containing the other fish and placed into a quarantine tank, given antibiotics and were monitored. After trials, fish were returned back to their housing trough and were monitored for signs of stress or disease. Fish that exhibited either disease or stress were not reused in the experimental protocol.

*Experimental design*

All trials occurred at the Experimental Stream Research Facility of the University of Michigan Biological Station in Pellston, MI. To investigate the interactive effects of predators with different hunting modes and flow environments on prey behavior, a fully factorial 2 x 3 experimental design was conducted with flow environment and hunting mode as the two factors. The flow environment treatment consisted of two different conditions: a flow environment and a
no flow environment. The predator treatment had three different conditions that were based on the predator’s hunting mode: no predator, active predator (largemouth bass), and sit-and-wait predator (channel catfish). Each combination of flow and predator treatments consisted of 11 replicates. Four crayfish were used in each trial (see below) and each crayfish was used only once. A total of 264 crayfish were used in this experiment. Given the limited number of predators, some fish were used in more than one trial. To account for this, trial number was used as a random factor within the statistical treatments.

Experimental arenas

Four experimental arenas (122 cm x 122 cm x 41 cm L:W:H; Fig. 1) were created using cinder blocks (30.5 cm in length) and 4 mil polyethylene plastic sheeting. All arenas and tests were conducted outside on the Experimental Stream Research Facility’s concrete pad. A 5 cm layer of pea gravel covered the bottom of the arena. Egg crating sheets (30.48 cm x 30.48 cm L:W) divided the arena into the multiple sections. Each arena included one predator section and four prey sections (Figure 1). Each prey section was 30.5 cm x 30.5 cm (L:W) and were located in the four corners of the arena. The rest of the arena (4389.12 cm²) was the predator section. One shelter and a piece of food was placed in each prey section (shelter and food described below) (Figure 1). A total of four identical experimental arenas were constructed where two had flow water and two had no flow of water.

All arenas were supplied with unfiltered water from the Maple River that was pumped into a 208.2 L head tank. The water input pipe to the head tank was covered with nylon fabric which filtered out a majority of the detritus and sand being pumped from the river. Water flowed from the head tank to the upstream upper part of the arena through three 1.6 cm diameter hoses in each flow arena. Water flowed through the flow arenas at a rate of 0.1 L/s. The starting water
levels in both the flow arenas and no flow arenas was 20.3 cm. Due to evaporation, the water level of no flow arenas was 12.7 cm by the end of the trial.

A large frame was constructed over the arenas and held the lighting and recording equipment. The wooden structure was covered with two 9.1 m x 9.1 m black tarps to provide consistent lighting conditions for treatments throughout the trials and to protect recording equipment. Trials were recorded using low light security cameras (Model # PRO-530) that were mounted on wooden frames 1.8 m above the arenas. The cameras were wired to a SWANN DVR4-3000 model DVR and a monitor that were housed in the Experimental Stream Research Facility building. This configuration allowed a view of the arenas while trials were running. Videos were recorded at 2 frames per second. Eight 25W A19 transparent red light bulbs with aluminum reflectors were mounted the wooden frames to provide lighting. Since crayfish cannot visualize red light, the effect of the light would have a minimal effect on their behavior (Tomba et al., 2001). Research has found that colored light is less effective at attracting fish including longer wavelength light like red, than white light (Marchesan et al., 2005).

Shelter and food

Shelters for the prey sections were constructed from PVC pipes (8.25 cm x 7.6 cm L:D) cut in half and attached to a Plexiglas® base with silicone. Previous studies have shown that crayfish will use half cut PVC pipes as shelters (Martin III and Moore 2008; Jurcak and Moore 2014). One shelter was placed in each of the four prey areas. Food consisted of 2.5 cm x 2.5 cm x 1.0 cm (L:W:H) squares of Pollock fillets (Walmart ® brand fish) which were placed in a mesh pouch (8.9 cm x 7.6 cm). The food pouches also held a 3.8 cm x 5.1 cm ceramic tile to provide weight so that the pouches would not move or float. Previous studies have shown that crayfish will readily consume Pollock fillets (Jurcak and Moore 2014). One food pouch was placed in
each prey section of the arena, at a diagonal from the shelter in each section. Fresh Pollock pieces were used for each trial.

*Experimental protocol*

All trials lasted for 21 hours. Shelters and food pouches were placed in each of the four prey sections. Both chelae of each crayfish were painted with white correction fluid in order to aid in visualization for video analysis and recording (Bergman et al. 2003; Fero and Moore 2008; Martin III and Moore 2008). Each crayfish was attached to a tether/lead system to prevent the animal from climbing out of the arena or entering the predator section. A strip of Velcro® was glued to the dorsal portion of the carapace with Scotch® liquid super glue. A separate Velcro® piece was then attached to a 20.0 cm lead made of 12 lb strength fishing line which was tied to three stacked 3.8 cm x 1.0 cm tiles. The two Velcro® pieces were joined prior to introducing the crayfish to the arena. The tether weight was placed in the center of the prey section, allowing the crayfish to move about the prey section without the capability of escaping the arena or entering other sections of the trial arena. Previous predator-prey research has used tethers on crayfish as prey and have shown minimal impacts to behavior (Englund and Krupa 2000; Kuhlmann et al. 2008). Crayfish were placed in each prey section 15 minutes before the trial was started for an acclimation period. Fish were not provided an acclimation period to reduce the time that crayfish were exposed to predatory odor without the trial being filmed. Trials started at 16:00:00 and finished at 13:00:00 the next day. At 16:00:00, the predator for each treatment (i.e. largemouth bass for active predator treatments and channel catfish for sit-and-wait predator treatments) was placed into the predator section of the arena. After the trials were complete, all fish, crayfish, and food pouches were removed from the arena. To flush the arenas, the flow arena treatments were allowed to sit for three hours. A single 1.6 cm diameter hose was placed into the no flow arenas.
Flow and no flow arenas were flushed at a rate of a minimum of 3.75 L/min for three hours. Thus, 675 L of clean water flowed through the arenas between trials.

Data analysis

For all trials, videos were analyzed to calculate the amount of time in seconds crayfish spent in each of the four zones of the prey section during the trial: shelter opening, shelter, free, and food. The free zone was defined as area which was “free” of resources and not located at the opening of the shelter. Shelter opening and free areas are considered spatial areas, while shelter and food areas are considered resource areas. Time started when the carapace of the crayfish entered or left each zone (Figure 2). The total number of area transitions crayfish made in the trial were also calculated. For fish behavior, videos were analyzed to calculate the amount of time in seconds fish spent in each of the five zones of the predator section during the trial. The five zones of the predator section were: upstream, downstream, east, west, and middle. The upstream zone was the area where flow was introduced into the arena, while the downstream zone was the area where the outflow was located. Time started when the anterior half of the fish entered or left each zone.

We performed outlier and collinearity tests (Zuur et al. 2009) as a pre-conditioning step for the data for both the fish and crayfish data. We found no outliers to remove and there was no discernable collinearity in any of the response or predictor variables. For the fish data, linear mixed models (LMM) followed by analysis of deviance tables using Type II Wald Chi Square tests (Zuur et al. 2009) were used to determine the effect of flow environment (flow, no flow) and predator treatment (active, sit-and-wait, no predator) on the response variables association with fish movement and location. Differences of least squares means (‘difflsmeans’) from the lmerTest package (Kuznetsova et al., 2015) in R was used as a post hoc test to discern which
factors were responsible for significant differences within main effects detected by the mixed models. Models were constructed using predator treatment and flow environment as fully interaction terms and as fixed effects and arena number as a single random effect. For the crayfish data, linear mixed models (LMM) followed by analysis of deviance tables using Type II Wald Chi Square tests (Zuur et al. 2009) were used to determine the effect of flow environment (flow, no flow), predator treatment (active, sit-and-wait, no predator), and two different crayfish location indicators (upper and lower as well as left and right) on crayfish zone use in the lme4 package (Bates et al. 2015) in R statistical software (version 3.3.0) (R Development Core Team, 2016). Models were constructed using predator treatment, flow environment, and crayfish location as fixed effects with full interaction terms and trial number and arena number as random effects. Differences of least squares means (‘difflsmeans’) from the lmerTest package (Kuznetsova et al. 2015) in R was used as a post hoc test to discern which factors were responsible for significant differences within significant main effects detected by the mixed models. For crayfish behavior, shelter opening, free space, and transitions, did not demonstrate any statistical significance and were dropped from subsequent presentation and discussion. For the fish behavior, east, west, and middle did not show any statistical significance and were removed from graphical presentation and discussions.

Results

Prey behavior in shelter area

The interaction of the presence of a predator and flow environment altered the way in which crayfish used shelters. When an active predator was present, crayfish in both the upstream and downstream areas spent significantly more time in the shelter in flow environment than no flow environments (Fisher-LSD, $p = 0.03$, $p = 0.0003$, Figure 3). In addition, crayfish in this
same treatment condition (active predator and flow) spent significantly more time in the shelter area than when a sit-and-wait predator was present with flow conditions (Fisher-LSD, p = 0.02, Table 2). Crayfish in the downstream area of no flow environments spent significantly less time in the shelter arena when an active predator was present than when no predator was present (Fisher-LSD, p = 0.03). Crayfish in the upstream area in flow environments with no predator spent significantly more time in the shelter area than in no flow environments (Fisher-LSD, p = 0.01, Figure 3). Crayfish in the upstream area in flow environments spent significantly less time in the shelter when a sit-and-wait predator was present compared to when no predator was present (Fisher-LSD, p = 0.0072, Table 2).

**Prey behavior food area**

When an active predator was present, crayfish in the downstream areas spent significantly more time in the food area in no flow environments than in flow environments (Fisher-LSD p = 0.02, Figure 4). In the no flow environments, crayfish in the downstream area spent significantly more time in the food area when an active predator was present than when there was no predator (Fisher-LSD p = 0.03, Table 3). When no predator was present in flow environments, crayfish in the upstream areas spent significantly more time in the food area than crayfish in the downstream areas (Fisher-LSD, p = 0.02, Figure 4).

**Fish behavior**

We detected a significant effect due to the flow environment (SS = 0.4429, df = 1, F = 5.00, p = 0.03 and interaction of the predator treatment and flow environment (SS = 0.6000, df = 1, F = 6.77, p = 0.012) on the behavior of the two different fish species in the upstream area. The catfish, a sit-and-wait predator, spent significantly more time in the upstream area in flow treatments as opposed to the no flow treatments (Fisher-LSD pairwise Comparisons, p = 0.007,
Conversely, the sit-and-wait predators spent significantly less time in the upstream area in the no flow treatment treatments ($SS = 0.4222, df = 1, F = 5.03, p = 0.03$). The sit-and-wait predators also spent significantly more time in the upstream area under flow conditions when compared to active predators (Fisher-LSD pairwise Comparisons, $p = 0.03$, Figure 5). We found a trend for sit-and-wait predators to spend more time in the downstream area of the no flow arenas than flow arenas (Tukey Multiple Comparisons for proportions, $p = 0.11$, Figure 5). Sit-and-wait predators also spent more time in the downstream area in no flow arenas than active predators (Tukey Multiple Comparisons proportions, $p = 0.11$ Figure 5). Finally, the active predators did not alter their behavior as a result of changes in the flow treatment.

