Landscapes of fear and safety: the integration of two different sensory landscapes determines behavioral responses in the crayfish *Faxonius rusticus* and is mediated by chemical cues

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Abstract

Research into predator–prey interactions has focused on the landscape of fear and nonconsumptive effects that result from prey responses. Prey behavior is influenced by predator presence and the location and quality of foraging resources in habitats. These areas have been fruitful, but the role of prey refuges has lagged. We investigated how refuge spatial distribution and quality influence prey behavior. To determine the role of the landscape of safety (LOS) in prey decision-making, we altered spatial relationships between refuges, refuge quality, and predation threats in mesocosms. Mesocosms were constructed such that prey only received predatory chemical cues. We employed a behavioral assay including largemouth bass (*Micropterus salmoides* (Lacepède, 1802): predator) and virile crayfish (*Faxonius rusticus* (Girard, 1852): prey). Crayfish shelter use was significantly influenced by quality and spatial relationship of shelters to predatory threats, and the interaction of these two factors. Particularly, crayfish used high-quality shelters more often when located closer to predatory cues than farther away and did not use low-quality shelters more than controls. High-quality shelter usage decreased as threat level (measured by gape ratio) decreased. These results support the idea that prey utilize an LOS, and information contained in these two landscapes may alter behavioral decisions.

Key words: rusty crayfish (*Faxonius rusticus*), largemouth bass (*Micropterus salmoides*), predator–prey interactions, landscape of fear, refuges

Introduction

Predator–prey ecology has been broadly categorized into two distinctive categories: consumptive and nonconsumptive effects (Lima 1998). Consumptive effects arise when a prey animal is directly consumed by a predator, while nonconsumptive effects describe the changes (i.e., behavioral or physiological) an animal may exhibit simply because of a predator’s presence (Lima and Dill 1990). Certainly, consumptive effects directly alter prey abundances and are important factors to these interactions (Matassa and Trussell 2011). However, nonconsumptive effects may more strongly influence these dynamics (Preisser et al. 2005; Matassa and Trussell 2011) and remain crucial pieces to predator–prey ecology. Nonconsumptive effects have been shown to alter prey morphology, behavior, and even their physiology despite the lack of direct interaction with predators. From the large body of these results, the theory of landscape of fear (LOF) arose (Gaynor et al. 2019).

The LOF theory states that prey make decisions based off of predatory stimuli present within the sensory landscape (Lima and Dill 1990; Laundré et al. 2001, 2010). The LOF also sheds light onto how animals use their habitat in the presence of predators (i.e., increased shelter use, movement to a different altitude, etc.). This theory arose from the classic studies of wolves (*Canis lupus* Linnaeus, 1758) and elk (*Cervus elaphus* Linnaeus, 1758) in Yellowstone National Park where elk foraging habits and movement patterns were altered in wolf presence but has also been observed in a broad group of prey items (Schmitz et al. 1997; Laundré et al. 2001; Ripple and Beschta 2004). The nonconsumptive effects that arise as a result of the LOF have been shown to have cascading effects for different trophic levels (Brown et al. 1999; Haggerty et al. 2018). Beyond the changes in prey behavior, trophic effects and changes in ecological processes have been seen in nutrient cycling and effects have been seen for generations beyond the initial predator–prey interactions (Ngai and Srivastava 2006).

For the last several decades, the LOF has been used to describe relationships between predators and prey as well as the many physiological, evolutionary, and behavioral changes
that ensue due to these interactions (Holopainen et al. 1997; Chivers et al. 2008). It is well documented that prey animals make decisions about habitat usage based around the level of threat they are experiencing (Werner et al. 1983; Bonnot et al. 2013). For instance, snowshoe hares (Lepus americanus Erxleben, 1777) are known to alter foraging behaviors due to the risk of predation by lynx (Lynx canadensis Kerr, 1792) (Hik 1995). Behavioral shifts in prey may induce large-scale changes to communities and community dynamics by altering plant assemblages, energy flow through systems, and even nutrient cycling (Evans 1984; Schmitz et al. 2010). However, until recently, less attention has been given to the landscape of safety (LOS), which is centered around the idea that organisms may be fleeing to safety in the form of shelters, burrows, or dens as opposed to moving away from threat. Some work even suggests that the use of shelters and burrows in the presence of predation threats may lead to energy deficits in prey. Here, the idea is that the lost foraging time and the energy lost during this time may be a larger cost to pay in the long run than seeking refuge (Peckarsky et al. 1993; Orrock et al. 2013). The details of when the costs of seeking refuge outweigh the costs of continued foraging are complex and depend upon the type and degree of threat, resource distribution, resource quality, and prey physiology (Downes 2001; Brown and Kotler 2004; Finstad et al. 2007). This leads to an interesting question of what factors prey use to make decisions on which behaviors (social interactions and refuge use) to enact under different levels of threat.

