INTRODUCTION

Crayfish are keystone species in aquatic ecosystems because of their polytrophic ecological role (Phillips et al., 2009; Olden et al., 2011). Crayfish act as both primary consumers (Garvey et al., 1994; Hein et al., 2006) and prey to aquatic predators such as small mouth bass, Micropterus dolomieui (Stein and Magnuson, 1976; Stein, 1977). Many of these ecological decisions are mediated by chemical cues in their environment (Hazlett, 1994, 1999; Hazlett and Schoolmaster, 1998). In natural environments, crayfish may be simultaneously exposed to a multitude of chemical cues such as food odors, predator odors, alarm odors from conspecifics, and pheromones (Hazlett, 1994, 1999; Hazlett and Schoolmaster, 1998; Tomba et al., 2001). This complex sensory environment can be termed a sensory landscape (Wilson and Weissburg, 2013).

We define a sensory landscape as the spatial and temporal distribution of all of the stimuli within an organism’s environment. This includes both attractive (mates, food and shelters) and aversive (poisons, predators and competitors) stimuli, as well as sensory pollution and noise. The spatial and temporal structure of information contained within the sensory landscape is determined by the interaction of various stimuli and noise sources with the physical and chemical properties of the environment. For example, biotic structures (trees that provide shading and sound barriers) and flow of background media can influence how these sensory stimuli are distributed throughout the landscape in both space and time (Evenden et al., 2000; Abrahams et al., 2007; Fairhurst et al., 2013; Wilson and Weissburg, 2013).

In addition to movement and transmission of stimuli within a landscape, the motivations of the prey may alter the value of different stimuli (or aspects of the sensory landscape). During periods of predation pressure, darkened areas of a landscape may be valued by prey as a form of avoiding predators, whereas bright areas of the landscape may be used during visual foraging. Through adaptation, sensory systems and behavioral responses, organisms have evolved to operate within complex and often conflicting sensory landscapes (Moore and Crimaldi, 2004; Cappe et al., 2010).

The Umwelt, i.e., the totality of a sensory landscape, is constantly changing throughout space and time, e.g., light levels throughout a day, alterations in background sound levels, natural movements of prey and predators (Endler, 1987; Lehtiniemi, 2005). As stimulus sources change location or intensity, the environmental stimuli of the sensory landscape changes as well (Bytheway et al., 2013). Organisms, in these overlapping individual sensory landscapes, use the information extracted from each cue along with the cost and benefits of different actions to make behavioral decisions (Derby and
Crayfish could make context dependent cost-to-benefit analysis based on the sensory landscape present in their environment. Indeed, neural experiments and models demonstrate that the underlying architecture is present in order to make these types of calculations (Edwards, 1991). Thus, extracting the spatial location of different odor sources may provide the necessary risk and reward information for accurate cost-to-benefit analyses (Keller et al., 2001; Tomba et al., 2001). In addition, crayfish are capable of determining risk of different sensory sources and measuring this risk has been shown to be context dependent (Keller and Moore, 2000).

During predator-prey interactions, crayfish make decisions about the relative risk of predation compared to the relative reward of resource acquisition and often these decisions are made from information extracted from the dynamic sensory landscapes within natural habitats (Dill, 1987; Keller et al., 2001; Tomba et al., 2001; Corcoran et al., 2013). In order to investigate how prey animals make decisions about resource acquisition within dynamic sensory landscapes, we challenged crayfish, Orconectes virilis (Hagen, 1870) with three distinct landscapes that featured overlapping and conflicting sensory signals. These stimuli included two attractive signals (a visual and tactile resource (shelters), and chemically detected resource (food)), and an adverse chemical stimuli (alarm odor). We hypothesized that crayfish will make behavioral decisions based on a hierarchy of stimuli. Within this hierarchy, crayfish will base their spatial movement on alarm odor first, shelter second, and food third even if food and shelter stimuli are presented together.