**Discussion**

We had hypothesized that the crayfish would increase their time in the shelter area in the presence of an active predator compared to a sit-and-wait predator. Our results showed this to be true, specifically in the flow environments. In addition, we had hypothesized that the flow environment would have a greater influence on the crayfish response, such as shelter usage, to the sit-and-wait predator. However, there was no significant differences on the amount of time crayfish spent in the shelter area when a sit-and-wait predator was present between flow environments. Conversely, the interaction of the flow environment and presence of the active predator altered crayfish shelter use, with crayfish spending more time in the shelter area in the flow environments than no flow environments.

In order to fully understand the NCEs of predators, researchers need to incorporate the formation of the reaction space (Sih et al. 2010; Weissburg et al. 2014). To reiterate, the reaction space is the temporally and spatially dynamic space in which the predatory signal is of sufficient intensity that NCEs are produced within prey. Within this reaction space is the sensory
information used by prey to assess predation threats and alter their behavior, physiology, or morphology (Lima and Dill 1990; Lima 1998; Devereux et al. 2006). As our study has shown the interaction of different factors (environmental flow or hunting mode) changes the behavioral responses of prey to predatory cues. The results presented here are parsimonious with the idea that the reaction space within our test arenas were altered by these two factors. Within an ecological context, these same factors should determine the spatial and temporal extent of the reaction space and any NCEs produced by the interactions between predator and prey.

The interaction of the predator treatment and flow environment affected prey behavior, particularly when active predators were present. When an active predator was present, crayfish increased shelter usage and spent less time in the food area in flow than no flow environments (Figures 3 and 4). This is a typical behavioral response as crayfish increase shelter use in the presence of predatory fish (Rahel and Stein 1988). Yet, these same changes in prey behavior were affected by environmental and predator treatment interactions, were absent with the sit-and-wait predator in both the flow and no flow conditions (Figures 3 and 4). Crayfish did not significantly change the amount of time in the shelter area or food area when a sit-and-wait predator was present in either environment. Along this line, despite fish being three times larger than the largest crayfish, some of the prey might not have perceived a critical threat from the predators. Thus, the behavioral changes seen in the crayfish may not be a threat response. The flow environment could have been the main driver of shelter use, since the time in the shelter area of the predator treatments did not significantly differ from the predator treatments and the no predator treatments. However, the only treatments with the significant difference in shelter usage between environments was in the active predator treatments. Therefore, the flow environment coupled with the stimuli from the active predator altered crayfish behavior. Since
there are multiple ways in which crayfish can alter their behavior to predator stimuli, measures such as walking speed, time moving, and time stationary, together with shelter usage may better elucidate other effects of flow environment and predator treatments. It is also possible that the different hunting modes of the predators may change the effectiveness of the shelters placed within the experimental arenas. Catfish, being benthic predators, may be more effective at consuming crayfish in shelters used in this study which may have altered the crayfish response to this particular predator.

Although we did not analyze the temporal dynamics of this predator-prey interaction, prey can alter their responses to predatory stimuli, such as habituation, if there are no direct attacks being performed. Given the constraints of the experimental design with cages surrounding the crayfish, there were no possible attacks or consumptive events that occurred during the trials. An interesting aspect to add for further studies would be an analysis of the interaction of predator movement, environmental conditions, and temporal properties of predatory cues. Certainly, with chemical signals, environmental habitats with slow or low flow conditions would allow chemical signals to linger in the environment for longer periods of time (Dusenbery 1992). This lingering may cause either sensory adaptation (the lack of response from the sensory system despite the presence of stimuli) or behavioral habituation (Wilson 1998). These aspects could be additional factors to add into the reaction space model with further research. Indeed, the reaction space is a complex concept to measure. In addition to these temporal properties of sensory and behavioral systems, predatory diet and size are important factors in the production of stimuli. The types of diets consumed by predators has been shown to influence the reaction of prey to predatory odors (Scherer and Smee 2016). In addition, size and age of the predator may alter the perceived threat (Lundvall et al. 1999). Finally, if predatory
cues cause changes in the location and activity of prey, predators may need to alter their diet which in turn would alter the perception of threat. After all of these factors are taken into consideration (both experimentally and conceptually), a much deeper melding of the landscape of fear with sensory ecology can produce a more robust idea of how reaction spaces influence NCEs on an ecosystem level.

Crayfish use chemical signals for predator detection and the movement of chemical signals within environments is highly dependent upon the flow conditions of the medium (Moore and Crimaldi 2004). Thus, the most parsimonious explanation for the above results is that a larger reaction space within which prey are expected to respond was created by the combined effects of increased predator movement and ambient flow conditions. A sit-and-wait predator with lower activity levels does not create as large of a reaction space. The spatial distribution of signals from a sit-and-wait predator may be more concentrated to the area the predator is in, while a broader distribution of stimulus is created as an active predator moves throughout the habitat. An active predator moving throughout the environment will create a larger reaction space than sit-and-wait predator, as the movement will extend the area the predatory stimuli occupies. An active predator in an environment with flow will have a greater reaction space compared to a no flow environment as the interaction of the movement and flow will create a larger dispersion of the chemical stimuli.

Another interesting aspect of these findings could be the influence of native habitat flow conditions on the responses of the prey. Crayfish from hydrodynamically different flow regimes (lentic and lotic) respond to the presence of chemicals signals differently while orienting (Moore et al. 2015) and within agonistic contests (Bergman et al. 2006). All of the crayfish used in this study were collected from a lentic environment and it is entirely possible that crayfish collected
from lotic conditions may be more responsive to chemical signals. Given that the distribution of chemical signals is significantly different in these two systems, typical reaction spaces of prey may be dependent upon flow conditions within their native habitats. Regardless of this side point, the stimulus distribution from the two types of predators (active and sit-and-wait) will also be different in lotic and lentic environments.

This explanation is further supported by our results which showed that the behavior of the predatory fish significantly changed, with location of the predator changing in response to alterations in flow. Sit-and-wait predators spent significantly more time in the upstream area in flow environments, but significantly more time in the downstream area in no flow environments (Figure 5). There was no difference between the flow environments in the amount of time active predators spent in the upstream and downstream areas. Thus, changes in environmental conditions can alter fish movement behavior (Liao 2007). Given that visual and chemical signals produced by predatory fish are dependent on movement and flow, sit-and-wait predators will have reaction spaces that are unique for each habitat. Although their movement did not change in response to flow, the fact that flow will alter the distribution of chemical signals, active hunters will create different reaction spaces under flow and no flow conditions. The movement of fish to upstream or downstream sections of the experimental arena are most likely a response to the limited environmental size. Yet, within stream reaches, there is always an up- and downstream location of predators and prey. Thus, the interpretation of fish being upstream or downstream of the crayfish in our setup is applicable to prey located in similar situations in flowing systems.

Another aspect of the reaction space is the movement of predators which would create a larger distribution of stimuli. Predatory hunting mode or activity level can significantly alter prey behavior and ecosystem functions (Huey and Pianka 1981; Schmitz, 2008; Preisser et al. 2007;
Woodcock and Heard 2011; Miller et al. 2014). In terrestrial habitats where active hunting spiders were present but could not consume grasshoppers, the NCEs of the hunting spiders led to alterations in grasshopper behavior that elicited an increase in nitrogen mineralization and a decrease in plant diversity. Inversely, the introduction of sit-and-wait spiders caused a foraging shift in the grasshoppers, initiating an increase in plant diversity and decrease in nitrogen mineralization (Schmitz 2008). The ecosystem effects differ based on the movement of the predator and predator stimuli creating different landscapes of fear. Often, these studies examine the interaction of hunting mode and habitat domain, defined as the spatial area used by predators (Schmitz 2005; Schmitz 2007; Woodcock & Heard 2011; Miller et al. 2014). Active predators have larger spatial domains, moving through more of the microhabitat, than sit-and-wait predators, which have narrow habitat domains (Miller et al. 2014). While these studies have clearly showed that predator movement can alter NCEs, what is absent from these studies is a consideration of how the predator’s movement throughout a habitat creates predatory cues which alter prey behavior.

As previous work has shown, the interaction of predator signals, predator movement activity, and stimulus transmission within habitats creates the landscape of fear for prey (Keller et al. 2001; Schmitz 2008; Ferner et al. 2009). As an active predator moves throughout the broad area of the environment, there will be an increase in the exposure of visual cues to prey across the environment when compared to a sit-and-wait predator. Within the chemical senses, the generation of predator cues from active hunters will result in a large spatially, but diffuse distribution of cues as opposed to sit-and-wait predators, which will be the intensive, but spatially constrained (Weissburg & Zimmer-Faust 1993; Keller et al. 2001; Ferner et al. 2009). Clearly, the spatial and temporal distribution of this fearful landscape will be different, resulting
in spatial distinct NCEs. When these landscapes of fear are combined with differing prey sensitivities to signals, the result is a complex landscape of non-consumptive effects. Prey with more sensitive sensory capabilities will have larger reaction spaces and may be more heavily impacted by non-consumptive effects which has been defined as a reaction space. Prey with a large reaction space (possibly due to greater sensory sensitivities) would be exhibiting an anti-predator response at a great distance from the predator and for longer periods of time than prey with a small reaction space. Thus, focusing only on predatory movement or cue generation missed the important aspect of prey sensory biology.

Furthermore, within a single habitat, stimulus transmission properties are spatially and temporally distinct. For example, due to the influence of air temperature on sound transmission, nocturnal temperatures create a tunneling effect that allowed the calls of male bladder grasshoppers to travel significantly farther (van Staaden and Romer 1997). The same effect would occur for predatory cues. Not only do environmental conditions influence the transmission of the signals, different spatial locations within the environment can also alter the sensory landscape. The hunting calls of bats differ based on the type of environment that bats were hunting insects in with calls of long duration and lower frequencies in open areas and shorter call durations with higher frequencies near forested vegetation (Neuweiler and Fenton 1988; Schnitzler and Kalko 2001). For chemical signals, flow dynamics are critical in distributing chemical signals. Certainly, the results from this study show that some chemically-mediated NCEs may be hidden unless environmental dynamics are taken into consideration (Figures 3 and 4). Taken together, these studies show that environmental conditions influence the transmission of signals which creates the sensory landscape from which prey extract threat information. The environmental transmission can potentially increase the range of the predatory stimuli, thus
enlarging the prey’s reaction space. As a result, the exact structure of the reaction space is a result of the interaction of the movement of the stimulus source (predator) and the transmission mechanics of the environment.