Similarly to the LOF, where animals utilize the spatial distribution of predatory stimuli to make decisions, animals may be using some aspect of spatial maps associated with refuges to create an LOS (Kramer and Bonefants 1997; Lutt beg et al. 2020). In this way, organisms may be provided with the spatial aspects of their environment as it pertains to shelters and possible refuges (MacKay et al. 2021). The LOS may provide increased fitness to organisms, as they are able to pass on genetic information to offspring, and, more broadly, balance to ecosystem functioning as prey populations will be less likely to fluctuate and cause imbalances (Sih and McCarthy 2002). If prey have access to the LOS, comparing the LOF and LOS to gauge costs and benefits of foraging to refuge seeking may be possible.

Recent work has shown that prey animals are able to utilize the LOS to seek refuges in threatening situations (MacKay et al. 2021). The ability to successfully locate and utilize a refuge when predators are present allows for profitable evasion tactics by prey species (Kauffman et al. 2007). However, prey usage of shelters within the LOS may be altered due to the spatial relationship between the source of threat (predator) and the refuge as well as the quality of the shelter. From a time and energy perspective, behavioral budgets are a zero-sum game (Gallagher et al. 2017). Time spent in a refuge being protected from predation is measured against less time spent foraging, competing for mates, and expanding territories (Bleicher and Dickman 2020). Even movement from foraging resources and shelters can be considered energetically costly and indicates that prey may need information about the spatial distribution of predators, refuges, and foraging resources.

Given the interaction of these external factors in prey decision-making, the spatial distribution (or sensory landscapes) of important sources of information needs to be investigated in more detail. For instance, a high-quality shelter that is situated near the aversive stimuli may become less attractive to prey than a shelter of medium quality or low quality located farther away from the threat. In the same way that giving up densities plays critical roles in the decision-making paradigm and determination of an organism’s patch use, information on the quality and spatial distribution of refuges may also be important in how prey use these refuges (Brooker et al. 2013). However, there is a gap in our current understanding of how the interaction between refuge quality and spatial location relative to predation threats is used by prey to make decisions within the LOF. Thus, the work presented here sought to uncover how prey animals make decisions about shelter use when shelter quality as well as spatial aspects involving predator and shelter location are modulated.

**Materials and methods**

**Experimental design**

To determine how both the quality of a shelter and its location relative to a potential predator impacted crayfish behavior, we created a 4 × 2 design experiment. The first factor consisted of shelter quality. Two different PVC half pipes were used as shelters, and two different shelters were constructed. The first shelter had a single opening (high quality) and the second shelter had four openings (low quality). The definition of shelter quality is based on previous shelter choice experiments where shelters with less openings (less entry points for predators) are more attractive than shelters with multiple openings (multiple entry points for predators) (Martin and Moore 2008; Chibucos et al. 2015). The second factor was shelter location. Every trial utilized both shelters, but in one case, the low-quality shelter was placed upstream (hence closest to the predator and termed low close) and the other condition used the high-quality shelter closer to the predator (termed high close). The third factor was the presence or absence of a potential threat, in this case, a predator. Large-mouth bass were chosen as the predator in these instances because these predators and crayfish co-occur in the surrounding habitats and have been shown to be effective predators on crayfish (Wood and Moore 2019; Wood and Moore 2020). In line with this, largemouth bass are also gape-limited predators, meaning they will not consume any prey item larger than the gape of their mouth. Because of this, larger bass (which have larger gapes) generally present a higher level of threat than smaller bass. When comparing the gape of the bass to the carapace width of each crayfish, we calculated the gape ratio. Thus, each trial with bass present had a gape ratio calculated. Finally, the fourth factor was the social status of the crayfish. Crayfish naturally engage in agonistic interactions and through these interactions form a dominance relationship. With two crayfish presence in each trial, one
crayfish will become dominant and the second one will be subordinate. The determination of social status is explained below.

A total of 57 trials were run with two unique crayfish for each trial. Trials (Ns) were divided among the treatments in the following manner. Trials were only removed from analysis if the crayfish escaped from the mesocosm during the experiment.

