A Comparative Study on the Escape Behaviors of Orthopterans in San Juan de Peñas Blancas, San Ramón, Costa Rica

Josie Starnes, Jared Salin, Crystal Myers, and Melanic Osegueda

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Abstract. A total of three trials were conducted to compare the escape behaviors of the various Orthopterans *Abracris flavolineata* (De Geer, 1773), *Orophus sp.* (Saussure, 1861), *Silvitettix sp.*, *Leptomerinthoprora brevipennis* (Rehn, 1905). Since many Orthopterans use visual stimuli and react in similar ways to threats, the selected trials tested different kinds of escape behaviors. The first trial testing an approaching vertical stimulus showed no significance in escape behavior between *A. flavolineata* and *Orophus sp.* Trial two with a horizontal stimulus also showed no significance, this time between all four species. When looking at solely the grasshopper species in a contingency test from trial two, there was indication that they behaved similarly. Trial three was held outdoors with an approaching downwards angled stimulus, also showing no significance between the four species. When comparing the indoor trials to the outdoor trials, the results were significant, indicative that the specimens reacted faster indoors. A large factor in the results showing insignificance can be traced to the lack of enough specimens to test.

Keywords: *Abracris flavolineata, Orophus sp., Silvitettix sp., Leptomerinthoprora brevipennis,* behavior

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Insects have compound eyes composed of many ommatidia, which are clusters of photoreceptor cells, thus allowing for a mosaic image to be perceived (Snyder 1979). Insect vision is most sensitive to changing patterns and movements. Knowing this, it came into question whether Orthopterans will always react the same as one another upon perceiving a predator/stimulus. Escape behaviors are fast and robust, which can allow for a good understanding of the neural basis of behavior (Card, 2012). In orthopterans, jumping and flight are a major means of escape, therefore it was interesting to assess the differences between species that are not cryptic versus those that are (Hochkirch, 2002). In prior studies with cryptic grasshoppers, it was found that they tended to escape to background that were seemingly more cryptic, thus remaining hidden (Eterovick, 1997). Other studies have revealed that there is an example of coevolution between behavior and morphology is that crypsis is associated with motionlessness (Ioannou, 2009). In a test to determine whether slow moving defense helped the survivorship of lubber grasshoppers, the slow-moving lubbers suffered less predation than those that reacted at a faster rate (Hatle, 1997). When grasshoppers were approached repeatedly in other experiments, their responses changed in terms of flight pattern, using different tactics such as camouflage or habitat usage (Bateman, 2014). When jumping, the legs are usually in sync, but can be asynchronous, propelled by one hind leg often (Burrows, 2010). This information led to an anticipation that there would be differences among various Orthopteran species in terms of reaction to stimuli.

Materials and Methods

Vertical stimulus.

Using 2 leftover cardboard boxes (19in x 19.5in x 16in.), a tall box was constructed. Alternating species between *Abracris flavolineata* and *Orophus sp.*, each was placed one at a time in the center of the box to habituate for five minutes. The species were kept in a collapsible insect cage manufactured by BioQuip (Rancho Dominguez, CA, USA) with 12 x 12 x 12-inch dimensions. Using a stimulus constructed out of a funnel, a cardboard circle, string, rocks, and tape (see Fig. 1) that were found in the Soltis Center in Costa Rica, the stimulus was lowered vertically down off a two-foot metal pole. Four microscope lights were zip tied to each corner so that the box was illuminated. The box was lined with markers one inch apart (see Fig. 2). The stimulus was lowered at a rate of three inches per second, monitored with a timer, until the Orthopterans showed an escape behavior, classified by walking or jumping away. The behavior was recorded along with the distance the stimulus was away from the specimens. A Shapiro-Wilk test for normality was conducted using an online generator from GraphPad Software (San Diego, California, USA), as well as a Mann-Whitney U test from GraphPad Software (San Diego, CA, USA).

Horizontal stimulus.

Using the same carboard box, a hole was cut in the side for entrance of a stimulus horizontally. Using the two-foot metal pole, a flat foam square cut out was attached with tape to act as a stimulus. Alternating between four specimens, *Abracris flavolineata*, *Orophus sp.*, *Silvitettix sp.*, and *Leptomerinthoprora brevipennis*, an individual was placed in one at a time and allowed to habituate for five minutes. The horizontal stimulus was moved toward the specimen at a constant rate until an escape behavior of walking or jumping occurred. The behavior and distance from stimulus to specimen was collected for each specimen. A Shapiro-Wilk test for normality was run from GraphPad Software (San Diego, CA, USA), and then a subsequent Kruskal-Wallis test was performed with GraphPad Software (San Diego, CA, USA). A contingency test was also run to test the behaviors of those insects that reacted with GraphPad Software (San Diego, CA, USA).