Even understanding the interactions between predator movement and environmental transmission still leaves a gap in knowledge. The final missing piece of the puzzle is the sensory ecology (sensitivity, modality, thresholds) of the prey. Not all landscapes are perceived equally by prey items. Invasive crayfish in Italy and Australia responded to alarm odors from heterospecifics and conspecifics compared to native species which only responded to conspecifics (Gherardi et al. 2002; Hazlet et al. 2003). The increased sensitivity to multiple types of chemical information may be advantageous, aiding in additional information of predation risk, thus increasing survival. Prey may also be sensitive to differences in the stimulus from the predator, such as predator hunger level and predator diet. Tadpoles significantly decreased their activity when presented with dragonfly predator cues that had consumed tadpoles, compared to dragonfly predator cues alone (van Buskirk and Arioli 2002). Given the above studies, we can conclude that prey sensitivity is imperative to the construction of the reaction space and further research is necessary for understanding the role sensitivity has in the reaction space.

Our results show that the environmental conditions of the medium (in particular, flow or no flow) coupled with the movement of predators within that medium together influence the reaction space of prey. Additional work needs to examine prey sensory modality and sensitivity to further expand on the concept of the reaction space. Furthermore, research investigating the interaction of the type of sensory stimulus from the predator and the environmental transmission will also advance the understanding of the reaction space construction. Thus, knowing information that prey are using for decision making will allow us to better understand the
behavioral, physiological, morphological changes, and potential ecological consequences these behavioral changes will have on the ecosystem.
CHAPTER III. DIFFERENT SIZE REACTION SPACES OF TWO PREY SPECIES TO VARIOUS CONCENTRATIONS OF PREDATOR ODOR

Introduction

Non-consumptive effects (NCEs) of predators may have a larger effect on prey behavior and population structure than direct mortality or consumptive effects (Brown et al. 1999; Turner and Peacor 2012). NCEs are defined as alterations in prey behavior, physiology, or morphology elicited by the presence of a predator (Lima 1998; Schmitz et al. 2004; Weissburg et al. 2014). Examining the consequences of NCEs has led to the creation of the landscape of fear concept, where the perception of the threat of predation is intensive enough to elicit changes in the prey movement and foraging behavior (Laundré et al. 2001; Ripple and Beschta 2004; Matassa and Trussell 2011). If NCEs are driving prey behavioral choices, then cues from a predator which alert prey of potential risk must be of sufficient intensity to be detect by their sensory systems.

Prey use different types of sensory stimuli to extract information regarding predation threats. To distinguish predators from other organisms, fiddler crabs use the angular position and angular size of an organism relative to the horizon (Layne et al. 1997). Caterpillars used substrate-borne vibrations to detect the presence of predatory threat as well as distinguish the type of predator (Castellanos and Barbosa 2006). Predator odor from green crabs elicited a reduction in the number barnacles consumed by snails compared to snails foraging in an environment without cues from green crabs (Trussell et al. 2003). Oysters grew significantly stronger shells when exposed to odor from blue crabs that were fed live oysters than aged oyster tissue (Scherer et al. 2017). Prey use a variety of stimuli to detect predatory threat and use the information within the stimuli to respond. Thus, the sensory ecology of the prey organism
determines which signal modality is used to detect predatory threats and the sensitivity of that modality to the spatial distribution of signals within the environment.

Organisms may vary in their sensitivity to different types of stimuli. These sensitivity differences to stimuli may result between species exposed to the same predator stimuli. Great black-backed gulls and herring gulls when presented with the same set of various predator vocalizations and alarm calls showed significant differences in their responses. Although herring gulls showed no variation in response, great black-backed gulls changed their response to the stimuli with the greatest response to human voice (Maclean and Bonter 2013). Organisms within the same species may vary in their sensitivity depending on their geographical location. When presented with alarm cues, hard clams from Georgia reduced their pumping behavior whereas hard clams from Maine did not alter their behavior (Smee and Weissburg 2008). Difference in sensitivity to certain predatory stimuli will be revealed in an anti-predator response, such as a behavior, which would result in various size reaction spaces. A greater sensitivity will lead to a larger reaction space than prey which are less sensitive, leading to various responses and NCEs.

Sensory modality and sensitivity are two aspects that determine the distance at which prey respond to predatory cues which ultimately determines the spatial area in which prey can detect threats. We have previously defined the spatially and temporally dynamic area where predatory stimuli are intense enough to elicit an anti-predator response as the reaction space. This concept is an extension of the sphere of influence of the predator by Turner and Montgomery (2003). Instead of focusing on the production of cues by the predator which creates a predator-centric view, the sensory capabilities of the prey need to be considered. The NCEs are dependent on the structure of the sphere of influence, which is reliant on factors such as predator density, predator movement, sensory modality, and medium of which predatory stimuli are
distributed (Turner and Montgomery 2003). From a sensory ecology perspective, four key elements determine the size and scope of the reaction space: stimulus modality, predator movement, environmental transmission, and prey sensory ecology. The stimulus modality in the reaction space includes the manner at which the prey perceives information from the predator (i.e. visual, auditory, olfactory). The second element, movement of predators (i.e. hunting mode), determines the spatial dimensions involved in the distribution of aversive signals. The third element of the reaction space is the environmental transmission of the predatory stimuli. The type of environment the stimuli are in (i.e. flowing vs non-flowing, open vs. forested) can affect the movement and cause alterations in the stimuli properties of the stimuli and cause alterations. Finally, the last element of the reaction space is the sensory ecology (sensory thresholds) of the prey. Each element and the interaction of elements together influence construction of the prey’s reaction space and their response.

In this study, we focused on two elements of the reaction space, the stimulus modality and prey sensory ecology. The purpose of this work was to determine the sensitivity thresholds of different prey species to a similar predator odor. If the thresholds differ, this difference creates the potential for varying sizes of reaction spaces for prey species which would be evident through differing behavioral responses. For our experiment, two species of crayfish were chosen as prey, *Orconectes virilis*, (virile crayfish) and *Orconectes rusticus* (rusty crayfish) and *Micropterus salmoides* (largemouth bass) was chosen as the predator. Largemouth bass are a common crayfish predator, native to the area where this study took place, as well as, being a known predator to both species of crayfish (Garvey et al. 1994; Stein 1977; Zorn et al. 2002). Crayfish rely mainly on their chemical senses for gaining information for behavioral decision making (Hazlett 1999; Keller & Moore 1999). Virile crayfish are native to Michigan, while rusty
crayfish are extremely invasive in the Great Lakes region, being introduced and spread most likely by anglers as bait (Lodge et al. 2000). Rusty crayfish are successful at displacing native species due to their aggression (Lodge et al. 2000, Olden et al. 2006). However, both virile and rusty crayfish coexist in Burt Lake, where they were collected, for at least 25 years (Hazlett 2003).

Additionally, research has shown that successful invasive species may use a larger array of chemical information of predator risk than native prey species (Hazlett 2000; Hazlett et al. 2003; Gherardi et al. 2002). Multiple studies exposed native and invasive crayfish to alarm odors from conspecifics and heterospecifics (Hazlett 2000; Hazlett et al. 2003; Gherardi et al. 2002). Across studies, the invasive crayfish responded to alarm odors from all species where native crayfish responded primarily to conspecifics (Hazlett 2000; Hazlett et al. 2003; Gherardi et al. 2002). We hypothesized that the rusty crayfish would be more sensitive to the different concentrations of predator odor than the virile crayfish. Thus, if rusty crayfish respond to a broader range of chemical information, they may be more sensitive to chemical stimuli than virile crayfish, having a larger reaction space.

**Materials and Methods**

*Animal collection and holding*

Male and female crayfish, *Orconectes virilis* and *Orconectes rusticus*, were collected from Maple Bay in Burt Lake in Cheboygan County, MI (N 45.48°, W 84.70°). All *O. virilis* and *O. rusticus* crayfish were in the non-reproductive form (Form II). The average carapace length of all crayfish used in the experiment was 2.88 ± 0.03 cm and only crayfish with intact sensory appendages were used in the trials. Crayfish were housed in individual containers (18.1 cm x 16.2 cm x 7.3 cm L:W:H) placed in either a flow through galvanized steel trough (237.5 cm x...
86.4 cm x 60.1 cm L:W:H) or one of two flow through streams (304.8 cm x 81.3 cm x 40.6 cm L:W:H ) built from cinderblocks (30.5 cm in length), lined with 4 mil plastic sheeting. Unfiltered river water from the Maple River was pumped into the trough and artificial streams to provide fresh oxygenated water as well as detritus for food. Crayfish were used only once in the trials. After trials were completed, crayfish were returned to their individual containers.

*Micropterus salmoides*, largemouth bass, were purchased from Harrietta Hills Trout Farm in Harrietta MI. Both male and female largemouth bass used were 12.7-25.4 cm standard length. Thirty bass were collectively housed in two metal troughs (237.5 cm x 86.4 cm x 60.1 cm L:W:H) and were provided PVC tubes for shelters. Fifteen bass were placed in each trough. Unfiltered river water from the Maple River was pumped into the trough to provide fresh oxygenated water. In order to mimic natural settings and protect the bass from any potential injury from the bottom of the metal trough, a mixture of sand and gravel substrate was provided. A 244 cm x 91 cm sheet of window screening covered the top of the trough to protect the fish from potential predators. Fish were fed Purina Aqua Max Sportfish/Grower 600 pellets every day.

*Monitoring health and safety of the fish*

All procedures with largemouth bass were approved by research protocols (856543-2 BGSU) and PRO00006840 (Michigan). Bass were removed immediately from the odor collecting barrels during collection or from their collective housing if the fish exhibited any signs of stress. The fish were put into a quarantine tank to monitor stress levels. Fish that exhibited signs of disease were removed from tanks containing the other fish and placed into a quarantine tank, given antibiotics and were monitored. After odor collection (described below), fish were returned back to their housing trough and were monitored for signs of stress or disease.
Experimental design

All experimental trials occurred at the University of Michigan Biological Station's Experimental Stream Research Facility in Pellston, MI. To investigate the interactive effects of predator odor concentration and prey sensitivity, a fully 2 x 4 factorial experimental design was run with predator odor concentration and prey species as factors. Predator odor concentration had four conditions: control (no predator odor), low (1 bass per 50L), medium (2 bass per 50L), and high (3 bass per 50L). Prey species had two different conditions: native (*O. virilis*), and invasive (*O. rusticus*). *O. virilis* control and low had an N = 9 and *O. virilis* medium and high had an N = 10. *O. rusticus* control and low had an N = 10, *O. rusticus* medium had an N = 9, and *O. rusticus* high had an N = 8. One crayfish was used in each trial and each crayfish was used only once. A total of 76 crayfish were used in this experiment.

