Preferential Foraging Behavior of the Terrestrial Slug Orange Banded Arion *(Arion fasciautus)* to Familiar and Novel Food Resources

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Abstract

The Orange Banded Arion *(Arion fasciautus)* is a terrestrial slug that is known to inhabit damp areas across the United States. In massive numbers, they can destroy agriculture crops and lower canopy of deciduous forests. This experiment tests the behavior and consumption of familiar and novel foods of slugs from sugar maple and conifer habitats. The familiar foods consist of leaf litter from their established habitat and wild-caught earthworms. The novel foods are store-bought mealworms and romaine lettuce. The hypothesis was that slugs will tend to spend more of their time eating novel food items in the animal and plant trials. The location mean index of the slugs in the plant trial, show preferences for the natural leaves found in the habitats.

Slugs from both habitats prefer mealworms rather than the earthworms. The slugs consumed (mean selectivity index) the familiar food resources in both habitats. *Arion fasciautus* in conifer habitats in low numbers, and they need another habitat adjacent to it to obtain the nourishment they need. This terrestrial slug is widely abundant based on the quality of vegetation and organic matter presented within its habitat.

Keywords: Slug habitat, consumption, *Arion fasciautus*, Orange Banded Arion, slug pests, foraging, earthworm, mealworm, novel foods, familiar foods, selectivity index, location index.

Introduction

Slugs are detritivores of any habitat that consists of dead plant matter with frequent rain patterns to keep this area moist including tropical and temperate forests, and agricultural fields. Although many people take little notice of them, they are classified as macrofauna that break down plant detritus and release nutrients back into the soil. Their role as detritivores is essential within the food chain and allows energy to flow throughout the ecosystem.

Slugs are well-known for destroying agricultural crops, weeds, broadleaf plants and grasses. Their presences as pests in agricultural areas have documented as early as the 10th century AD; most of the farmers from that time would use the dust of hot lime in order to keep them out of their fields. At the beginning of the 20th century, pesticides such as lime, salt, soot and ashes were made available for people to buy at local grocery stores. Today, large agricultural

areas use chemical dusts, sprays, and baits. The main concern using chemicals to decrease the slug population is the harmful effects to the nutrients found within the soil and chemicals that don't percolate into the ground are left on the crops (Barker, 2002).

Orange Banded Arion (*Arion fasciautus*) is a terrestrial slug that was introduced into North America from Europe, and inhabits damp areas, such as wet meadows adjacent to streams. They are most commonly found among the leaf litter, along woody debris, and moving around the trunks of trees (Haro, 2004). During the months of May and June, *Arion fasciautus* eats more earthworms and arthropods in search for a richer protein source as the months of slug activity decline due to colder temperatures (Jennings, 1975). Moreover, slugs prefer to forage on fresh plant leaves due to the small soft dermal hairs that allow them to eat effortlessly. Although they feed on dead plant material from the forest floor, when presented with the fresh plant material they feed without hesitation.

The objective of this experiment is to test the preferences of wild-caught slug, *Arion fasciautus* between familiar and novel food items. The familiar food items are fresh earthworms and dead leaf matter; novel food items include store-bought mealworms and romaine lettuce. Food items were paired into plant trials and animal trials to test the preference for each type of food; the amount of time spent with a given food item and the selectivity, comparing which food item was consumed more than the other, were measured. The hypothesis was that slugs will tend to spend more of their time eating novel food items in both trials. Thus they should favor the romaine lettuce and the mealworms over the leaf litter and the earthworms.

Materials and Methods

Study Area- This study was conducted at the University of Notre Dame Environmental Research Center (UNDERC) in the Upper Peninsula of Michigan. *Arion fasciatus* inhabits the

conifer forest on property; tree species that are used to identify conifer habitat, include Easter White Pine (*Pinus strobus*), Red Pine (*Pinus resinosa*), Black Spruce (*Picea mariana*), and White Spruce (*Picea glauca*). Along with the conifer areas sampled, sugar maple habitat was included to compare the feeding preferences among the *Arion fasciatus*. One hundred and eighty four slugs were sampled during this experiment and were then split into the two different trials for food preferences. The sampled sites were chosen due to the abundance of slugs that inhabit these areas. The following habitats were surveyed but had a limited population size; sphagnum bogs, marshes, vernal pools, and habitats adjacent to the lakes.