**MATERIALS AND METHODS**

**Animal Collection and Holding**

Male and female *O. virilis* were collected from Maple Bay in Burt Lake in Cheboygan County, MI, USA (45.48°N, 84.70°W), during the summer of 2013. All 355 crayfish used in this study were in the non-reproductive form (Form II). Crayfish were housed in metal horse troughs and were provided detritus for food. Crayfish were used only once in the trials. The carapace width of all crayfish used in the experiment was measured and only crayfish with intact appendages were used in trials (average size 3.69 ± 0.03 cm). Crayfish were returned to separate metal horse troughs after trials were completed.

**Experimental Arena**

An experimental arena (122 cm × 122 cm × 41 cm L × W × H) was created using cinder blocks (30.5 cm in length) with a 4 mm polyethylene plastic sheathing. A 5 cm layer of pea gravel covered the bottom of the arena. A 1.3 cm thick wooden T structure (30.5 cm across and 62.2 cm down) was placed in the arena to create a Y-maze with two different arms (Fig. 1). Four Y-maze arenas were constructed side by side.

Unfiltered water taken from the Maple River was pumped into the troughs to provide fresh oxygenated water as well as detritus for food. Crayfish were used only once in the trials. The carapace length of all crayfish used in the experiment was measured and only crayfish with intact appendages were used in trials (average size 3.69 ± 0.03 cm). Crayfish were returned to separate metal horse troughs after trials were completed.

**Experimental Design**

All trials occurred at the Experimental Stream Research Facility of the University of Michigan Biological Station in Pellston, MI, USA. To investigate the habitat choices crayfish make in dangerous environments a fully 3 × 3 × 2 factorial experimental design was run with resource type, alarm odor location, and sex as the three factors. Resource type had three different conditions: shelters, food, and shelters and food combined. Alarm odor location consisted of three different conditions: no alarm odor, alarm odor in the habitat with resources, and alarm odor in the habitat without resources. The third factor was sex of the crayfish. Each combination of resource type, alarm odor location, and sex consisted of 10 replicates.

**Resource Types**

Shelters.—Shelters were constructed from 7.6 cm diameter PVC pipes cut in half and attached to a Plexiglas base with silicone. Two shelters were 11.4 cm long and the third shelter was 13.9 cm long. The first small shelter was placed at the end of the wooden divider and the second small shelter was placed 39.4 cm away from the first shelter, along the wooden divider. The larger shelter was placed across from the two smaller shelters, along the testing arena wall. Shelter use was based on Martin and Moore (2008).

Food.—Food consisted of squares (2.5 cm × 2.5 cm × 1.0 cm L × W × H) of pollock filets (Walmart® brand fish; Hazlett, 2003). Pollock fillets were placed within a mesh pouch (8.9 cm × 7.6 cm) containing a 3.8 cm × 5.1 cm tile to provide weight so that the pouches did not move or float. Food pouches were placed in the same set up as the shelters, as mentioned earlier.

![Fig. 1. Y-maze setup for decision making in changing sensory landscapes experiment. One arm would have resources, either solely shelters, solely food, or a combination of food and shelter (as seen in the figure) depending on the treatment. The other arm would not have any resources. Depending on the treatment, the alarm odor would either be in the same arm as the resources, in the arm without resources, or not present in the Y-maze.](image-url)
Fresh pieces of Pollock filets were used for each trial. The last food pouch was placed across from the two food pouches, along the testing arena wall.

Combination of Shelters and Food.—The combination trial set included one side with three food squares of Pollock filets and three shelters (two small and one large). The shelters were placed in the same set up as the shelters only trials. The three food pouches were placed directly across from the shelters (Fig. 1). Shelter construction and use were based on Martin and Moore (2008) and food use was based on Hazlett (2003).

Alarm Odor

In order to simulate a predation event, a single female crayfish had both chelae clipped off with a wire cutter (Hazlett, 2000). The focal and the clipped female crayfish were placed in a 40.6 cm long mesh bag in the arena to allow the crayfish being tested to sense alarm odors from an injured conspecific. For each trial including an alarm odor, a new female crayfish was used. Female crayfish (3.6 ± 0.1 cm carapace length) were used for all of the female as well as male trials so all tested crayfish were exposed to the same type of alarm odor.