Field test.

Using the same horizontal stimulus, the specimens were transported outside in Falcon tubes manufactured by BioQuip (Rancho Dominguez, CA, USA), and their reactions were tested in the grass. The four insect species were tested one at a time and allowed to habituate in the grass for one minute each. The insect was approached head-on with the stimulus at a downward 45-degree angle until jump behavior occurred. The distance from the stimulus to the origin of the insect was recorded, along with the jump distance. A Shapiro-Wilk test for normality was run with GraphPad Software (San Diego, CA, USA), as well as a Kruskal-Wallis test with GraphPad Software (San Diego, CA, USA).

Comparison of indoor vs. outdoor trials.

Using data collected from prior trials, GraphPad Software (San Diego, CA, USA) was used to conduct the Shapiro-Wilk tes, as well as a Mann-Whitney U test.

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Figure 1 The initial vertical stimulus used in Trial 1.

Figure 2 Box lined with markers one inch apart

Figure 3 Horizontal stimulus

Results

Vertical Stimulus.

In this trial the two species *Abracris flavolineata* and *Orophus sp.* were observed and compared in terms of distance (inches) of the visual stimulus from the specimen until reaction. To generate statistics for this test, the Shapiro-Wilk test for normality was performed using an online generator from GraphPad Software (San Diego, California, USA) that followed the following equation.

$$W = \frac{\left(\sum_{i=1}^{n} a_i x_{(i)}\right)^2}{\sum_{i=1}^{n}(x_i - \overline{x})^2}$$

Subsequently, since the results were non-parametric, the Mann-Whitney U test was conducted using an online generator from GraphPad Software (San Diego, California, USA) to determine if there was a significant difference between the two means of *A. flaveolineata* and *Orophus sp.* The equation is seen to the right.

$$U_1 = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1$$

$$U_2 = n_1 n_2 + \frac{n_2(n_2+1)}{2} - R_2$$

This statistical analysis revealed that p=0.64552, with a standard deviation of 10.0386, therefore there was no significant difference between the two species.

The results from the experiment are as follows in Fig. 4.

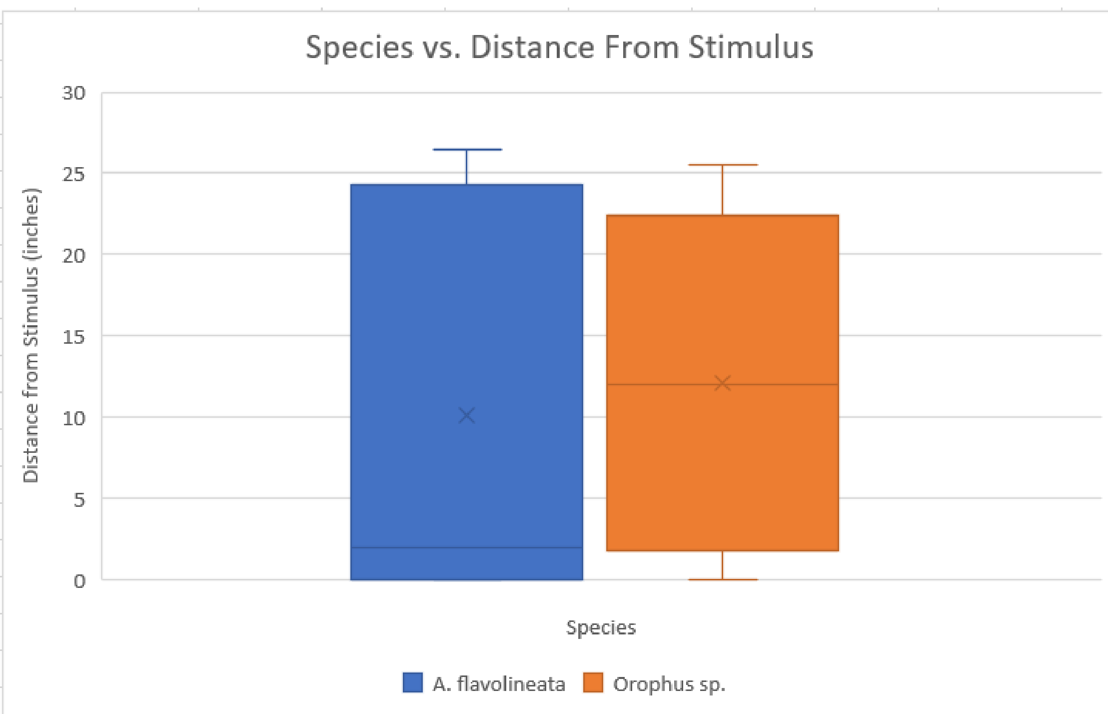


Fig. 4. *Results from Vertical Stimulus trial indicated that while A. flavolineata appeared to react while the stimulus was further away, there were no significant differences when the statistics were run.. n = 11, Mean=10, SD= 10.529, W=0.845*

Horizontal Stimulus.