<table>
<thead>
<tr>
<th>Spatial quality treatment</th>
<th>Predator treatment</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low close</td>
<td>Bass present</td>
<td>11</td>
</tr>
<tr>
<td>Low close</td>
<td>Bass absent</td>
<td>17</td>
</tr>
<tr>
<td>High close</td>
<td>Bass present</td>
<td>11</td>
</tr>
<tr>
<td>High close</td>
<td>Bass absent</td>
<td>18</td>
</tr>
</tbody>
</table>

The Ns represented here are trials and not crayfish. Each system had two crayfish, and this was controlled for in the statistical analysis by using a mixed model. Crayfish escaped more often when bass were present in the upstream arena, but the number of escaping crayfish when bass were present compared with the control conditions was not significant (Chi squared = 1.45, p = 0.22). The field season ended before it was possible to raise the Ns of the two different bass-present trials to the levels of the controls.

Collection and housing of animals

A total of 114 form II (nonreproductive) female rusticus crayfish (*Faxonius rusticus*) (carapace width = $1.23 \pm 0.01$ cm (mean ± SEM)) were collected from Maple Bay, Burt Lake in Cheboygan County, Michigan, USA (45.4873°N, 84.7065°W) using modified hand nets. All crayfish used in the experiments had intact appendages and walking legs. A flow-through steel cattle trough (200 cm $\times$ 60 cm $\times$ 60 cm: $l \times w \times d$) was used as a communal holding tank for all crayfish. The communal tank was fed with water from the East Branch of the Maple River (45.5280°N, −84.7738°W) delivered through a PVC pipe (7.6 cm diameter) covered with nylon stockings to filter out macroinvertebrates and excess detritus. A standpipe at the far end (from the water entry) of the tank kept water depth at approximately 60 cm. Netting covered each trough to ensure fish would not escape and PVC shelters (7.6 cm diameter) were provided for housing. Fish were fed a daily diet of commercial fish food pellets (Sportsman’s Choice® Trophy Fish Feed High-Protein Multispecies Fish Formula). All tanks were kept under natural daylight/darkness regime for the entirety of the experiment. Upon completion of trials, all bass were euthanized following approved IACUC protocol methods (see below).

**Ethical approval**

Largemouth bass were maintained and handled following established animal care and use procedures; the use of vertebrate animals was approved by the Institutional Care and Use Committee at University of Michigan (Protocol: PRO00008892) and by the Institutional Care and Use Committee at Bowling Green State University (Protocol: 1411240-6).

**Experimental mesocosms**

Four flow-through stream mesocosms (162.56 cm $\times$ 121.92 cm $\times$ 40.64 cm: $l \times w \times d$) were constructed using cinderblocks lined with 4-mil black polyethylene sheeting (Fig. 1). Water was delivered to each of the mesocosms by a 208 L plastic drum that served as a constant head tank. The constant head tank was fed with unfiltered water from the East Branch of the Maple River and the same water was used to house the crayfish and bass. Water entered the drums via 7.6 cm PVC pipes that were fitted with nylon stockings to filter out macroinvertebrates. Water was fed from the drum to mesocosms through two garden hoses per mesocosm for a...
total of eight hoses on a single drum. The garden hoses had diameters of 1.9 cm (flow rate = 0.1 ± 0.05 L/s (mean ± SEM)). Largemouth bass are present within the Maple River, but previous work has shown that using unfiltered and unaltered Maple River water in control situations is applicable as a control. The concentration of predatory cues as altered in the experimental setup results in a significant change in antipredatory behavior (Beattie and Moore 2018).

Each mesocosm was composed of two identically sized sections: a predator arena and a prey arena. Predator arenas were located upstream of the prey arena and were covered with mesh sheeting to prevent bass escape. The predator and prey arenas of all mesocosms were each measured at 81.28 cm × 60.96 cm × 40.64 cm (l × w × d). The predator section had no substrate, while the prey section was lined with a sand substrate to a depth of 7.6 cm. This same construction technique has been used successfully in previous predator–prey experiments (Wood et al. 2018; Wagner and Moore 2022). Water flowed into the upstream predator section of each mesocosm before overflowing through a screened opening (28 cm × 12 cm opening with 1 mm × 1 mm screening) in a partial wall into the downstream prey section. The wall served to separate the prey from the predator and removed any visual or mechanical signals (Wood and Moore 2020).