Experimental Protocol

All trials were run from 09:00 to 17:00 and recorded using a DVR system. Shelters and food pouches were placed in one arm of the Y-maze arena prior to the beginning of the trial. Each experimental treatment had 5 trials with the resources in the left arm and five trials with the resources in the right arm. Before each trial, crayfish were randomly selected from holding tanks and marked with white out (Bic® White Out brand) to increase visibility for subsequent post trial video analysis.

Crayfish were placed at the downstream end of the Y-maze arena (neutral area) and allowed to acclimate for 15 minutes. Once the acclimation period finished, the crayfish was removed from the arena. An injured crayfish (described above) was placed in a 40.6 cm long mesh bag. The mesh bag with the injured crayfish was clipped onto the back part of wood divider with a plastic tarp clip and hung on either side of the upstream end Y-maze with the injured crayfish under the water.

Once the injured crayfish was placed into the arena, the tested crayfish was placed back into the beginning of the Y-maze by the outflow. The 15-minute trial began once the tested crayfish entered the arena for a second time. The tested crayfish was free to move about the arena and make a choice on which habitat to enter. Once the 15-minute trial was over, the crayfish was removed from the arena as well as the injured crayfish. There was one hour in between trials in order for all odors from previous trials to be flushed out.

Data Analysis

For all trials, videos were analyzed to calculate total amount of time in seconds that each crayfish spent in each section of the Y-maze testing arena (resource, non-resource, and neutral areas). Time was started when the carapace of the crayfish entered a section and time was stopped when the carapace of the crayfish left a section. Effect of predator location on average time spent in each section of the Y-maze, resource type, and sex were statistically analyzed using a mixed-design MANOVA because of the interdependency of the two arms of the Y-maze. This model had one within group (Y-maze arm) with three between group factors (sex, resource type, and alarm odor presence). If the initial analysis showed any significance, this test was followed with a Fisher-LSD to test for significant differences across variables (Statistica 9.0 StatSoft, Tulsa, OK, USA).

RESULTS

Overall Effects

The presence of an alarm odor altered crayfish behavior significantly ($F_{2.54,0.05} = 3.4, p < 0.05$) and there was a significant interaction between the alarm odor and resource type ($F_{4.63,0.05} = 3.2, p < 0.05$). There were no significant differences between sex, alarm odor treatment, sex and alarm odor treatment, sex and resource type, and sex, alarm odor treatment, and resource type.
without resources, crayfish spent significantly more time in the resource area with shelters compared to just food and a combination of resources (Fisher-LSD, $p < 0.05$). Crayfish spent $512.0 \pm 78.0$ s in the resource area with shelters, $334.0 \pm 79.6$ s in the resource area with food, and $351.5 \pm 77.5$ s in the resource area with a combination of resources.

Effects of Sex on Crayfish Choice.—When the alarm odor was in the resource area, there were no significant differences between the amount of time female and male crayfish spent in the resource area by resource types (Fisher-LSD, $p > 0.05$). Females spent $195.7 \pm 108.0$ s and males spent $326.2 \pm 136.1$ s in the resource area with shelters. Females spent $163.9 \pm 108.1$ s and males spent $149.6 \pm 93.73$ s in the resource area with food. Females spent $288.2 \pm 125.6$ s and males spent $111.3 \pm 62.3$ s in the resource area with a combination of resources.

Sex Differences on Time Spent in Different Areas of the Y-maze Testing Arena

Female crayfish spent significantly more time in the non-resource area compared to male crayfish (Fisher-LSD, $p < 0.05$; Fig. 4). Females spent $320.8 \pm 37.0$ s and males spent $250.5 \pm 35.8$ s in the non-resource area. There was no difference between the amount of time that female and male crayfish spent in the resource area (Fisher-LSD, $p > 0.05$). Females spent $275.2 \pm 37.0$ s and males spent $263.6 \pm 37.0$ s in the resource area. There was no difference between the amount of time that female and male crayfish spent in the neutral area of the Y-maze (Fisher-LSD, $p > 0.05$). Females spent $301.6 \pm 32.8$ s and males spent $386 \pm 37.8$ s in the neutral area.