Experimental Procedure –Trapping: Metal Sherman traps missing one door were placed in these habitats and were baited with oats, the traps were set in the evening and were collected the next morning. The ideal time to collect slugs was during the early morning between 7am to 9am after a severe rain fall. The rain fall allows these gastropods to perform their adhesive locomotion, a movement driven by periodic muscle contractions and thin layer of mucus to move in terrestrial areas (Iwamoto et al. 2014). Two mason jars were labeled for the area they were caught in, and then were transported back to the lab.

Medium sized plastic containers with four mesh screens were used to house the slugs once they were brought back from the field. Large wet sponges were placed in the center of the containers, to keep them from dehydrating. The approximate starvation time of the slugs, was one to two days before they were ready to be used for experimental purposes (Paustian, 2012). Days of starvation did not extend past two days, as this increases the mortality rate of the slugs. Earthworms were collected using a mustard concentration, 40 grams of mustard powder to 3.8 litters of hot water (Szabo, 2012). The earthworms captured were placed in the freezer overnight and were set in the refrigerator to defrost, before the trials started.

Experimental Procedure- Plant & Animal Trials: Petri dishes were used during the trials to enclose the slug in a small area to reach the food in small distances. Slugs tend to move along sides of large containers that have a greater surface area, the petri dishes limits this behavior. A line was drawn on the outside of the petri dish to indicate the area where the slug spends most of its time. The petri dishes were numbered; each individual slug was recorded based on the number of the dish. Each of the trials were checked every 10 minutes to indicate which side the slugs were on, if they were located on the line between them they were recorded as the middle location. Each of the trials lasted for 2 hours.

Prior to the start of each trial, slugs were weighed and measured in order to adjust for the amount of food intake. Each food item had a standard weight; the romaine lettuce weighed 0.25 grams and the novelty foods weighed 0.20 grams. The mealworms weighed 0.35 grams and the earthworms weighed 0.40 grams. Small droplets of water were added to the dried foods and in open areas of the dish to assist the slugs in movement. The mealworms and the leaves were moistened before the trial in order for the slugs to digest the food easier. After two hours, all tested slugs were placed in another plastic container with a wet sponge and small amounts of oats were given to them. Slugs that were not used for the experiment after the two day period of starvation were placed with the tested slugs to recover. In order to avoid recapture of individuals tested previously, the sites were only used twice.

Statistical Analysis:

Note: the mean values that have positive values represent the novelty food items (lettuce and mealworms) and the negative values represents the familiar food items (leaves and earthworms).

We used SYSTAT to run our entire statistical analysis. The location index represents the amount of time each slug spent on the side of the petri dish. The location index was calculated by

the log of the ratio of the number of times the slug was on the novel food divided by the number of times the slug was on the familiar food type. Because this ratio would be mathematically undefined if the slug was not observed on the familiar food type, a small value (0.00001) was added to each datum. Mean values that are positive represent preference for novelty food items (lettuce and mealworms) and the negative values represents preference for the familiar food items (leaves and earthworms). Two-sample t-tests determined differences in the preference between the two habitat types, and a one-sample t-test determined if slugs showed a preference for one food type over the other. Different tests were conducted for vegetative and animal food types.

Consumption was also analyzed using a selectivity index that indicates which resources were eaten more relative to its abundance. Selectivity was calculated for each trial, by the proportion of the food eaten (difference divided by before weight) divided by the abundance of available food options, including the proportion of the novel foods subtracting the familiar foods. In addition, abundance of available food differs between the plant and animal trial. The plant trial had a consist weight of 0.25 grams for the before trials, so the abundance of available foods was 0.5. In the animal trials, the abundance of available food is calculated by adding the before weights of the novel and familiar foods together (Cramer, 2014). One two-sample t-test was used to compare the habitats to the selectivity of the food items eaten. Then two one-sample t-tests determined if food preference was zero in both the leaf and animal trials. All results are reported as means ± standard errors.