Flightless *Silvitettix sp.* and *Leptomerinthoprora brevipennis* were introduced to the existing *A. flaveolineata* and *Orophus sp.* The species were compared in terms of distance (inches) from stimulus until reaction. The Shapiro-Wilk test for normality was used again, revealing that the data was non-parametric. Since there were more than two specimens being observed, the Kruskal-Wallis test was performed using an online generator by GraphPad (San Diego, California, USA) which used the following equation to determine whether the samples originate from the same distribution.

$$T = (N-1)\frac{\sum_{i=1}^{k} n_i(\bar{r}_i - \bar{r})^2}{\sum_{i=1}^{k}\sum_{j=1}^{n_i}(r_{ij} - \bar{r})^2}$$

The Kruskal-Wallis test determined that the p-value was 0.19207 with a standard deviation of 3.1283, therefore there was no significant difference among the species.

The results of the trial are as follows in Fig. 5.

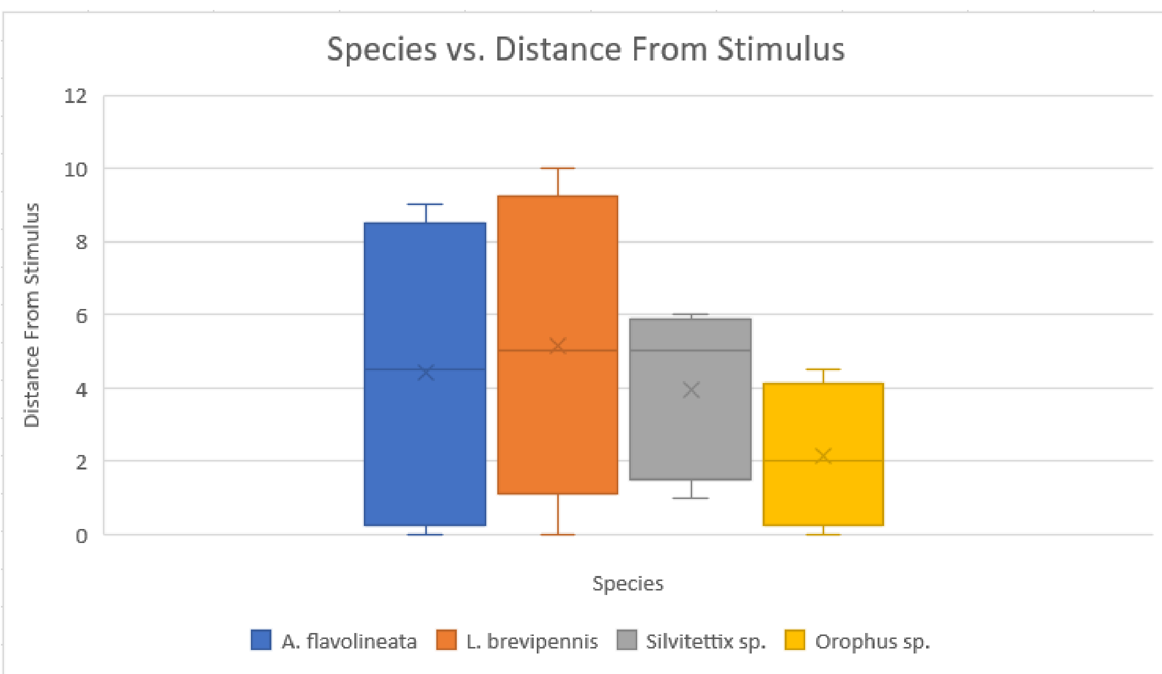


Fig. 5. Results for horizontal stimulus trial indicated that while the grasshopper species appeared to react while the stimulus was further away than the katydid, there was so significance found. n=29, Mean=4.22, SD= 3.18, W=0.924.

Comparing between species.

A contingency test was run using an online generator by GraphPad (San Diego, California, USA) to test the behaviors solely between those individuals that reacted.