DISCUSSION

The results from this study show that crayfish make decisions based on information obtained from their sensory landscape. In our study, the cost was the predation risk versus the benefit of a food and/or shelter, similar to Edwards (1991) model of crayfish behavior. Our results have shown that the perceived value of resources change as the sensory landscape changes. Crayfish modulated their use of shelter and food in response to the alarm odor. In the absence of an aversive signal (alarm odor), crayfish preferred food resources over shelter resources (Fig. 3). When the alarm cue was present, crayfish switched their behavioral preferences from a food resource to the shelter resource. Thus, the presence of an alarm odor (potential costs) is changing the cost-to-benefit analysis, which is context dependent. These results showed that crayfish choices and subsequent behaviors are dependent upon the sensory landscape present in the testing arena.

The mix of resource and alarm sensory cues within our experimental design challenged the crayfish to perform a multimodal (visual and chemical) analysis in order to make behavioral choices. In natural systems, the complex symphony of sensory signals can be thought of as a sensory landscape. Within this landscape, organisms need to extract meaning from the signals (danger from alarm signals, food from Pollock fillets) and extract some concept of spatial relationship of these different signals in order to make an effective decision. Previous research has shown that crayfish are capable of making cost-to-benefit analyses for behavioral decisions (Edwards, 1991; Liden et al., 2001). The results from our study suggest that crayfish modulate their resource use and spatial location based on a cost-to-benefit analysis using the information extracted from their sensory landscape. In most cases, that cost-to-benefit analysis results in organisms selecting habitats with increased protection from predation even if those habitats
Previous work has shown that the behavioral impact of alarm odors on crayfish depends upon the spatial location of other sensory stimuli in the test arena (Hazlett, 1994, 1999, 2000; Mitchell and Hazlett, 1996; Bouwma and Hazlett, 2001; Tomba et al., 2001). Virile crayfish reached food sources significantly less when an alarm odor was present with a food source compared to the food source alone (Tomba et al., 2001). Behavioral decisions in the face of complex sensory landscapes are driven by information extracted from not just an averse stimulus, such as predator odor or injured conspecific, but from different attractive stimuli, such as food shelters or mates, and their perceived value. The combination of both aversive and multiple attractive stimuli placed crayfish in a situation where the cost-to-benefit analysis includes context dependent assessment of resource value. This cost-to-benefit analysis that crayfish make was based on information on costs and information on benefits that changes as the information in sensory landscapes changes. Previous work has shown that risk-reward behavioral choices are context dependent in crayfish (Keller and Moore, 2001). The value crayfish placed on resources may change as the sensory landscape was altered, such as the values of shelters increasing over food when a predator is present. The changing values from the cost to benefit analyses in the sensory landscape drove the behavioral decisions of crayfish.

We suggest using a sensory landscape concept in future work, because crayfish use the entire umwelt of stimuli to guide their decision on habitat and resource use. In natural systems, organisms are faced with a host of sensory stimuli containing information about a number of different ecological decisions (Moore and Crimaldi, 2004). In addition, natural habitats have visual signals that contain information about shelters, predators, and other ecologically important resources (Hughes, 1996; Murphy, 2006). The sensory landscape of odors (Vickers, 2000; Weissburg, 2011) and visual signals (Endler, 1987) is constantly changing in space and time. To begin to predict the behavioral decisions of crayfish and other organisms based on cost to benefit analyses, the entirety of the stimuli present within natural systems must be accounted for. Not only does the interaction between predator and prey change the layering of these landscapes, but other factors such as resources, conspecifics, and heterospecifics also affect the landscape.

Knowing the sensory landscape of organisms is important when studying decision making within predator-prey relationship. By recognizing the sensory landscapes, researchers will be able to understand how the environmental stimuli are presenting information to the organism. The information within the environmental stimuli drives the decision making of organisms. Just as animals need to gather the best set of information in order to make the best set of decisions, researchers need to understand how the sensory environment relays information to the organism in order to make the most accurate predictions on animal decision making in behavior.

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REFERENCES


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