A wooden frame sat on top of the mesocosms and held an infrared DVR camera (Zosi ZR08ZN10) 1.1 m above the water surface of each mesocosm to record crayfish nocturnal behaviors. Each camera was set at a frame rate of 30 fps, which is high enough to capture crayfish movement (Moore et al. 2021). A single low-intensity red light bulb (Great Value brand: Model A19045 LED Lamp, 9 W, 145 mA, 120 V, 60 Hz, RED) was used to illuminate two of the mesocosms that allowed analysis of the crayfish behavior. The presence of red light does not impact crayfish behavior (Cronin and Goldsmith 1982; Bruski and Dunham 1987).

Shelter resources

Two different types of shelters were constructed to be placed in the prey section of the mesocosms. Since crayfish prefer shelters with fewer openings (Martin and Moore 2008; Chibucos et al. 2015), low-quality and high-quality shelters were constructed and used in each experiment. The high-quality shelter consisted of a single PVC half-pipe shelter (10 cm × 8.5 cm × 4 cm: l × w × h) with one opening. The low-quality shelter was a single PVC half-pipe cross shelter (10 cm × 8.5 cm × 4 cm: l × w × h) that consisted of four openings at 90° angles to each other. Shelters were painted black and marked on top with WiteOut® to improve visibility during behavioral trials.

Experimental protocol

The experimental design was a fully factorial design with two factors and each factor having two conditions producing a 2 × 2 design. The first factor was predator presence, which consisted of two conditions: presence or absence of a single bass placed in the upstream arena. The second factor was the spatial arrangement of the shelter types. In one condition, the high-quality shelter was closer to the predator arena with the low-quality shelter downstream. The second condition switched the shelter placement leaving the low-quality shelter closer to the predator arena and the high-quality shelter downstream. In addition, by selecting different sized crayfish and bass we were able to alter the gape ratio across all of the trials. Here, we define gape ratio as the gape width of the bass (in cm) divided by the width of the carapace of the crayfish (in cm; Wood and Moore 2019). Bass are gape-limited predators meaning that something larger than the gape of the fish cannot be preyed upon. In our gape ratio, as the ratio increases, bass can more easily shallow and prey upon crayfish. As gape ratio diminishes, crayfish should be free from the threat of predation. Previous work has shown that gape ratios around 0.8 and above mean that bass can consume the prey (Wood and Moore 2019).

All trials were run for a period of 23 h beginning on 20 June 2022 and ending on 1 August 2022. Trial cycles began at approximately 0900 when the previous night’s setup was stopped, and the bass and crayfish were removed. To reset the next set of mesocosms, bass were selected from one of the three holding tanks and then measured (total length) on a fish measuring board to the nearest 0.1 mm. Once measured, the bass were then randomly placed into one of the four upstream predator arenas. Next, size-matched (within 10% of carapace length) crayfish were selected from the holding tank. Size matched individuals were used to ensure that there was not an initial competitive imbalance between the two crayfish. Animals of similar sizes (within 10% of carapace length) are considered to be equally matched (Moore 2007). Upon selection, crayfish carapace length and width were recorded to the nearest 0.1 mm with calipers before the pair of crayfish were placed into each downstream prey arena. Previous work has focused on the use of shelters and resources with single crayfish under various predation threats (Wood et al. 2018; Wood and Moore 2019, 2020; Wagner and Moore 2022). Yet, in natural systems, crayfish occur in high densities and have social hierarchies that control access to resources (Capelli and Munjal 1982; Figler et al. 1999; Klar and Crowley 2012), so we wanted to expand our work by using multiple crayfish to add social interactions into predator–prey interactions. Crayfish were marked with a white patch on their carapace before each trial using a nontoxic correction pen (BIC® WiteOut® 2-in-1 correction fluid) to improve visibility in behavioral video assessments. The behavior of crayfish is not altered by the presence of WiteOut® application (Fero and Moore 2008; Martin and Moore 2008; Jurcak and Moore 2018). Shelters were rotated in their spatial array and reset into a mesocosm.

At 2300 that night, an automatic light timer activated the red lights positioned above the mesocosms and 30 min later, the cameras above each mesocosm began recording the nocturnal behaviors of the crayfish. The cameras shut down at 0323 when behavioral recordings were complete. Previous work has shown that this time frame provides enough data on behavioral choices and social interactions (Jackson and Moore 2019). In addition, this time frame has been shown to be sufficient when observing the nocturnal foraging and shelter use behaviors of crayfish (Wood and Moore 2019, 2020) and provides a good balance between data collection, data...
analysis efforts, and data storage. Trials commenced on the following day at approximately 0800 with crayfish removal from the system. Thus, trials were considered to have ended when crayfish were removed.