Table 1. Number of species reacting during Trial 2.

	Jumped	Walked away
A. flaveolineata	8	1
L. brevipennis	5	4
***Silvitettix* sp.**	6	1
***Orophus* sp.**	1	3

The contingency test for those that reacted reflected a p value of 0.0732, which does not show significance between the behaviors. Among the grasshopper species the p value was 0.1979, meaning that they behaved similarly.

When doubling the numbers (keeping the same ratio), the p value became 0.003, which does show significance.

Field test.

To determine if the cryptic species reacted differently when outdoors, this trial was run comparing the distance (inches) from the stimulus before each species reacted. Using the Shapiro-Wilk test, the results were indicative to be non-parametric. The Kruskal-Wallis test was then performed. The p value was calculated to be 0.43987 while the standard deviation was 2.274. The results are as follows in Fig. 6.

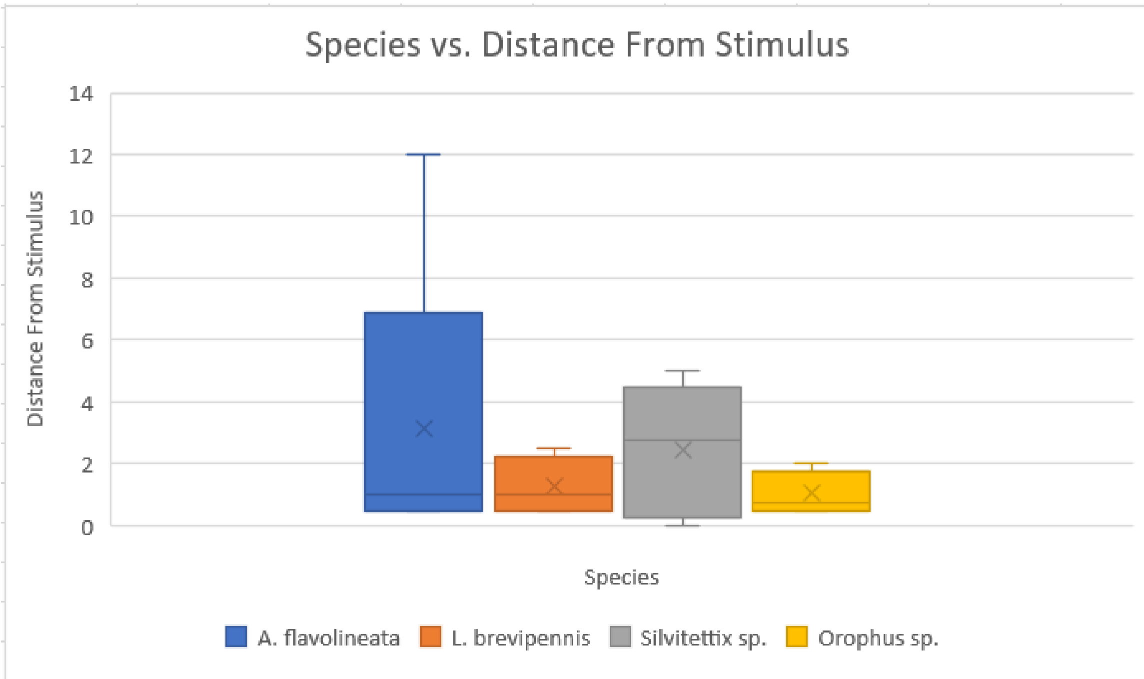


Fig. 6. *Field test results indicated that while the grasshopper species seemingly reacted while the stimulus was further away, there was no significance found between the species. n=31, Mean= 1.8387, SD= 2.274. W=0.63699.*