Results

We tested 40 slugs from the conifer habitat and 48 slugs from the maple habitat. The amount of time the slugs spent on each food type for the plant trials did not differ between

habitats (Conifer: -1.321 ± 0.702, Maple: -1.492 ± 0.590, t=0.187, df=86, p=0.851; Figure 1). In addition, the amount of time spent on the plant material regardless of habitat was not equal to zero, indicating strong preference for maple leaves over romaine lettuce (mean: -1.414 ± 0.450, t= -3.138 , df=87 , p=0.002; Figure 3).

We tested 39 slugs from the conifer habitat and 40 slugs from the maple habitat. A two-sample t-test compared the differences between the conifer and maple areas for the animal trails; and found no significant differences (Conifer: 0.683 ± 0.618, Maple: 1.131 ± 0.548, t=-0.0541, df= 77, p=0.589; Figure 2). There was no difference in preference in the conifer and sugar maple habitats. The slugs spent more time with mealworms than the earthworms (mean: 0.910 ± 0.410, t=2.216, df=78 p=0.029; Figure 3).

We tested 14 slugs each from conifer and maple habitats. Selectivity between the slugs found in the conifer and sugar maple habitat in the leaf trials was significantly different (Conifer: 0.194 ± 0.054, Maple: -0.205 ± 0.102, t=3.456, df=26, p=0.001; Figure 4). The slugs in the conifer area prefer the lettuce (mean: 0.194 ± 0.054, t=3.586, df=13, p=0.003; Figure 6), whereas the maple slugs show no preference (mean: -0.205 ± 0.102, t=2.01, df=13, p=0.065; Figure 6).

We tested 18 conifer slugs and 19 maple slugs for the animal selectivity trial. Selectivity for worms was significantly different based on habitat (Conifer: -0.711± 0.065, Maple: -0.451 ± 0.089, t= -2.332, df=35, p=0.025; Figure 5). There was a difference in magnitude of response. Conifer slugs had a strong preference for earthworms (mean: -0.711 ± 0.065, t= -10.916, df=17, p<0.001; Figure 6) than maple slugs (mean: -0.451 ± 0.089, t= -5.073, df=18, p<0.001; Figure 6).

Discussion

Location Index –Explanation: Slugs in the plant trial were found more often in the leaf area than the romaine lettuce area. During the trials, a majority of the slugs would quickly move to the leaf cover areas and then would rarely move to the lettuce area. *Arion* generally prefer woody areas over herbaceous areas with little cover, and previous study suggests that the slugs were prone to seek cover if they were exposed in an open area (Fusser et al, 2016). Although the overall weight of the leaves were about the same there would generally be more amounts of leaf litter because they had a lighter weight. The romaine lettuce had a heavier weight within a small surface area, due to the amount of water held in the lettuce. Moreover, *Arion* are unable to hide underground like other slug species and would instead find cover under dead wood and plant matter (Fusser et al, 2016). Therefore, the slugs may spend more time on the leaves regardless of the habitat because they provide better cover.

The slugs in the worm trial also showed that the preference was the same between habitats for the location index regardless of habitat. However in the one sample t-test, the slugs spent more time in the mealworm area. Mealworms can be found in the forest under dead logs, but their life cycle stage in its larval form occurs during late autumn (Penn State College of Agriculture Science). Therefore, foraging slugs have never experienced mealworms, because they hibernate during periods of colder temperatures (below 45º F). The results of the location index may indicate that slugs will investigate a new food type that they have never encountered (BBC News). As slugs encounter mealworms, they will find that they are rich in protein (contains up to 75% of their dry weight), including amino acids such as phenylalanine, tyrosine, and tryptophan that has a nutritious advantage over earthworms (Megido et al, 2016).

Selectivity Explanation-Consumption: Slugs from conifer habitats consume greater amount of romaine lettuce whereas slugs from sugar maple habitat preferred leaves. The abundance of populations of slugs in the maple habitat affects foraging preference of the other habitats because slugs have more options to eat food resources. Slugs rarely seek to forage in the conifer habitats due to the limited options, compared to a sugar maple forest. The slugs would most likely choose the options from the plant trials in this order; romaine lettuce, maple leaves, and conifer needles. Slugs are more likely to feed on plant matter with soft dermal cell than plant matter with harder dermal cells (Jennings, 1975), which may explain why slugs from the conifer consumed more lettuce than conifer needles.