Data analysis

Videos were retrieved from the DVR and analyzed for behavioral time budgets by a single observer to keep consistency of behavioral definitions across treatments and trials. Two separate behaviors were recorded for the crayfish: shelter use and fights. Other behaviors such as exploring, climbing walls, and stationary waiting were not included in the analysis. We chose to include only those behaviors that are involved in resource use or social behavior. A total of 415 discrete sets of behaviors occurred across all treatments with 241 of those behaviors being shelter use and 174 being fights. Shelter use was defined as the crayfish either on top of or inside of the shelter. At least 50% of the carapace had to be inside of the shelter for the crayfish to be considered inside of a shelter. Several variables were recorded and consisted of when a crayfish exhibited shelter use, the crayfish that was using the shelter, the shelter being used (high- or low-quality), the shelter’s location (close or far), and the length of time of shelter used. A fight was defined as an agonistic interaction that occurred beyond two crayfish touching chelae (crayfish may exhibit this behavior in a more explorative rather than an agonistic manner) or walking over or past each other (Wofford et al. 2015). When two crayfish exhibited agonistic behavior, the length and maximum intensity of the interaction were recorded along with the winner of the interaction. The fighting behavior of the crayfish was examined for the maximum intensity reached for agonistic interactions. Maximum intensity was determined using an 11-point ethogram (Jackson and Moore 2019). Winning an interaction was defined by the losing crayfish’s behavior: if a crayfish retreated for 3 s or tail flipped away, the opposing crayfish was considered the winner.

Statistical analysis

R was used for all data analysis (R Core Team 2022). Data conditioning followed those steps that are performed before running a mixed model analysis (Zuur et al. 2009). Cleveland dot charts were used to determine whether the data contained any outliers and there were none, so, all of the behavioral data were included in the analysis. The second step included the production of histograms, qqplots, and normality tests (Shapiro–Wilk) of the behavioral variables for length of shelter use, length of agonistic interactions, and maximum intensity of fights. If the data were not normally distributed, the R function “BestNormalized” was used to select the transformation of the data and the transformed data were rechecked for normality (Peterson 2021). The time spent in a shelter by crayfish, the length of agonistic interactions, and maximum intensity were not normally distributed, and time in shelter and length of interactions were transformed using an arcsine transformation, while maximum intensity was transformed using an orderNorm transformation. The different transformations were chosen to produce normality in the different data sets. After transformation, data were normally distributed. Finally, to check for collinearity within both the behavioral variables, independent regressions were performed between the dependent (behavioral) variables. None of the behavioral measures showed any significant correlations.

Because multiple behavioral measures occurred within single trials and to control for possible mesocosm effects, all statistical models were performed using generalized linear mixed models (Zuur et al. 2009). All models run in R used the lmer function from the lme4 package (Bates et al. 2015; R Core Team 2022). Following model construction, the outputs were extracted using the anova function from the car package (Fox and Weisburg 2019). For the models, the behavioral measure served as the dependent variable and the shelter quality (high or low), spatial orientation (close or far), bass (presence or absence), and occupant status (dominant or subordinate) were used as the independent measures. Gape ratio was not placed in the original models because the control trials did not have any bass present and thus did not have a gape ratio. If the initial models showed a significant effect due to the presence of bass, the data were subset to include only those trials where bass were present. Subsequently, mixed models were created using shelter quality, spatial orientation, and gape ratio (the average gape ratio of both crayfish was used) as the independent measures. Post hoc analyses of comparisons were carried out on categorical variables (shelter quality, spatial orientation, bass presence, and occupant status) using the “emmeans” function (Lenth 2022). The statistical effect of each model was determined by extracting Cohen’s D (Cohen 1988) using the effectsize package (Ben-Shachar et al. 2020). Cohen’s D is a statistical measure that indicates the strength of the apparent relationship indicated by the statistical analysis and can be used to interpret the importance of statistical significance. Values for Cohen’s D can generally be interpreted as small (<0.2), medium (0.5), or large (0.8).