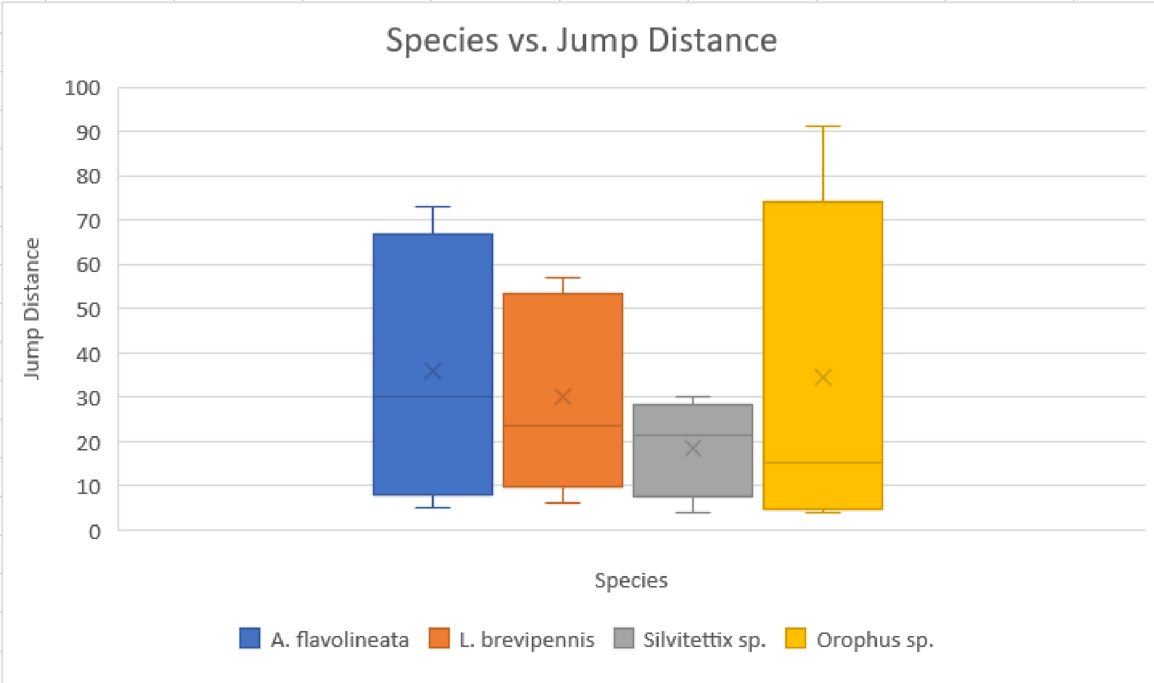


Fig. 7. *When comparing Jump distance vs. Species, while the katydids appeared to have the highest distance, there was no significance found in the statistical tests. SD=22.4127, p=0.51392.*

Comparison of trials

To determine if the species reacted differently indoors vs. outdoors, this test was run. The results were non-parametric, per the Shapiro-Wilks test. The Mann-Whitney test revealed p=0.00398, which reflected significance. The species reacted faster indoors. The SD for each species is as follows:

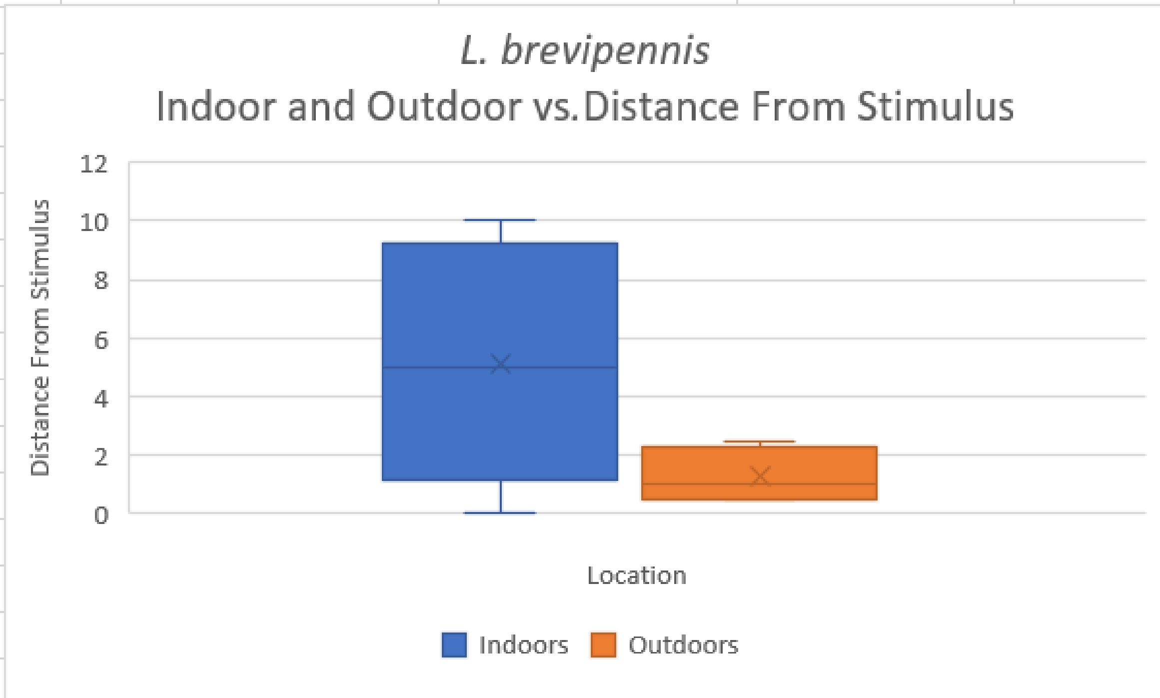
A. flavolineata $\sigma=4.1419$

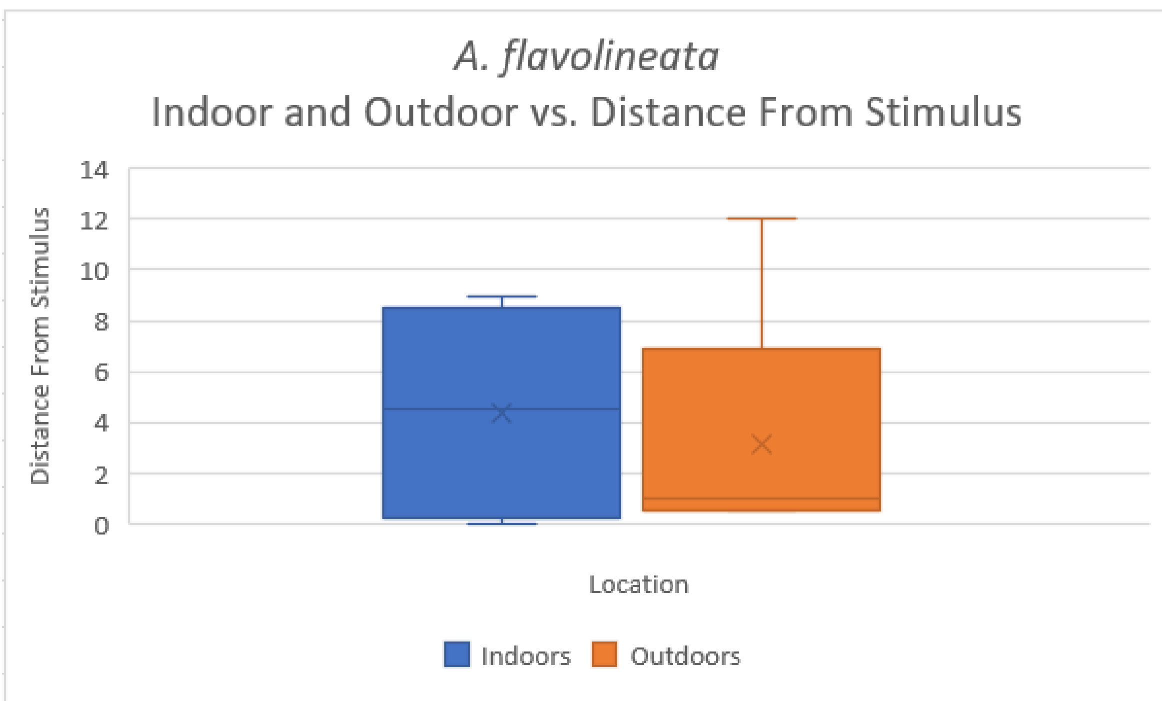
L. brevipennis $\sigma=3.3175$

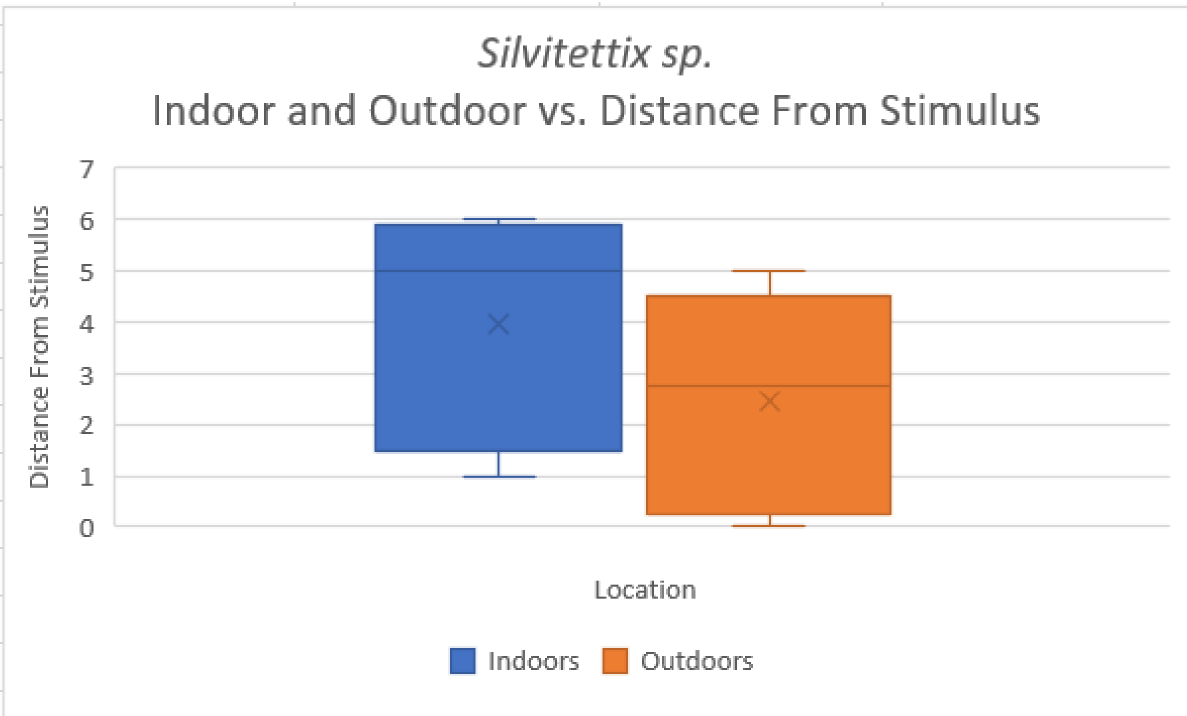
Silvitettix sp. $\sigma=2.1325$

Orophus sp. $\sigma=1.4239$

The data and a graphical representation of the species indoors vs. outdoors is below in Fig. 11.


L. brevipennis
Indoor and Outdoor vs.Distance From Stimulus