Experimental arenas

Four experimental arenas (82 cm x 61 cm x 41 cm L:W:H; Fig. 1) were created using cinder blocks (30.5 cm in length) and 4 mm polyethylene plastic sheeting (Figure 6). A 5 cm layer of sand covered the bottom of the arena. Untreated plywood boards (71.1 cm x 30.5 cm, L:H) were placed at the downstream end of the stream as outflow boards. Each board was drilled with six 8 mm holes, with each hole fitted with pipette tips. The boards were placed behind plastic sheeting that lined the streams. A total of four identical experimental arenas were constructed. All arenas were supplied with water taken from the Maple River that was pumped into a 208.2 L constant head tank which allowed detritus to filter. Water flowed from the head tank to the upper part of the arena through one 1.6 cm diameter hoses, fitted with a pvc elbow to prevent the water flow from going straight down into the substrate. Water flowed through the
arenas at an average rate of 0.07 L/s. The water level in the arenas was 20.32 cm. The arenas were flushed for at least an hour and a half to remove any odors from the previous trial.

Four low light security cameras (Model # PRO-530) were mounted on wooden frames above the arenas and were wired to a SWANN DVR4-3000 model DVR and monitors housed in the Stream Research Facility building to view arenas while trials were running. Videos were recorded at 8 frames per second. The wooden structure was covered with two 9.1 m x 9.1 m black tarps to provide consistent lighting conditions for treatments throughout the trials and to protect recording equipment. Four 25W A19 transparent red light bulbs with aluminum reflectors were mounted on the wooden frames to provide lighting.

Experimental protocol

All trials took place between 09:00 – 15:00 and were video recorded. Both chelae of each crayfish and the carapace were painted using white correction fluid in order to aid in visualization for video recording. Sponges soaked in bass odor (described below) were placed in the upstream area of the arenas in front of the hose inputting stream water at the start of the 15 min acclimation period. After the sponge was placed in the arena, crayfish were placed in a 12.5 cm x 12.5 cm x 12.5 cm box constructed out of egg crating, in the back of the downstream area for the acclimation period. This allowed the crayfish to stay in the downstream area until the trial began, while still being able to perceive odors. Both a shelter and a food cap (described below) were placed in the resource area of the arena at the start of the acclimation period.

After the 15 min acclimation period, the crayfish was removed from the box and the trial began. Crayfish had 15 min to move in the arena. After the trials were complete, crayfish, food caps, and sponges were removed from the arenas. The food cap was weighed directly after the trial had ended. Crayfish were returned to their individual Tupperware containers after the trials
were complete. Sponges were removed and wrung out. All arenas were flushed for at least an hour and a half to remove any odors from the previous trial.

**Food and shelter**

Food for the trials consisted of fish gelatin made from 46 g of canned sardines, 28g of Knox unflavored gelatin, and 600 ml of boiling water (Wolf et al. 2004; Lahman et al. 2015). All ingredients were combined in a blender and homogenized. The mixture was poured into 3 cm x 3 cm circular caps and were refrigerated for at least 12 hrs. After refrigeration, all caps were wrapped with parafilm to prevent the fish gelatin from drying out and placed in the refrigerator until use. All food caps were weighed before the start of each trial. Previous studies have shown that crayfish will use half cut PVC pipes as shelters (Martin III and Moore 2008, Jurcak and Moore 2014). PVC pipes (8.25 cm long x 7.6 cm diameter) were cut in half and attached to a Plexiglas base with silicone to construct shelters.

**Odor collection and odor prep**

Largemouth bass odor was collected in four 208.2 L barrels cut in half. All barrels were filled with 50 L of water from the Maple River and contained a 25.4 cm air stone connected to an Aqua culture brand air pump to oxygenate the water. All barrels were covered with window screening similar to those covering the collective housing troughs. The number of bass placed in each barrel corresponded to the three bass concentrations (1 bass per 50 L, 2 bass per 50 L, and 3 bass per 50 L). Fish were transferred from the housing trough to odor collection barrels in a bucket filled with water from their housing trough to reduce stress in transportation. Bass were placed in the barrel for 24 hours. Fish were returned to their collective housing after spending 24hrs. in the odor collection barrels and were acclimated for 20 minutes before introduction into their trough. Water from the four barrels, which were all of the same concentration, were then
transferred to a single 208.2 holding barrel containing eight hose valves. Water with odor from
the holding barrel was replaced every 6-7 days.

The odor used in the trials were prepared by transferring four liters of the odor water
from the holding barrel into five gallon buckets. A sponge was used for a momentumless odor
source delivery, in order to mimic the odor dispersal of a fish predator. Six 12.2 g weights were
attached to the bottom of a 20.3 cm x 3.8 cm 10.8 cm sponge, so the sponge would sit on the
bottom of the arena and stay stationary. The sponge used for the trial was placed in a 5 gallon
with the odor and was soaked for an hour and a half before the trial started. Sponges used in
control trials were only soaked in stream water and were not used in trials that contained bass
odor.

Data analysis

For all trials, videos were analyzed using EthoVision Noldus XT 8, a motion tracking
software. EthoVision Noldus was used to track a single point on the carapace of the crayfish at
each second of the trial. X,Y coordinates were generated for each second of the trial and used to
analyze multiple behavioral measures. Previous work has used x and y data points to calculate
movement and behavioral measurements (Wolf et al. 2004; Lahman et al. 2015; Kamran and
Moore 2015). The arena was divided into four zones and the time spent in each zone was
determined from the x, y data points. The empty zone was defined as the area furthest from the
sponge. The resource zone was defined as the area which contained the food cap and the shelter.
The area before the sponge and next to the resource zone was defined as the predator odor zone.
The area behind sponge was defined as the upstream of predator zone. Because the zones are
unequal in size, time spent in zones was corrected based on the size of the zone by dividing the
total time of the trial by the relative area of each zone. This produced a proportion of time
expected (given a random walk) spent in each zone. This data was natural log transformed for normality. Additionally, time spent walking or stopped within a zone was normalized for the total time spent in that zone. This produced a proportion which was transformed using an arcsin square root transformation.

The x, y data points were also used to calculate walking speed. Crayfish were considered walking when its moving speed was greater than 0.5 cm/s (Lahman et al. 2015). Crayfish with a walking speed lower than 0.5cm/s were considered stationary. The walking speed in each zone was then calculated for each crayfish. The total time spent walking and stationary were recorded in each of the four zones. When the crayfish was touching the food cap, this was defined as time with food behavior.

Statistical analysis

We preformed tests (e.g. cooks distance, normal qq, residuals vs fitted, etc.) to the data as a pre-conditioning step to identify outliers (Zuur et al. 2009). Two trials were consistently identified as outliers across tests and were removed from the analysis. Linear mixed effect models followed by analysis of deviance tables using Type II Wald Chi Square tests (Zuur et al. 2009) were used to examine the effect of crayfish species and bass odor concentration on crayfish behavior using the lme4 library (Bates et al. 2015) in R statistical software v. 3.3.2 (R Core Team 2016). The Difference of Least Square Means (‘difflsmeans’) post hoc test from the LmerTest package (Kuznetsova et al., 2015) in R was used to distinguish facts responsible for significant differences. The models were created using prey species (rusty, virile,) and predator concentration (control, low, medium, high) as the fixed effects, and tank (Tank 1, 2, 3, 4) as a random effect. There were no significant differences in the time spent with food so the analysis was dropped from the presentation of the results in this study.
Results

Overall effects

There were significant interaction effects of predator concentration and prey species ($F_{(10,162,0.05)} = 2.204, p < 0.002$) as well as main effects due to prey species ($F_{(10, 55, 0.05)} = 7.247, p < 0.0001$) and predator concentration ($F_{(30,162, 0.05)} = 1.74, p = 0.016$).

Interaction effects of time spent in each zone

Rusty crayfish spent a significantly higher percentage of time in the Predator Odor zone when exposed to the low predator concentration than when no predator odor was present (Fisher LSD, $p = 0.030$, Fig 7). Rusty crayfish also spent marginally significantly higher percentage of time when exposed to the high predator concentration compared to when there was no odor present (Fisher LSD, $p = 0.053$, Fig 7). There were no significant differences across concentration in the Predator Odor zone with the virile crayfish. Between species, virile crayfish spent a significantly higher percentage of time in the Predator Odor zone than rusty crayfish (Fisher LSD, $p < 0.01$, Fig 7).

In the Resource Zone, rusty crayfish spent a marginally significantly less percentage of time when no predator was present, compared to the low predator concentration (Fisher LSD, $p = 0.08$, Fig 10). Again, there were no significant differences between across concentrations in the Resource Zone with virile crayfish. Between species, virile crayfish spent a significantly higher percentage of time in the Resource Zone than rusty crayfish when no predator odor was present (Fisher LSD, $p < 0.01$, Fig 10) and when a medium predator concentration was present (Fisher LSD, $p = 0.02$, Fig 10). In the Empty Zone, there were no significant differences across concentrations for both species. Virile crayfish spent a significantly higher percentage of time in
the Empty Zone than Rusty crayfish when exposed to the medium (Fisher LSD, p = 0.01, Fig 10), and high predator concentrations (Fisher LSD, p = 0.02, Fig 10)

*Interaction effects of time spent stopped in each zone*

In the Predator Odor Zone, virile crayfish spent a significantly higher percentage of time stationary when exposed to no predator odor than the medium predator concentration (Fisher LSD, p = 0.02, Fig 8). Virile crayfish also spent a significantly higher percentage of time stopped when exposed to the low predator concentration than both the medium (Fisher LSD, p < 0.001, Fig 8) and high predator concentrations (Fisher LSD, p = 0.03, Fig 8). There were no significant differences in rusty crayfish stopping behavior across concentrations. Between species, virile crayfish spent a significantly higher percentage of time stationary in when no predator odor was present (Fisher LSD, p = 0.01, Fig 8) and in the low predator concentration (Fisher LSD, p < 0.01, Fig 8) than rusty crayfish.

In the Resource Zone, virile crayfish spent a significantly lower percentage of time stopped when exposed to the high predator concentration than the lower predator concentration (Fisher LSD, p < 0.01, Fig 11) When exposed to the medium predator concentration, virile crayfish also spent a significantly lower percentage of time stopped than in the low predator concentration (Fisher LSD, p < 0.01, Fig 11). As seen in the Predator Zone, there were also no significant differences across concentrations in the rusty stationary behavior. Between species, rusty crayfish spent a significantly lower percentage of time stationary in the no predator (Fisher LSD, p = 0.03, Fig 3) and low predator concentrations (Fisher LSD, p < 0.001, Fig 11) than virile crayfish.

In the Empty Zone, virile crayfish spent a significantly lower time stationary when no odor was present than when a low (Fisher LSD, p = 0.01, Fig 14) and medium predator
concentration was present (Fisher LSD, p = 0.03, Fig 3). Virile crayfish also spent a marginally significant lower percentage of time stopped when a high predator concentration was present compared to a low predator concentration (Fisher LSD, p = 0.1, Fig 14). Unlike, the Predator and Resource Zones, rusty crayfish significantly differed in their stationary behavior in the Empty zone. Rusty crayfish spent a significantly higher proportion of time stationary when exposed to the medium concentration of predator odor compared to no predator odor (Fisher LSD, p = 0.03, Fig 14), low (Fisher LSD, p = 0.02, Fig 14), and high predator concentrations (Fisher LSD, p < 0.01, Fig 3). Between species, rusty crayfish spent a significantly lower percentage of time stopped when exposed to the low (Fisher LSD, p < 0.001, Fig 14) and high predator concentrations (Fisher LSD, p < 0.01, Fig 4) than virile crayfish.