Results

Shelter use overall model

Crayfish spent significantly greater time in the high-quality shelter when it was located closest to the bass arena when bass were present (Table 1; Fig. 2). The linear mixed model showed that the amount of time spent in the shelter was dependent upon the interaction of all four categorical variables (shelter quality, shelter location, bass presence, and occupant status). Dominant crayfish spent the highest amount of time in the high-quality shelter when it was located closest to the bass. The amount of time spent in the shelter under this condition was significantly different than all other shelter, location, bass presence, and occupant status conditions (emmeans post hoc analysis, p < 0.001 for all comparisons except for the comparison of the high-quality shelter located close to the bass used by subordinate crayfish). The amount of time spent in the high-quality shelter when it was upstream was 6-fold greater than any other condition regardless of the social status of the crayfish using the shelter.
Shelter use as a function of gape ratio

Examining shelter use using gape ratio as a factor for only trials where bass were present produced significant interaction effects of spatial location, shelter quality, and gape ratio (Table 1). Linear regressions (Fig. 3) showed that shelter use increased as gape ratio increased when the high-quality shelter was in the close position and the low-quality shelter was in the far position. There were no significant effects of the spatial arrangement shelter was in the farthest location. All other regressions were not significant (Table 2).

Fig. 2. Mean (±SEM) of the time that crayfish (*Faxonius rusticus*) spent in a shelter during trials with bass (*Micropterus salmoides*) present (A, top graph) and during controls when bass are absent (B, bottom graph). Trials are grouped based on whether it was the dominant crayfish (tan bars with crosshatch) or subordinate (solid green bars). The shelter quality and location are along the x-axis. Bars with different capital letters indicate a significant difference using a mixed model (statistical values in text) followed by using an emmeans post hoc test.

Fig. 3. Linear regressions of shelter use as a function of the gape ratio between crayfish (*Faxonius rusticus*) and predatory bass (*Micropterus salmoides*) when bass were present. Graph displays best-fit line and 95% confidence interval (shading) of treatments where the high-quality shelter was close (black squares) or far (blue triangles) and when the low-quality shelter was close (green upside down triangles) or far (red circles). A linear mixed effects model produced a significant interaction between shelter quality (high or low), spatial position (close or far), and the gape ratio ($p = 0.024$, Table 1).

**Table 1.** Model results using a linear mixed model to analyze shelter use (only significant results are displayed).

<table>
<thead>
<tr>
<th>Model results</th>
<th>$F$</th>
<th>$p$</th>
<th>Cohen’s D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelter quality × bass × shelter location × occupant status</td>
<td>$F_{1,232,0.05} = 8.57$</td>
<td>$p &lt; 0.006375$</td>
<td>1.66</td>
</tr>
<tr>
<td>Subset to include only bass presence trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupant status × shelter location × gape ratio</td>
<td>$F_{1,218,0.05} = 5.7$</td>
<td>$p = 0.018$</td>
<td>0.75</td>
</tr>
<tr>
<td>Shelter quality × gape ratio</td>
<td>$F_{1,207,0.05} = 19.7$</td>
<td>$p &lt; 0.0001$</td>
<td>0.83</td>
</tr>
</tbody>
</table>

**Table 2.** Regression fits showing shelter use as a function of the gape ratio between crayfish and bass.

<table>
<thead>
<tr>
<th>Model</th>
<th>Slope</th>
<th>$r^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-quality shelter in the close position</td>
<td>−718.45</td>
<td>0.015</td>
<td>0.22</td>
</tr>
<tr>
<td>Low-quality shelter in the far position</td>
<td>251.9</td>
<td>0.014</td>
<td>0.76</td>
</tr>
<tr>
<td>High-quality shelter in the far position</td>
<td>398</td>
<td>0.17</td>
<td>0.038</td>
</tr>
<tr>
<td>Low-quality shelter in the close position</td>
<td>−99</td>
<td>0.018</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Fight length overall model

Crayfish only altered their fights when bass were present in the upstream arenas (Table 3; Fig. 4) although the effect size was quite small ($D = 0.17$). When bass were present in the upstream arena, fights lasted $6.9 \pm 0.6$ s (mean ± SEM) and when bass were absent, fights were longer ($9.2 \pm 0.8$ s). There were no significant effects of the spatial arrangement.
Table 3. Model results using a linear mixed model to analyze fight length use (only significant results are displayed).

<table>
<thead>
<tr>
<th>Model results</th>
<th>F</th>
<th>p</th>
<th>Cohen’s D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bass</td>
<td>$F_{[1,38,0.05]} = 5.2$</td>
<td>$p = 0.028$</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Fig. 4. Mean (±SEM) of the total length of time the crayfish (*Faxonius rusticus*) pair spent fighting within a trial. A one-way significant effect showed a significant difference between the predator present (left hand bar) and control (right hand bar) trials ($p = 0.028$). There were no differences in fight length as a result of the spatial arrangement of shelters. Asterisk indicates a significant difference using a mixed model (statistical values in text) followed by using an emmeans post hoc test.