Slugs from the conifer habitat have a strong preference for lettuce, ignoring the conifer needles. Slugs from the maple habitat ate a small amount of lettuce, but took greater interest in the sugar maple leaves. *Arion fasciatus* are abundant in pine and spruce litter due to its rich ground vegetation, however these slugs require areas that have deciduous trees; such as the sugar maple for feeding purposes if they are limited. Since slugs depend on the tree species presented in their habitat, the quality of food is crucial. The slugs in the conifer area would prefer romaine lettuce due to its characteristic of holding water and easier digestibility. A maple slug would prefer maple leaves or lettuce because they are similar; the familiar and novel factor plays a role in preference (Beyer & Saari, 1977).

. Slugs in the sugar maple and conifer habitats both show a significant increase in consumption of earthworms compared to mealworms. A majority of the earthworms were cut into sections in order to meet the standard weight for the experiment. When the slugs encountered the earthworms they had soft flesh, blood was exposed and minerals were found within the gizzard. The presentation of the earthworms had an advantage for the slug to increase

their food intake, as opposed to the dried meal worms that were soaked in water but had no exposed flesh.

The result of this test was significant, meaning regardless of habitat all the slugs had interest in consuming more earthworms than mealworms. In the one-sample t-test for the mean selectivity index, the maple slugs had no preference and the conifer slugs would consume greater amount of the romaine leaves. Therefore, *Arion fasciatus* would prefer vegetation with soft epidermal cells such as the maple leaves and romaine lettuce. They would consume earthworms due to how they were presented in the dish, it would be rare to find an earthworm carcass in the forest- a bias control over the animal trial.

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Figures

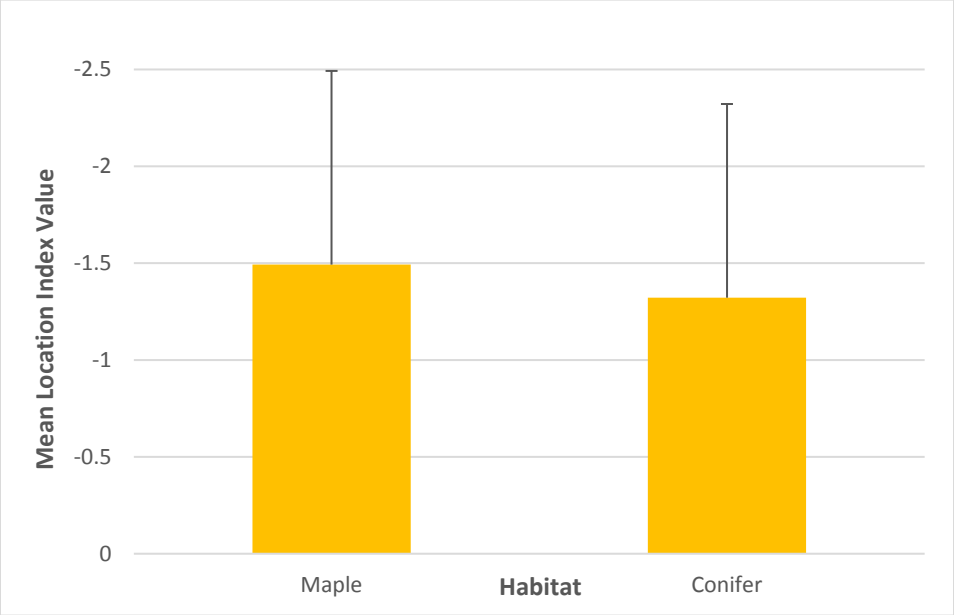

Figure 1. Location Index of sugar maple and conifer habitats spent on leaves. A two-sample t-test, used to compare the difference between the maple and conifer habitats to reveal that there is not a significant difference between the mean location index (Conifer: -1.321 ± 0.702, Maple: -1.492 ± 0.590, t=0.187, df=86, p=0.851).