Interaction effects of walking speed in each zone

In the Predator Odor Zone, virile crayfish walked significantly faster when exposed to the medium predator concentration than no predator (Fisher LSD, p < 0.001, Fig 9), low (Fisher LSD, p < 0.001, Fig 9), and the high predator concentrations (Fisher LSD, p < 0.01, Fig 9). Rusty crayfish walked significantly faster when exposed to the high predator concentration than the medium predator concentration (Fisher LSD, p < 0.05, Fig 9). When exposed to no predator odor, rusty crayfish walked marginally significantly faster than in the medium predator concentration (Fisher LSD, p = 0.09, Fig 9). Between species, there were significant differences in walking speed across all concentrations. Rusty crayfish walked significantly faster than virile crayfish when no odor (Fisher LSD, p < 0.001, Fig 9), low (Fisher LSD, p = 0.02), and high predator odor concentrations (Fisher LSD, p < 0.01). Conversely, virile crayfish walked significantly faster than rusty crayfish when exposed to the medium predator odor concentration (Fisher LSD, p = 0.04, Fig 9).
In the Resource Zone, there were no significant differences in walking speed across concentrations for the virile crayfish. However, rusty crayfish walked significantly faster when exposed to the high predator concentration than when no predator (Fisher LSD, p = 0.02, Fig 12), low (Fisher LSD, p < 0.01, Fig 12), and medium predator concentrations (Fisher LSD, p < 0.001). Between species, virile crayfish walked significantly slower than rusty crayfish when exposed to the low (Fisher LSD, p < 0.01, Fig 12) and high predator odor concentrations (Fisher LSD, p < 0.001). Additionally, virile crayfish walked marginally significantly slower than rusty crayfish when no predator odor was present (Fisher LSD, p = 0.07, Fig 12).

**Discussion**

The results of this study show differing significant effects of predator concentration on the movement behavior of two different prey species. Virile crayfish spent significantly more time downstream when exposed to higher concentrations of predatory odor when compared to the rusty crayfish (Figure 8,9). Additionally, virile crayfish spent significantly more time stationary than did the rusty crayfish (Figure 8,11,14). Rusty crayfish walked significantly faster when exposed to the highest concentration compared to the lower concentrations (Figures 9,12). The results from our study show that these two different prey species may vary in their sensitivity and behavioral response to predatory odor.

Both species used different behavioral strategies when responding to the predatory cues. The rusty crayfish only altered its behavior in the presence of the highest concentration of predatory odor, whereas the virile crayfish responded to lower concentrations. Once the rusty crayfish detect and respond to the predatory odor, their behavioral strategy is significantly different than the virile crayfish. The rusty crayfish moves upstream, past the source of the odor as quickly as possible, whereas the virile crayfish does the exact opposite behavior. This species slows down its walking rate and spends significantly more time stationary. The virile crayfish
also behaviorally responded to more of the predatory odor concentrations than the rusty crayfish. Thus, the rusty crayfish is less sensitive (behaviorally) to the multiple concentrations of predatory odor than the virile crayfish, leading to the virile crayfish having a potentially larger reaction space.

The presence of the predator odor created behavioral responses in the crayfish, yet these responses differed in both their temporal and spatial components. The odor concentrations at which an anti-predatory response is evoked might be driven by diverse detection thresholds across species. Organisms with lower thresholds will respond at lower odor concentrations, which will translate into anti-predator behaviors changes, while those with higher thresholds would respond at higher concentration. This is seen in the present study. Differences in sensory thresholds would be shown through behavioral changes. This means that the same predator could have differing ‘landscapes of fear’ for one prey species that may vary in their detection of predation threats. The impacts and size of these landscapes are dependent of prey sensory ecology.

Predicting the extent and intensity of NCEs is dependent upon knowing response thresholds of prey, which may vary both among and within species. For example, male crickets exhibited a greater increase in metabolic rates compared to female crickets exposed to the same predator cue (Lagos and Herberstein 2017). Likewise, cow elk exhibited increases in vigilance behavior compared to bull elk in the presence of predation risk by wolves (Winnie Jr. and Creel 2007). Certainly, across species, NCEs and reaction spaces will be different. The southern lapwing displayed greater flight initiation distances than other species from potential threats approaching directly in front of them (Fernández-Juricic et al. 2005). Bull frog, leopard frog, and tree frog tadpoles exhibited significant spatial avoidance to caged mudminnows, whereas wood
frog, green frog, and American toad tadpoles did not (Relyea 2001). Variations in the size of reaction spaces within and between species will drive a multitude of NCEs. These examples illustrate differential behavioral changes in prey species to the presence of predatory stimuli that may be driven by sensory thresholds.

Another potential driver of the differences seen in the study is inherent behavioral differences in each species. In particular, rusty crayfish are highly invasive in the Great Lakes region and is successful in displacing native crayfish, such as the virile crayfish (Lodge et al. 2000, Hazlet 2003, Olden et al. 2006). Invasive species tended to be bolder, more exploratory, and more aggressive than native species (Juette et al. 2014). This could explain why rusty crayfish increased movement compared to virile crayfish, and less behavioral changes, across concentrations. However, bolder individuals have been subjected to higher predation rates. In the field, bolder roach (*Rutilus rutilus*) were found to be preyed upon more by cormorants (*Phalacrocorax carbo*) (Hulthén et al. 2017). Whether due to differing sensory thresholds or inherent behaviors, these variations in behavioral responses to predation threat can lead to a range of NCEs.

Behavioral changes in prey can lead to many ecological consequences including the spatial distribution of organisms within an environment and ecosystem processes (Hernández and Laundré 2005; Schmitz et al. 2004; Hawlena et al. 2012). If prey differ in the size of their reaction space and behavioral response, the influence of NCES on the environment may vary. NCEs of wolves in Yellowstone National park caused habitat and diet shifts in elk where these same NCEs were not seen in bison (Hernández and Laundré 2005). The presence of active hunting spiders led to foraging shifts in prey eliciting increases in aboveground net primary production (ANPP), where NCEs of sit-and-wait predators led to a decrease in ANPP (Schmitz
By understanding the construction of the reaction space, the mechanisms leading to the ecological consequences from NCEs will be elucidated.

In order to fully understand NCEs as they dictate prey response, research is needed on how the sensory ecology of prey creates reaction spaces. There has been a call for a greater understanding in the role of sensory ecology within predator-prey interactions and NCEs (Weissburg et al. 2014; Sih et al. 2010). The perception of sensory cues from predators is what drives the behavioral, morphological, and physiological changes in prey. As the results of this study imply, the size of reaction spaces is prey dependent. The variation in the structure of reaction spaces can lead to a diversity of NCEs even from a single predator, which could lead to multiple and complex ecological consequences. This study focused on just the prey sensory ecology component of the reaction space. Future research also needs to consider the interaction of prey sensory ecology with the other components of the reaction space, including the stimulus modality, predator movement, and environmental transmission of stimuli.
CHAPTER IV. EFFECT OF ANTHROPOGENIC CHEMICALS ON THE REACTION SPACE OF PREY

Introduction

Behavioral ecology is concerned with the decisions animals make in ecological settings including when to forage, avoid predation, and whom to mate with (Dill 1987). To make appropriate decisions, animals extract information from in their environment (Dill 1987; Keller and Moore 1999). Information comes from stimuli which is comprised in the sensory landscape which is the spatially and temporally dynamic distribution of various stimuli of an organism’s environment (Wilson and Weissburg 2013; Jurcak and Moore 2014). Stimuli within the environment includes both attractive stimuli such as resources or mates (Martin III and Moore 2008; Wilgers and Hebets 2012) and aversive stimuli such as predatory signals and alarm signals (Chivers et al., 2001; Hazlett 2000, Jurcak and Moore 2014). As such, animals make decisions in an environment with conflicting stimuli.

Aversive stimuli within the sensory landscape from predators contribute to the creation of the reaction space. The reaction space is the spatially and temporally dynamic area where predatory stimuli are intensive enough to elicit anti-predator responses. Responses include behavioral, physiological, and morphological changes (Jurcak and Moore, in review). The reaction space is composed of four factors: sensory stimulus, predator hunting mode, environmental transmission, sensory ecology of the prey. Each factor and the interaction of these factors influence the prey’s anti-predator response. The response of prey to information of the predator within the reaction space, lead to the non-consumptive effects (NCEs) of predators, such as behavioral, physiological, and morphological changes, driven by the predatory stimuli.
The reaction space is exclusively composed of aversive signals, but prey’s behavioral responses are also determined by positive stimuli like the availability and reward of resources. This assessment leads to different behavioral decisions (i.e. whether to forage or seek shelter) which are driven by the types, intensity, and distribution of the sensory stimuli within the environment (Dill, 1987; Corcoran et al., 2013; Jurcak and Moore, 2014). An inability to detect and extract information could lead to inappropriate calculations of cost and benefit and maladaptive responses, and may ultimately lead to decreased survivorship, lack of acquisition of resources, and vulnerability to predators.

Pollution can influence the ability organisms to detect and extract information from their environment (Lurling 2012). Pollution, in particular chemicals, such as herbicides, and pesticides, pharmaceuticals, personal care products, and industrial chemicals, such as heavy metals can increase the mortality and alter behavior of non-target organisms (Carmago and Alonso 2006; Lurling 2012). The increasing prevalence of anthropogenic chemicals, especially in aquatic ecosystems, has led to negative impacts on organisms inhabiting these ecosystems (Carmago and Alonso 2006; Lurling 2012). *Echinometra lucunter* (rock boring urchins) exposed to biodiesel displayed inhibited growth in both their embryonic and larval stages (Leite et al. 2011). In addition to physiological effects, aquatic organisms exposed to anthropogenic chemicals have displayed alterations in their behaviors. Crayfish exposed to naproxen in dynamic flowing systems took longer to reach escalated fighting behaviors than unexposed crayfish (Neal and Moore 2017). *Pomatoschistus microps* showed erratic swimming behavior and were lethargic when exposed to concentrations of copper or mercury (Vieira et al. 2009). The ability to appropriately respond to predatory stimuli has also been affected by anthropogenic chemicals.
Anthropogenic chemicals in an environment can prevent the ability of prey to detect a predator, reducing or diminishing the reaction space. Chinook salmon exposed to diazinon showed reduced anti-predator responses to chemical alarm signals (Scholz et al. 2000). Similarly, copper exposed Colorado pikeminnow exhibited less fearful responses to homogenized skin from donor pikeminnow (Beyers and Farmer 2001). Crayfish increased their activity and walking speed in the presence of alarm signals when exposed to concentrations of metolachlor, in opposition to the expected decreased activity and freezing behaviors (Wolf and Moore 2002). The exposure of anthropogenic chemicals in these studies lead to behavioral responses that most likely would have decreased their ability to survive. Thus, the failure to properly respond to predatory threats would lead to decreased devastating fitness consequences.