Distance From Stimulus
0
2
4
6
8
10
12
Location
Indoors
Outdoors


A. flavolineata
Indoor and Outdoor vs. Distance From Stimulus
Distance From Stimulus
0
2
4
6
8
10
12
14
Location
Indoors
Outdoors



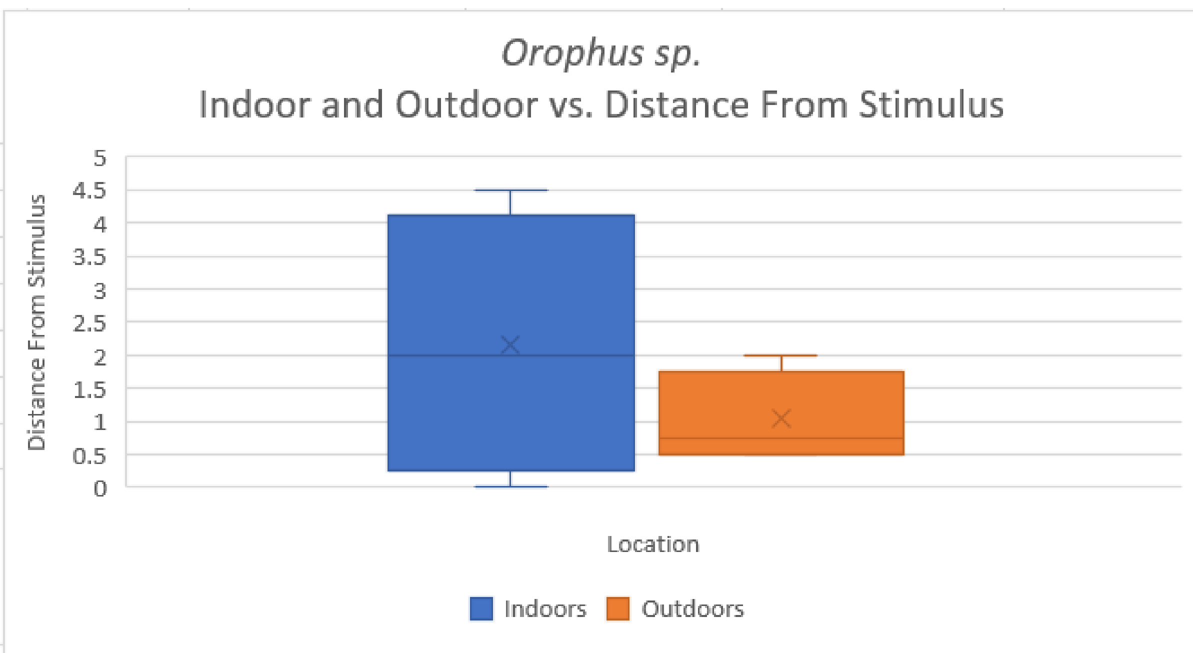


Fig. 11. *Each individual species can be seen above. The species can each be seen to react faster indoors as opposed to outdoors.*