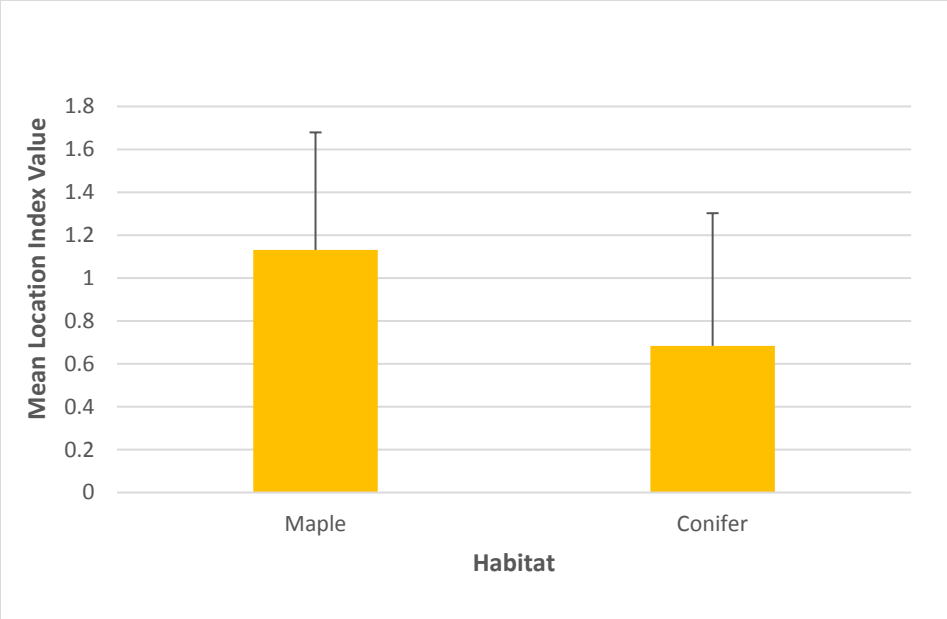

Figure 2. Location Index of sugar maple and conifer habitats reveals that there are no difference in preference between the mealworms and earthworms. A two-sample t-test, is used to compare the difference between the maple and conifer habitats to reveal that there is no significant difference between the mean location index (Conifer: 0.683 ± 0.618, Maple: 1.131 ± 0.548, t=-0.0541, df= 77, p=0.589).