Table 4. Model results using a linear mixed model to analyze maximum intensity of fights use (only significant results are displayed).

<table>
<thead>
<tr>
<th>Model results</th>
<th>F</th>
<th>p</th>
<th>Cohen’s D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bass</td>
<td>$F_{[1,170,0.05]} = 174$</td>
<td>$p &lt; 0.0001$</td>
<td>1.72</td>
</tr>
<tr>
<td>Bass × shelter location</td>
<td>$F_{[1,168,0.05]} = 6.7$</td>
<td>$p = 0.01$</td>
<td>0.56</td>
</tr>
</tbody>
</table>

of shelters on fight lengths ($p = 0.98$) or the interaction between bass presence and spatial arrangement ($p = 0.15$).

Fight length subset with gape ratio
There were no significant effects of gape ratio or its interactions with other variables on fight length.

Fight intensity overall model
The maximum intensity of the crayfish agonistic encounters was significantly decreased by the presence of bass in the upstream arena (Table 4; Fig. 5). Despite the significant interaction in the overall model, the post hoc test did not reveal any significant effects due to the interactions of the presence of bass and the spatial location of the different shelter qualities. The post hoc analysis of the one-way effects due to the presence of bass showed that the maximum fight intensity of encounters with bass decreased from 6.5 maximum intensity in the control treatments to 2.7 maximum intensity in the bass treatments (Fig. 4).

Fight intensity subset with gape ratio
There were no significant effects of gape ratio or its interactions with other variables on the maximum fight intensity.

Discussion
Crayfish antipredatory and aggressive behavior is influenced by the perception of both fear and safety cues within flow through mesocosms. Although previous work has shown that crayfish respond to both fear and safety cues (MacKay et al. 2021), the present findings add important details on how the LOS cues are used in conjunction with the LOF. Shelters perceived as high quality by crayfish are used more often in the presence of predatory cues, but only when that high-quality shelter is located closer to the source of the predatory cues (Fig. 2; Table 1). When this high-quality shelter is further away from the source of the predatory cues, the behavioral preference of this shelter disappears. A more detailed analysis shows that the use of the high-quality shelter decreases (independent of its location) as the gape ratio between the predatory bass and crayfish increases (Fig. 3; Table 1). Past work has shown that crayfish can accurately assess relative size relationships between themselves and bass using just chemosensory cues (Wood and Moore 2020). Since bass are gape-limited predators (Hambright 1991), an increase in the gape ratio is indicative of higher predatory threats as larger...
fish typically have larger gapes, and this could mean bass may more easily consume crayfish. In these studies, gape ratios ranged from 0.39 to 0.69. Previous work has shown that bass are able to reliably consume crayfish when gape ratios are 0.8 or lower; thus, all the predators could consume the prey in every trial (Hill et al. 2004). As gape ratio increased, the use of the high-quality shelter in the farthest position from the predatory cues increased, while the use of the high-quality shelter in the closest position decreased (Table 2). Thus, it appears as if crayfish are using the threat level of the predator (as indicated by the gape ratio), the quality of the shelter, and its spatial location in relation to the source of the predatory cues to make behavioral decisions on shelter use. In addition to shelter use, the length of aggressive interactions as well as their maximum intensity was reduced in the presence of predatory cues. While these differences may seem rather small to be detected by chemical senses, crayfish have shown the ability to detect small differences in concentrations of amino acids (Hatt 1989; Derby and Weissburg 2014).

In addition, crayfish have been shown to detect and use small changes in odor concentrations over time to orient to food sources (Moore et al. 2021). Thus, the suggestion that crayfish are measuring gape ratio as well as relative distances use chemical cues aligns with previous work. Fear or fearful responses can be viewed as one end of a continuum where safety may be placed at the other end (Bleicher and Dickman 2020). As such, an interesting question arises. As prey move away from areas of high threat, is that movement guided by decreasing fearful cues, increasing safety cues, or a combination of both? Often, the LOS is not considered in the theory of these decision-making models and in some cases, the decreasing gradient of fear aligns perfectly with an increasing gradient of safety. Thus, disambiguation of these two cues and the role that they play in prey decision-making remain elusive. While there has been some work on the role of safety cues in guiding prey behavior, most of that work has shown that threat or fearful cues are more important in prey decisions and safety cues may not play a role at all (Tolon et al. 2009; Bleicher 2017). Some studies even suggest that including safety cues in prey models may lead to less-than-optimal decision-making (Bleicher and Dickman 2020). The increased use of refuges in the presence of predatory threats may lead to decreased foraging, which then can lead to decreased growth and fecundity as well as other costs associated with fitness (Matassa and Trussell 2011; Teckentrup et al. 2018).