Discussion

Orthopterans are known to be cryptic insects, thus staying camouflaged in the wild. It is most efficient to maintain stoic when there is no need for movement, as it saves energy and keeps the insect out of the sight of predators. There is a risk of leaving resources, as well as a risk of being exposed that weighs against remaining cryptic with resources in the direct surroundings (Ydenberg, 1986). In other studies, cryptic frogs allowed predators closer than brightly colored ones (Broom, Ruxton 2005), as they have evolved to blend in to the environment around them. This allowed for the posing of the question of whether Orthopterans reflect these same patterns, as insect vision is most sensitive to changing patterns and movements (Keeley, 2011). This project was designed initially to compare a singular species of grasshopper (*A. flavolineata*) with a singular species of katydid (*Orophus* sp.) in terms of their escape behaviors. As the experiment evolved, four separate species were observed with the initial two, as well as the addition of *L. brevipennis* and *Silvitettix* sp..

The expectation for Trial one was that the grasshopper would react to the approaching vertical stimulus sooner than the katydid. There was no significant difference of escape behavior or reaction time between the grasshoppers and katydids. In part this could be due to the stimulus approaching from a vertical direction, thus not posing a threat. There were many errors encountered with the original design of the experiment. Initially, the insects were in a plastic holding container, but it was realized that the insects almost never reacted to the approaching stimuli. The length the stimuli would fall was increased to give the insects more time to react, by raising the design almost another two feet. Additionally, four microscope lights were added, as the tower of cardboard had caused the bottom to be very dark, but still there was almost no reaction seen. This could be because the lid of the carrying container was too visually distorting, or that the insects could rest on the lid as a solid barrier between them and the approaching stimulus. A new carrying container was constructed from a two-liter bottle, a plastic wrap lid, and a stick which lied at an angle to potentially give the insects a view of the stimulus. Once again, there was no reaction from the insects. It was decided to scratch the holding container, and instead allowed the insects to be free in the box, also increasing the stimulus size in hopes that this would illicit more reaction. While this design did finally make the insects react to the stimulus, it was a very uncontrolled and a variable setup. It had no control on the initial position of the insects, which means some would face towards the stimulus, others faced away, some would be in the center directly under the stimulus, and others near the corners. There was also a fair bit of trouble keeping the grasshoppers in the box at all, with many of them jumping through the hole in the plastic wrap ceiling and escaping. It was clear that this initial setup used for trial one was far too variable, so it was decided to cover the top completely, and simply approach the insects with a stimulus from the side, instead of above.

The expectation for Trial two was that the two flightless species would have slower reactions to the horizontal stimulus than *A. flavolineata*, but both would be more reactive than the katydid species. To try and account for the different positions in the box, doors were constructed on each side so the initial distance from the stimulus was relatively consistent. This final design, along with the field test, was much more consistent than previous setups. No significant difference between the species was observed in terms of reaction time. Running a contingency test solely for the insects that reacted also showed no significance, but among the grasshopper species there was significance, meaning they behaved similarly. When the ratios of insects that reacted were doubled, there was a statistical difference observed between the katydids and grasshoppers. This still indicated inconclusive data for the gathered results, as there were not enough specimens to work with.

The expectation for Trial three in the field was that 1) the two flightless species would have shorter jump distances than the other two species, and 2) the reaction to the stimulus would be slower in the field where insects are more hidden. Despite the statistics indicating insignificance, the two wingless species did have shorter median jump lengths than the winged species. There was no significant difference between the species regarding distance from stimulus before reaction.

When comparing the indoor and outdoor trials, the insects in the artificial environments reacted much sooner than those outdoors. This is likely due to cryptic behavior being possible in a natural environment, as opposed to a cardboard box.

Another challenge faced was the small number of samples collected, and the fact that specimens were being reused, which likely overly stressed/exhausted the insects from the repeated handling and testing. By the end of the last trials, almost half of the katydids had died, and at least one or two of every grasshopper species had also died. This created some difficulty when looking for statistical significance in any of the results. Had more specimens been collected and handled properly, the results could have been significant. There were limiting factors, such as the unpredictable rainy weather, so more time to conduct this experiment would have potentially led to more conclusive results.

Overall, this experiment was designed to compare escape behaviors between Orthopterans. Knowing how these species react outdoors could be beneficial for both detection and collection of them. This experiment also allows for the understanding of cryptic behavior in insects, thus providing insight as to how species have evolved to survive, and which are more likely to go undetected.

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