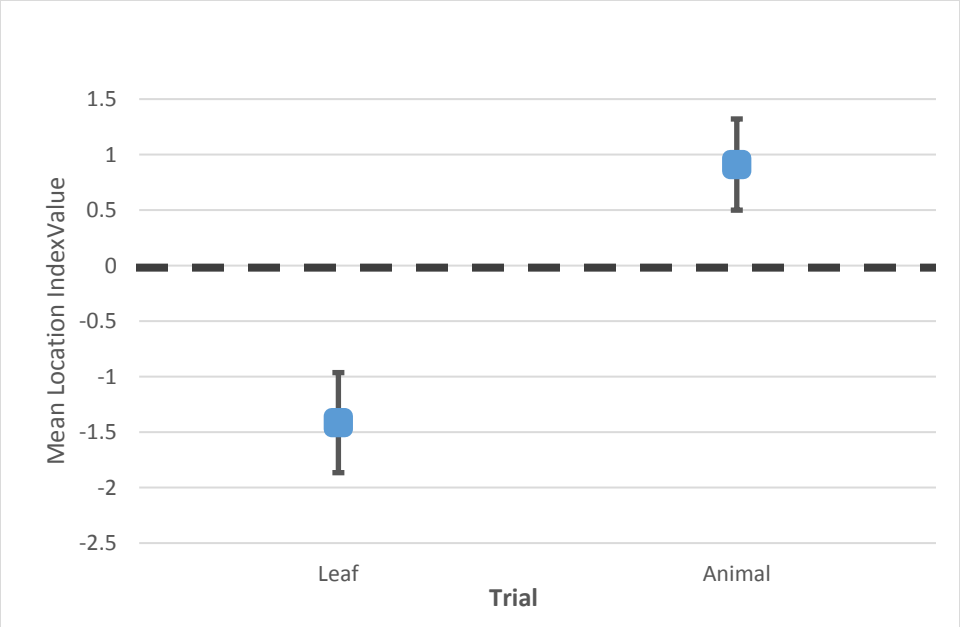

Figure 3. Location mean index for the leaf and animal trials. In both cases, a one-sample t-test was used to test for the zero to indicate the difference between the two. The dash lined represents the value of the index indicating no preference (Leaf trial mean: -1.414 ± 0.450, t= -3.138, df=87, p=0.002) (Animal trial mean: 0.910 ± 0.410, t=2.216, df=78 p=0.029).

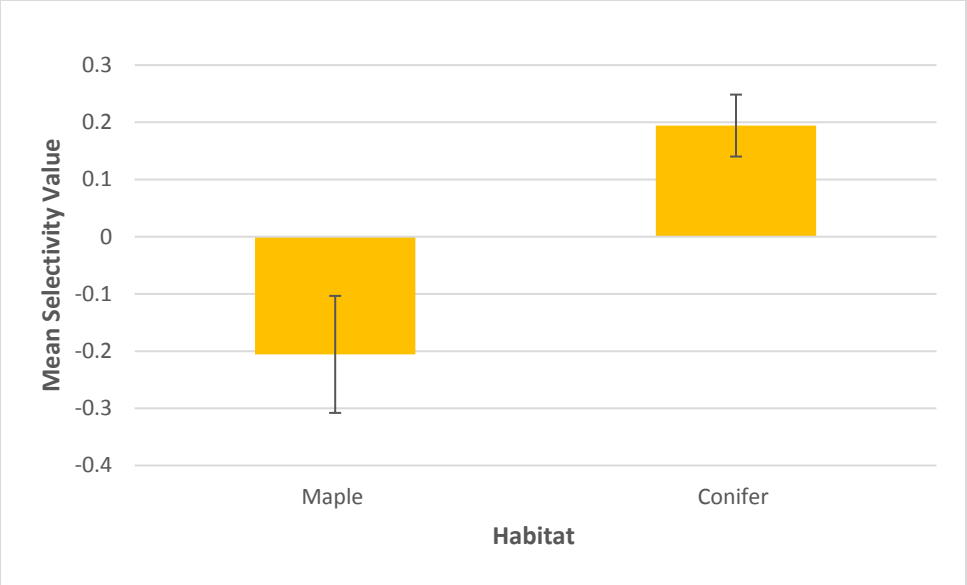

Figure 4. Two-sample t-test comparison between the conifer and maple habitats, based on the mean selectivity index on the leaf trials. Showing a significant difference, conifer slugs have a preference for romaine lettuce and the maple slugs have no preference for between the food resources (Conifer: 0.194 ± 0.054, Maple: -0.205 ± 0.102, t=3.456, df=26, p=0.001).