More recently, studies that carefully control for the spatial distribution of both predatory cues and safety cues have shown that information about the spatial distribution of safety cues is used by prey during threatening situations (Luttbeg et al. 2020). Under the threat conditions, prey are using cues about dens and burrows as predatory refuges (Smith et al. 2019). These results indicate that prey are potentially comparing the spatial distribution of predatory cues with the spatial distribution of safety cues/predatory refuges to make various decisions (Gaynor et al. 2019). The results from this current study support that same conclusion. Crayfish modulate their shelter use based on three distinct factors: shelter quality, shelter location relative to the predation threat, and the degree of threat (gape ratio). In this study, the presence of predatory cues increased shelter use and decreased the level of aggression. While these behavioral changes are not surprising, the increased shelter use is dependent on the quality of the shelter and the spatial location of that shelter relative to the spatial location of the threat. Combining the present findings with previous work (Mackay et al. 2021) indicates that crayfish are creating an LOS along with an LOF and possibly comparing these two landscapes before determining their behavioral response.

This conclusion does not mean that crayfish are capable of actually constructing and memorizing spatial maps. Many organisms navigate spatial landscapes without the ability to construct spatial maps (Van Hooser et al. 2005; Cheng 2012). Even if we constrain our view to predator–prey ecology as opposed to orientation or navigation, many organisms use the spatial distribution of resources and the quality of those resources to make foraging decisions (Stenberg and Persson 2005; Searle et al. 2007). Resource patchiness and giving-up densities of foraging resources are important elements of the sensory landscape that prey use to make decisions. Studies have shown that prey can compare the spatial distributions of food resources with the spatial distribution of predatory threats as two important sources of information (Searle et al. 2007, 2008; Iribarren and Kotler 2012). Since the LOS is likely readily available to organisms, incorporating concepts of safety, along with predators and food, into prey decision-making may lead to more predictive and robust models of prey behavior (Tolon et al. 2009; Matassa and Trussell 2011).

The use of refuges in the presence of predators is a complex decision that is influenced by food and refuge availability as well as its spatial distribution (Gaynor et al. 2019), the predator’s spatial distribution as well as their hunting mode (Schmidt and Kuiper 2015), and the mobility of the prey (Tolon et al. 2009). Recent work has called for the addition of refuge quality to be included among the factors that influence refuge use under predation threats (Hansson et al. 2016). In sessile organisms like barnacles, the refuge structure and food availability interact to form an assessment of refuge quality. In more mobile prey, these two factors are likely to be less conjoined and prey may have to access refuge quality and food availability on different decision axes. Within the crayfish system, studies appear to support the idea that the structural components of a refuge provide sufficient information for decision-making (Martin and Moore 2008; Mackay et al. 2021). Here, the spatial relationship of refuge and threat is an additional factor that prey are considering.

In reality, prey organisms have a number of different sensory landscapes available to them, including spatial and temporal distributions of food resources, competitors, potential mates, refuges, and of course, predators (Jordan and Ryan 2015; Gaynor et al. 2019). These landscapes vary across the different dimensions of the senses also as sensory stimuli are dispersed and move through environments at different spatial and temporal scales. Light stimuli are transmitted quickly in environments but dissipate as soon as the source of the signal moves (Denny 1993). Conversely, chemical cues move through environments rather slowly, but can remain in habitats for day or weeks (Moore and Crimaldi 2004). Thus, on top
of the information about the source of the stimuli (predator, refuge, food, mate) and the spatial distribution of these stimulus sources, the temporal nature of the landscape is an additional piece of information that prey have access to through sensory processing (Palmer et al. 2017).

The information from these three axes (source type and quality, spatial distribution, and temporal distribution) is likely integrated into how prey make decisions about resource use and movement patterns. A fuller understanding of prey decision-making in the presence of predatory threats needs to incorporate at least these three sources and potentially others. In the results presented here, the quality of refuges and their spatial distribution guide resource use in prey. Previous work has demonstrated that crayfish are able to fine-tune their antipredatory behavior based on the degree of threat by securing information about the gape ratio of predators (Wood and Moore 2019, 2020). Combining these studies shows that these prey consider the quality and location of refuges, the degree of threat (gape ratio), and the relative location of the threat to refuge. Searching for other sources of information that prey use to make antipredatory decisions will broaden predator–prey ecology beyond just the LOF.

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