The purpose of this study was to examine how exposure to an anthropogenic chemical, specifically carbaryl, would affect the ability of prey to detect a predator. Carbaryl is a widely used carbamate insecticide for home and garden and as well as agricultural crops (Xu 2000; Grube et al. 2011). As a cholinesterase inhibitor, exposure to carbaryl can result in high concentrations of acetylcholine in the nervous system, causing uncontrolled rapid movement, paralysis and death in organisms (Xu 2000). In particular, exposure to carbaryl has been shown to negatively affect the ability of crayfish foraging behaviors (Ludington and Moore 2017). This study also used two different prey species to examine any detrimental effects of anthropogenic chemicals to closely related prey species. For this experiment, the prey were *Orconectes virilis* (virile crayfish) and *Orconectes rusticus* (rusty crayfish). Many studies have used crayfish as model for studying anthropogenic chemical effects on behavior and have found that the chemically-mediated behaviors of crayfish have been negatively impacted (Browne and Moore 2014, Lahman et al. 2015; Neal and Moore 2017; Ludington and Moore 2017). Odor from
**Micropterus salmoides** (largemouth bass) was chosen because bass are common crayfish predators and consume both species of crayfish (Stein 1977; Garvey et al. 1994; Zorn et al. 2002). We hypothesized that both species of crayfish would be negatively affected by the exposure of carbaryl to accurately detect largemouth bass chemical stimuli. Also, virile crayfish would be more negatively affected by the carbaryl exposure, because they have been shown to be more sensitive to largemouth bass odor than rusty crayfish (Jurcak et al. *in prep*).

**Materials and Methods**

**Animal collection and holding**

Male and female crayfish, *Orconectes rusticus* and *Orconectes virilis*, were collected from Maple Bay in Burt Lake in Cheboygan County, MI (N 45.48°, W 84.70°). All crayfish were in the non-reproductive form (Form II) and had an average carapace length of (3.2 ± 0.03 cm). Only crayfish with intact sensory appendages were used in the trials. Crayfish were housed in flow through galvanized steel troughs (237.5 cm x 86.4 cm x 60.1 cm L:W:H) or in individual containers (18.1 cm x 16.2 cm x 7.3 cm L:W:H) placed in either one of two flow through streams built from cinderblocks (30.5 cm in length), lined with 0.1mm plastic sheeting. Unfiltered, oxygenated water from the Maple River was pumped into the troughs and artificial streams.

Largemouth bass, *Micropterus salmoides*, were purchased from Harrietta Hills Trout Farm in Harrietta MI. male and female largemouth bass used were 10-18 cm in total length. Fifty bass were collectively housed in two metal troughs (237.5 cm x 86.4 cm x 60.1 cm L:W:H) and were provided PVC tubes for shelters. Unfiltered, oxygenated water from the Maple River was pumped into the trough. A mixture of sand and gravel was provided for the bottom of the metal troughs to protect the bass injury and mimic natural substrate. Window screening sheets (244 cm
x 91 cm L:W) covered the top of the troughs to protect the fish from potential predators. Fish were fed either pellets made from *O. virilis* or *O. rusticus*, depending on the treatment.

**Monitoring health and safety of the fish**

All procedures with largemouth bass were approved by IUCAC research protocols (856543-5 BGSU) and (PRO00006840 Michigan). Bass were removed immediately from their collective housing or odor collection tubs (described below) if the fish exhibited any signs of stress. The fish were put into a quarantine tank to monitor stress levels. Fish that exhibited signs of disease were given antibiotics and were monitored. After odor collection (described below), fish were returned to their housing trough and were monitored for signs of stress or disease.

**Experimental design**

All experimental trials occurred at the University of Michigan Biological Station's Stream Research Facility in Pellston, MI. To investigate the interactive effects of carbaryl exposure and prey species, a fully 2 x 2 factorial experimental design was performed with carbaryl exposure, prey species, and amount of carbaryl exposure as factors. Carbaryl exposure had two conditions: exposed or non-exposed (control). Prey species had two different conditions: *O. virilis* and *O. rusticus*. *O. rusticus* control had an N = 16 and *O. rusticus* exposed had an N = 15. *O. virilis* non-exposed had an N = 19, and *O. virilis* exposed had an N = 16. One crayfish was used in each trial. A total of 66 crayfish were used in this experiment and each crayfish was only used once.

**Exposure streams**

Eight experimental streams (182.9 cm x 38.1 cm x 40.6 cm L:W:H; Fig. 1) were created using cinder blocks (30.5 cm in length) and 0.1 mm polyethylene plastic sheeting (Figure 1). A X cm layer of pea gravel covered the bottom of the arena. Untreated plywood boards (47.0 cm x 33.0 cm, L:H) were placed at the downstream end of the stream as outflow boards, which were
lined with plastic sheeting. Each board was drilled with nine 2.5 cm holes fitted with 1.27 cm diameter PVC pipe (L : 2.54 cm). Experimental streams were supplied with water taken from the Maple River that was pumped into a 208.2 L constant head tank, which allowed detritus to filter. Water flowed from the head tank to the upstream part of the stream through two 1.6 cm diameter hoses. A collimator constructed out of two layers of egg crating wrapped in window screening, was placed 30.5 cm from the top of the stream to aid in conditioning the flow. Water flowed through the streams at an average rate of .07 L/s. The water level in the arenas was an average of 14 cm.

Control or carbaryl solutions were contained in 22.7 L buckets located on top of cinderblocks at the upstream location of the stream. Each bucket contained a 0.4 cm (ID) aquarium tubing attached with a plastic connector at the bottom of the bucket in which the solution was delivered. Open Hoffman compressor clamps (1.9 cm) were used to control the flow rate of the chemical solution. The input of the solution was located 7.6 cm downstream of the collimator. Crayfish in the experimental stream was placed 50 cm downstream of the chemical solution input. A clay tile (5 cm x 1cm x 7cm) with a snap swivel attached to a 10 cm length braided fishing line with a 2.5 cm x 2.5 cm piece of Velcro was used to secure the crayfish. Each crayfish had a 2.5 cm x 2.5 cm piece of Velcro super glued (brand) to the carapace which attached to the Velcro piece on the tile (Ludington and Moore 2017; Neal and Moore 2017). Each bucket was covered with lids to prevent contamination or dilution of the carbaryl solution.

*Electrochemical measurements*

An Epsilon electrochemical detection system (Epsilon; Bioanalytical Systems, West Lafayette, IN) coupled with a three, 30-um carbon fiber microelectrode, was used to quantify the
concentration of carbaryl in the streams. A chemical tracer was used to measure the oxidation-reduction reactions to the microelectrode. Dopamine is an appropriate chemical tracer due to dopamine’s ability to accurately model concentration profiles for carbaryl, and dopamine’s relative ratio of advection and diffusion (Péclet number). The Péclet number of this system indicates that advection is more important than diffusion in the dispersion of chemicals (Denny 1993).

Microelectrodes were constructed in the lab (Wolf et al. 2009; Edwards and Moore 2014; Lahman and Moore 2015). The microelectrode was attached to a probe of a tripod which was lowered so the microelectrode was 3 cm above the substrate. This height mimicked the position of the crayfishes’ sensory appendages. The microelectrode was placed at 50 cm downstream of the chemical input, the same location where the crayfish would be secured during exposure. The setting for the Epsilon system was set to record with an applied potential at 500 mV with a 10 Hz noise filter for a total of 500 sec. A calibration of the microelectrode was made at five concentrations of dopamine. The microelectrode used to calculate dilution factor was calibrated using 5 known concentrations of dopamine (2, 4, 6, 8, and 10 µM). The concentration of the stock solution delivered to the artificial streams during Epsilon recordings was 31.16 µM. The dilution factor was calculated as 93 at 50 cm downstream from the chemical source where the crayfish would be located.

Exposure paradigm

Crayfish were exposed to 50 µg/l of carbaryl. Gardentech Sevin Concentrate Bug Killer was used to create the stock solutions. Crayfish were exposed for 23 hours in the experimental streams. Each stream had at least 24 hours in between trials to flush out any previous odors. Due
to the carbaryl being delivered through a gravity feed, the amount of carbaryl exposure ranged from 3.5-21 L over a 23 hour exposure period.

*Odor prep and collection*

Three largemouth bass were placed in one of four plastic containers containing 173 L of river water (189.3 L: Sterilite®) with an aerator. Each plastic container was covered with window screening to prevent anything from entering the odor collection containers, predation of fish, or fish leaving the containers. The container was covered with window screening and a tarp to prevent contamination from outside sources and any fish escapes. Odor was collected from the fish for 24 hours and fish were returned to their housing after odor collection. Trials where virile crayfish were used contained odor from fish that had consumed virile crayfish, and trials where rusty crayfish were used, odor was collected from fish that had consumed rusty crayfish. Fish were not fed in their housing tanks and not in the odor collection tubs. Crayfish were subjected to the predator odor only in the two current flume, after their exposure period in the streams.

*Two current flume*

Two 55 gallon barrels were located 104 cm above the ground on a wooden structure. Each barrel contained a pump (brand: Little Giant) which sat at the bottom of the barrel and pumped water/odor to a 5 gallon bucket sitting on top of the barrel. Each bucket had one inflow hole at the top of the which contained plastic hosing connected to the pump. An overflow hole on the opposite side in the same location connected to a pvc elbow and pipe which went back into the barrel. This was to provide a constant head inside the 5 gallon bucket. A hole on the front of the bucket near the bottom was connected to a y valve that split into two and had a switch. Connected to each valve was two hoses, one that was connected to another hose that went straight to the two-current flume, the other to the opposite bucket. Each bucket had hoses that
connected to the hoses that went into the flume so that the odor/water could be switched during the trial. One 55 gallon barrel contained fish odor and the other contained river water from the Maple River.

A two-current choice flume (Fig 15) was constructed from white Plexiglas, designed similarly to that in Jutfelt et al. (2016). The flume consisted of three sections: a conditioning section, a choice area, and an outflow section. In the conditioning section a center wall separated the area into two identical sides. Each side consisted of a wire screen covered in mesh window screening with a silicone baffle (13 cm downcurrent from the opening), another wire screen with mesh (12 cm downcurrent from the previous screen), an egg crating sheet (17 cm downcurrent from the previous screen) and finally three plastic honeycomb collimators (5 cm width, Plasticore brand) at 53, 71, and 91 cm from the opening. The total conditioning section was 91 cm x 61 cm L:W.