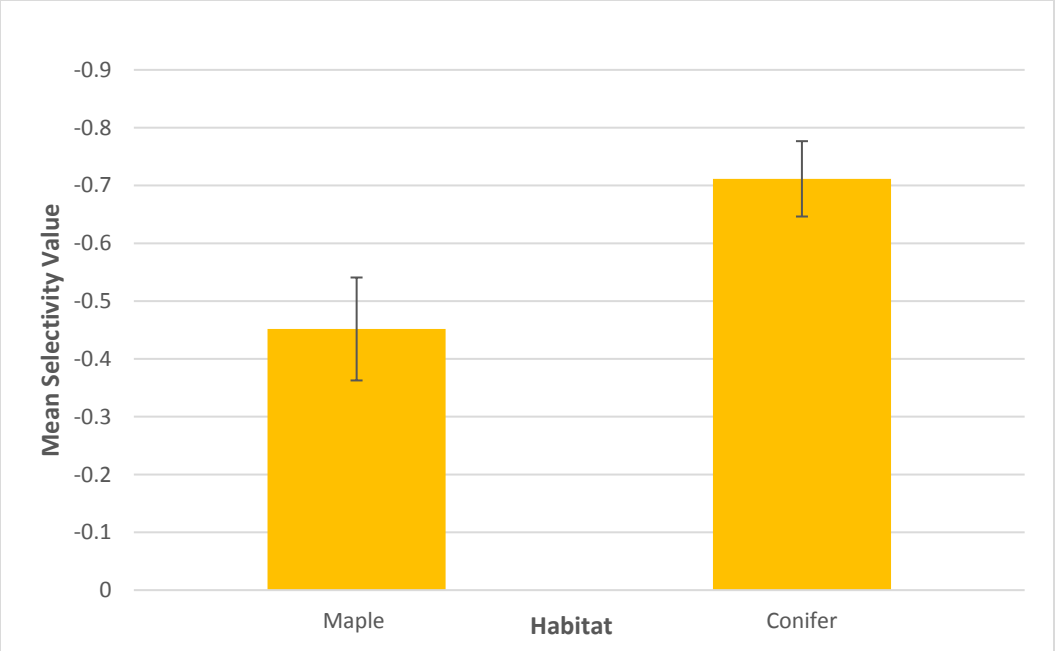

Figure 5. Two-sample t-test comparing the mean of selectivity in the animal trial based on each of the habitats. The sugar maple and conifer habitat were highly significant (Conifer: -0.711± 0.065, Maple: -0.451 ± 0.089, t= -2.332, df=35, p=0.025).

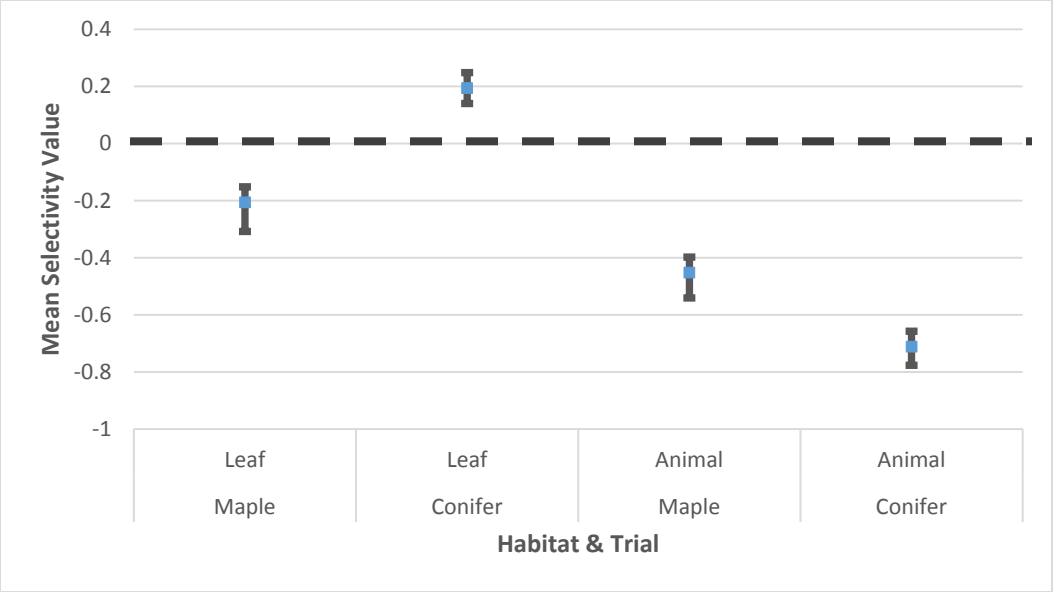

Figure 6. Four one-sample t-tests were used to compare the mean selectivity value that expected with no preference, shown by the dashed line. Maple and leaf trial is not significant, Conifer and leaf trial is significant, Maple and animal trial is significant, and Conifer and animal trial is significant (Maple/leaf mean: -0.205 ± 0.102, t=2.011, df=13, p=0.065), (Conifer/leaf mean: 0.194 ± 0.054, t=3.586, df=13, p=0.003),(Maple/animal mean: -0.451 ± 0.089, t= -5.073, df=18, p<0.001), (Conifer/animal mean: -0.711 ± 0.065, t= -10.916, df=17, p<0.00).

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