The choice area (64 cm x 66 cm) followed the conditioning section which contained two PVC half pipe shelters. A shelter was located on each side, along the wall, and 34 cm from the front of the choice arena. Egg crating covered with window screening divided the choice area from the outflow section. The outflow section contained a divider similar to the conditioning section which separated the section into two areas. Odor and river water flowed out of four outflow holes on each side of the divider. The entire two choice flume had a sand substrate glued to the flow. Sand was to provide a substrate for the crayfish to walk on. Dye tests were done to show mostly separate sides of odor and the time frame when the odor would be on each side. A wooden structure was constructed over the barrels and the two-current flume with a tarp covering the frame. This was to have consistent lighting and protect the flume and the video camera situated 130 cm above the choice area. The camera recorded all trials.
Experimental Protocol

Trials were run between 0:13:00 - 0:17:00 and were video recorded using a Sony handheld video camera above the flume. Before each trial, crayfish were acclimated for 15 minutes followed by an 11.5 minute trial. Previously exposed crayfish or control crayfish were placed at the downstream end of the choice area. At the beginning of the trial, the odors would start flowing. There were four time periods during the trial: pre odor (0-2:30) odor on the right side, river water on the left side (2:30-5:30), mixed (5:30-8:30), and river water on the right side, odor on the left side (8:30-11:30). At the 5 minute mark of the trial, valves were turned to switch the side the bass odor was on. This was done to prevent side bias. After trials, control animals were returned to their housing and exposed crayfish were euthanized. After each trial, the arena was drained and filled with fresh river water.

Video and statistical analysis

The behaviors of crayfish were analyzed for each trial. Behaviors included time spent on each side, time spent in shelter, climbing and lowered position. Crayfish were on the right side when the intersection of the cephalorax and abdomen of the crayfish was on the right side. Conversely, crayfish were considered on the left side when this intersection was on the left side. Time in shelter was defined when crayfish when the interaction of cephalo thorax and the abdomen was inside the shelter. Time climbing was defined when all of the walking legs of the crayfish were off the bottom of the flume and on the honeycomb or back egg crating wall. The lower position was defined when the tail started to curl and completely tucked under the body. Results were analyzed by running ANCOVAs to examine the effect of crayfish species, carbaryl concentration and amount exposed to on crayfish behavior.
Results

Time on side

There were no significant main interaction effects of exposure treatment and prey species on the time crayfish spent in the side with bass odor (ANCOVA: $F_{7,58} = 0.7662$, $p = 0.6178$, Figure 16). Similarly, there were no significant main interaction effects on the amount of time crayfish spent in the side with river water (ANCOVA: $F_{7,58} = 0.7662$, $p = 0.6178$, Figure 17).

Time in shelter

There were no significant main interaction effects of exposure treatment and prey species on the time crayfish spent in the shelter on the same side with bass odor (ANCOVA: $F_{7,58} = 0.784$, $p = 0.6034$. Figure 18). The time crayfish spent in the shelter on the same side as the river water also showed no significant main interaction effects (ANCOVA: $F_{7,58} = 0.6188$, $p = 0.7382$, Figure 19).

Time climbing

There were no significant main interaction effects of exposure treatment and prey species on the time crayfish spent in the climbing on the same side with bass odor (ANCOVA: $F_{7,58} = 0.9682$, $p = 0.463$, Figure 20). Similarly, the time crayfish spent climbing on the same side as the river water also showed no significant main interaction effects (ANCOVA: $F_{7,58} = 0.405$, $p = 0.8954$, Figure 21).

Time with tail tucked

There were no significant main interaction effects of exposure treatment and prey species on the time crayfish spent in with their tail tucked on the same side with bass odor (ANCOVA: $F_{7,58} = 1.321$, $p = 0.2602$, Figure 22). There were also no significant main interaction effects on
the amount of time crayfish spent with their tail tucked on the same side as the river water
(ANCOVA: F_{7,58} = 0.3182, p = 0.9428, Figure 23).

Discussion

We had hypothesized that the crayfish would be negatively impacted by the exposure of
carbaryl on their ability to detect and appropriately respond to a predatory threat. We also
hypothesized that the rusty crayfish would be more negatively affected by the exposure to
carbaryl than virile crayfish. However, our results indicated there were no significant effects of
exposure, species, or the interaction of exposure and species on the behavior of the crayfish.
These results were surprising since previous research has shown that carbaryl can alter
chemically mediated behaviors in crayfish (Ludington and Moore 2017).

While Ludington and Moore (2017) found that carbaryl negatively altered the foraging
behavior, their key finding was the effect of carbaryl was heavily influenced by the
hydrodynamics of the system and the structure of the chemical signal. Crayfish downstream of a
structure, such as a log, were impacted by the chemical exposure significantly more than crayfish
upstream of the log (Ludington and Moore 2017). Thus, the hydrodynamics and chemical
structure can lead to differing effects of exposure. Though the carbaryl exposure in my study did
not affect anti-predator behavior, there is a potential that by altering the hydrodynamics of the
system, this could have affected the exposure of the crayfish leading to significant behavioral
changes.

Despite the results of this study, research should continue to explore the effects of
anthropogenic chemicals on the sensory environment of predator-prey interactions. Prey
behaviors have been significantly influenced by exposure to anthropogenic chemicals (Scholz et
al. 2000; Beyers and Farmer 2001; Wolf and Moore 2002; Lurling 2012). Low concentrations of
fluoxetine led to decreased freezing behaviors mosquitofish and increased speeds of mosquitofish in entering the strike zone of a predator (Martin et al. 2017). Tadpoles in tap water showed a 49% decrease in activity when a predator cue was present, while tadpoles in wastewater effluent showed only a 24% decrease in activity in the presence of predator cues (Troyer and Turner 2015). The inability to detect and probably respond to predatory cues would lead to an increase in the probability of being preyed upon.

As the input of anthropogenic chemicals into the environment, especially aquatic environments, continue to rise, this will place greater stress on organisms. The understanding of the effects of anthropogenic chemicals in aquatic systems has been identified as an important area of future research in aquatic chemical ecology (Brönmark and Hansson 2012). An inability of prey to detect predatory threat and appropriately respond would have many fitness and ecological consequences altering both the non-consumptive and consumptive effects of predators.
CHAPTER V. SUMMARY AND GENERAL CONCLUSIONS

The field of predator-prey interactions has recognized the large impact that NCEs have on prey populations and entire ecosystems (Brown et al. 1999; Turner and Peacor 2012). From this recognition, the concept of the “landscape of fear” in ecology was created (Laundré et al. 2001; Altendorf et al. 2001). This work provided a wealth of insight on the response of prey to the presence of predators, but less insight on how the stimuli from predators elicit changes in prey. Recently, a call for a broader application of sensory ecology in the analysis of NCEs was made (Sih et al. 2010; Weissburg et al. 2014), with an additional call for understanding how anthropogenic chemicals could affect the chemical ecology of predator prey interactions (Bronmark and Hansson 2012). The research outlined in this dissertation aimed to provide answers to these calls in the field of predator-prey interactions. My research defined and empirically tested the reaction space, the spatially and temporally dynamic area where stimuli from predators alter the behavior, physiology, and/or morphology of prey. Exploring these features allowed for the comprehension of how each factor that comprises the reaction space influences the creation of the reaction space and the alteration of NCEs.

The results of the first aim of demonstrated that the interaction of factors from the reaction space led to different behavioral responses in prey. The environmental conditions of the medium together with predator movement affect the reaction space of prey. Crayfish increased shelter usage and spent less time in the food area in flow than no flow environments when an active predator was present. However, when a sit-and-wait predator was present in both the flow and no flow conditions, there were no significant differences in prey behavior. The variation in behavioral responses due to the interaction of environment and predator hunting mode may lead to differential NCEs. While research has found that the NCEs of predators of different hunting
modes lead to a variety of ecological consequences in ecosystem functioning (Schmitz 2008; Schmitz et al. 2004; Miller et al. 2014), the results from this work may provide the mechanism that is driving the ecological changes.

The results from the second aim of this research demonstrated that the behavioral response of two prey species varied across multiple concentrations of predator odor. Virile crayfish exhibited more stationary behavior than rusty crayfish, with the predator odor altering the virile behavior across concentrations, which was absent with rusty crayfish. Rusty crayfish also walked significantly faster than virile crayfish across concentrations, and the highest predator concentration having the greatest effect on rusty crayfish walking speed. Crayfish behavioral changes were also dependent on the location of the prey within the experimental arena. These results suggest that both species vary in their reaction space sizes, particularly the virile crayfish having a larger reaction space. Sensory threshold differences would be revealed through behavioral variances in species.

The results from the third aim of this research did not show any effect of pesticide exposure on prey behavior. Exposed crayfish of either species did not differ in their time in the odor or clean river water, shelter usage, climbing or lower posture than unexposed crayfish of either species. There are many factors which could contribute to these results, including the type of behaviors analyzed, type of anthropogenic chemical, concentration of chemical, amount of chemicals prey were exposed to, and the exposure period. Previous research has found that crayfish can be impacted by anthropogenic chemicals in a variety of chemically mediated behaviors such as foraging and fighting behavior (Lahman et al. 2015; Ludington and Moore 2017; Neal and Moore 2017) and that prey behavior can be affected by anthropogenic chemicals (Relyea and Edwards 2010; Peters et al. 2017). While the results of this study in the dissertation
does not match previous research, there is continued importance in understanding how anthropogenic chemicals can alter the reaction space of prey and further research needs to be done.

Overall, the results from this dissertation indicate that alterations in the sensory environment of predator-prey interactions, particularly the reaction space, lead to different prey behavioral responses. Variations in prey behavior due to the sensory stimuli of predators, environmental transmission, predator hunting mode and prey sensory ecology may result in a range of NCEs. Within the reaction space is where prey gain information about predators which drives the behavioral, morphological, and physiological changes in prey. While one of the studies in this research did not find effects of anthropogenic chemicals on the reaction space and prey behavior, this is an area that should be continued to be investigated due to the increase in anthropogenic chemicals in aquatic systems. Researchers need to take into account the reaction space and the sensory environment in order to better understand the mechanisms for NCEs and their ecological consequences.
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Figure 1. Experimental arena. Prey sections were in each corner of the arena. Upstream prey locations refers to the two prey sections closest to the inflow and the downstream prey locations refers to two the prey sections closest to the outflow. The remaining spatial area of the arena was the predator section. Water entered from three hoses at the top of the arena and exited at the outflow at the downstream end of the arena at a flow rate of 0.1 l/s. No flow arenas did not have hoses. Food, located in the corner closest to the predator section, Shelter, located in the corner along the wall, Shelter Opening, the area in front of the shelter, and Free, the area next to the shelter.
**Figure 2. Prey Section.** Divided into four areas (clockwise): Food, Shelter Opening, Shelter, and Free. Food, located in the corner closest to the predator section, Shelter, located in the corner along the wall, Shelter Opening, the area in front of the shelter, and Free, the area next to the shelter.
Figure 3. Shelter behavior. Mean (± SEM) proportion of time spent in the shelter area by prey in the upstream and downstream areas when there was an active predator, a sit-and-wait predator, and no predator in no flow (white) and flow (black) environments. Each bar contains N = 11. Bars with asterisks indicate differences. Comparisons are only for single predator/environment. (Fisher-LSD, p < 0.05)
Figure 4. Food behavior. Mean (± SEM) proportion of time spent in the food area by prey in the upstream and downstream areas when there was an active predator, a sit-and-wait predator, and no predator in no flow (white) and flow (black) environments. Each bar contains N = 11. Bars with asterisks indicate differences. Comparisons are only for single predator/environment. (Fisher-LSD, p < 0.05)
**Figure 5. Fish behavior.** Mean (± SEM) proportion of time spent by active and sit-and-wait predators in the Upstream and Downstream areas of the arena in no flow (white) and flow (black) environments. A total of N = 11 for each predator and flow environment. Comparisons are only for a single predator/environment (i.e. active in flow environment, upstream vs. downstream). Asterisks indicate differences. (LMM, Tukey Multiple Comparisons of Means post-hoc, p < 0.05).
Figure 6. Experimental arena. Water entered from a single hose at the top of the arena and exited at the outflow at the downstream end of the arena at a flow rate of 0.07 L/s. Food and shelter resources were located downstream from the predator odor source.
Figure 7. Time in Predator Zone. Mean (± SEM) percentage of time spent in the Predator Odor zone between virile crayfish (blue circle) and rusty crayfish (red square) across the control, low, medium, and high concentrations of predator odor. Asterisks indicate differences between species. Letters indicate significant differences.
Figure 8. Time Stopped in Predator Zone. Mean (± SEM) percentage of time spent stationary in the Predator Odor zone between virile crayfish (blue circle) and rusty crayfish (red square) across the control, low, medium, and high concentrations of predator odor. Asterisks indicate differences between species. Letters indicate significant differences.
**Figure 9. Walking Speed in Predator Zone.** Mean (± SEM) walking speed in the Predator Odor zone between virile crayfish (blue circle) and rusty crayfish (red square) across the control, low, medium, and high concentrations of predator odor. Asterisks indicate differences between species. Letters indicate significant differences.
Figure 10. Time in Resource Zone. Mean (± SEM) percentage of time spent in the Resource zone between virile crayfish (blue circle) and rusty crayfish (red square) across the control, low, medium, and high concentrations of predator odor. Asterisks indicate differences between species. Letters indicate significant differences.
**Figure 11. Time in Resource Zone.** Mean (± SEM) percentage of time spent stationary in the Resource zone between virile crayfish (blue circle) and rusty crayfish (red square) across the control, low, medium, and high concentrations of predator odor. Asterisks indicate differences between species. Letters indicate significant differences.
Fig 12. Walking Speed in Resource Zone. Mean (± SEM) walking speed in the Resource zone between virile crayfish (blue circle) and rusty crayfish (red square) across the control, low, medium, and high concentrations of predator odor. Asterisks indicate differences between species. Letters indicate significant differences.
Fig 13. Time in Empty Zone. Mean (± SEM) percentage of time spent in the Empty zone between virile crayfish (blue circle) and rusty crayfish (red square) across the control, low, medium, and high concentrations of predator odor. Asterisks indicate differences between species. Letters indicate significant differences.
Fig 14. **Time Stopped in Empty Zone.** Mean (± SEM) percentage of time spent stationary in the Empty zone between virile crayfish (blue circle) and rusty crayfish (red square) across the control, low, medium, and high concentrations of predator odor. Asterisks indicate differences between species. Letters indicate significant differences.
**Fig 15. Two current flume.** The flume was divided into three areas: flowing condition, choice arena, and water flow. Letters indicate different parts of the flume. A. inputs where odor and clean river water entered. B. baffle plates. C. Wire and egg crating colimnators D. honeycomb colimnators. E. Choice area F. two shelters G. egg crating divider from choice to water outflow area H. outflow tubes
Fig 16. **Time in Odor Side.** LS means (± SE) the time spent in the side with the odor between virile crayfish (red circle) and rusty crayfish (black square) between the control and exposed treatments.
Fig 17. **Time in Blank side.** LS means (± SE) the time spent in the side with the river water between virile crayfish (red circle) and rusty crayfish (black square) between the control and exposed treatments.
Fig 18. **Time in Shelter on Odor Side.** LS means (± SE) the time spent in the shelter on the side with the odor between virile crayfish (red circle) and rusty crayfish (black square) between the control and exposed treatments.
Fig 19. Time in Shelter on Blank Side. LS means (± SE) the time spent in the shelter on side with the river water between virile crayfish (red circle) and rusty crayfish (black square) between the control and exposed treatments.
Fig 20. **Time Climbing on Odor Side.** LS means (± SE) the time spent climbing on the side with the odor between virile crayfish (red circle) and rusty crayfish (black square) between the control and exposed treatments.
Fig 21. **Time Climbing on Odor Side.** LS means (± SE) the time spent climbing in the side with the river water between virile crayfish (red circle) and rusty crayfish (black square) between the control and exposed treatments.
Fig 22. Time Tail Tucked on Odor Side. LS means (± SE) the time spent in the lower position in the side with the odor between virile crayfish (red circle) and rusty crayfish (black square) between the control and exposed treatments.
Fig 23. Time Tail Tucked on Blank Side. LS means (± SE) the time spent in the lower position on the side with the odor between virile crayfish (red circle) and rusty crayfish (black square) between the control and exposed treatments.
## APPENDIX B. TABLES

### Table 1. Statistics for predatory fish behavior.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Fixed Effects</th>
<th>Test Statistic</th>
<th>Degrees of Freedom</th>
<th>F Value</th>
<th>P-Value</th>
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</thead>
<tbody>
<tr>
<td><strong>Upstream</strong></td>
<td>Predator</td>
<td>SS = 0.2168</td>
<td>1</td>
<td>F = 2.449</td>
<td>p = 0.1255</td>
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<td></td>
<td>Flow</td>
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<td>F = 5.002</td>
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<td></td>
<td>Predator*Flow</td>
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<td>F = 6.777</td>
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<td>Predator</td>
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<td>F = 1.059</td>
<td>p = 0.3096</td>
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<tr>
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<td>Flow</td>
<td>SS = 0.0860</td>
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<td>F = 1.024</td>
<td>p = 0.3176</td>
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<td>Predator</td>
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<tr>
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<td>Flow</td>
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<td>F = 1.142</td>
<td>p = 0.2916</td>
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<td>Predator*Flow</td>
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<td>F = 0.529</td>
<td>p = 0.4715</td>
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<td><strong>West</strong></td>
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<td>F = 2.822</td>
<td>p = 0.1007</td>
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<td>Flow</td>
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<td>Predator*Flow</td>
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<td>p = 0.0587</td>
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<tr>
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<td>F = 0.029</td>
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<td>Predator*Flow</td>
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<td>F = 0.000</td>
<td>p = 0.9952</td>
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Table 2. Statistics for the interaction of predator treatment, flow environment, and crayfish location on crayfish behavior.

<table>
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<th>Predator Treatment<em>Flow</em>Crayfish Location</th>
<th>P-value</th>
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<td>Shelter</td>
<td>Active<em>No Flow</em>Upstream – Active<em>Flow</em>Upstream</td>
<td>p = 0.0321</td>
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<td></td>
<td>– Active<em>No Flow</em>Downstream</td>
<td>p = 0.1498</td>
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<td></td>
<td>– Sit-and-Wait<em>No Flow</em>Upstream</td>
<td>p = 0.5745</td>
</tr>
<tr>
<td></td>
<td>– No predator<em>No Flow</em>Upstream</td>
<td>p = 0.9503</td>
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<td></td>
<td>Active<em>No Flow</em>Downstream – Active<em>Flow</em>Downstream</td>
<td>p = 0.0003</td>
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<td></td>
<td>– Sit-and-Wait<em>No Flow</em>Downstream</td>
<td>p = 0.3197</td>
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<tr>
<td></td>
<td>– No predator<em>No Flow</em>Downstream</td>
<td>p = 0.0047</td>
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<td></td>
<td>Active<em>Flow</em>Upstream – Active<em>Flow</em>Downstream</td>
<td>p = 0.9919</td>
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<td>– Sit-and-Wait<em>Flow</em>Upstream</td>
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<td></td>
<td>– No predator<em>Flow</em>Upstream</td>
<td>p = 0.6627</td>
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<td></td>
<td>Active<em>Flow</em>Downstream – Sit-and-Wait<em>Flow</em>Downstream</td>
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<td></td>
<td>– No predator<em>Flow</em>Downstream</td>
<td>p = 0.1055</td>
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<td>Sit-and-Wait<em>No Flow</em>Upstream – Sit-and-Wait<em>Flow</em>Upstream</td>
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<td>– Sit-and-Wait<em>No Flow</em>Downstream</td>
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<td>– No predator<em>No Flow</em>Upstream</td>
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<td>Sit-and-Wait<em>No Flow</em>Downstream – Sit-and-Wait<em>Flow</em>Downstream</td>
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<td>– No predator<em>No Flow</em>Downstream</td>
<td>p = 0.0643</td>
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<td>Sit-and-Wait<em>Flow</em>Upstream – Sit-and-Wait<em>Flow</em>Downstream</td>
<td>p = 0.4075</td>
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<td>– No predator<em>Flow</em>Upstream</td>
<td>p = 0.0072</td>
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<td>Sit-and-Wait<em>Flow</em>Downstream – No predator<em>Flow</em>Downstream</td>
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<td>p = 0.1788</td>
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APPENDIX C. ICUAC LETTER OF APPROVAL

DATE: December 22, 2014

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

PROJECT TITLE: [687097-2] Behavioral mechanisms of rusty crayfish, (Orconectes rusticus) used to determine between predator and prey of channel catfish (Ictalurus punctutas)

IACUC REFERENCE #: 12-016
SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: December 17, 2014
EXPIRATION DATE: March 24, 2016
REVIEW TYPE: Full Committee Review

Thank you for your submission of Continuing Review/Progress Report 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.

This project requires Continuing Review 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.
DATE: March 26, 2015

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

PROJECT TITLE: [687097-4] Behavioral mechanisms of rusty crayfish, (Orconectes rusticus) used to determine between predator and prey of channel catfish (Ictalurus punctatus)

IACUC REFERENCE #: 12-016
SUBMISSION TYPE: Amendment/Modification

ACTION: APPROVED
APPROVAL DATE: March 26, 2015
EXPIRATION DATE: March 24, 2016
REVIEW TYPE: Designated Member Review

Thank you for your submission of Amendment/Modification 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.

The following modifications have been approved:

• Add multiple species of fish to the protocol. Two species will be chosen based on availability from commercial fish farms.

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.
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.