

Coexistence in Burying Beetles: The Niche of Reproductive Temperatures

Abstract

Coexistence among species can occur in a variety of ways through niche partitioning. Two major niche components for many organisms are temperature and soil moisture and variation in these may occur with habitat heterogeneity. Near Gothic, Colorado at the Rocky Mountain Biological Laboratory, there are two habitats that pose differences in temperature and soil moisture: aspen forests and montane meadows. Two burying beetles species co-occur in these habitats (*Nicrophorus defodiens* and *Nicrophorus investigator*) and overlap significantly in their activity periods, diet, and reproductive behaviors. Temperature and soil moisture impact beetle development, adult flight, burial behaviors, and survival. Larvae are subject to temperature changes, where high temperatures lead to fatality and low temperatures slow development. Adults are subject to high temperatures and desiccation both aboveground and belowground. Aspen forests and montane meadows provide habitat heterogeneity (differing temperatures and soil moistures). I tested the hypothesis that each species has better reproductive success (higher fitness) in different habitats, thus providing a mechanism for their coexistence. I found that *N. defodiens* had a greater fitness (brood success, larvae number and larvae mass) in the aspen compared to *N. investigator*, and *N. investigator* had a higher fitness in the meadow compared to *N. defodiens*. *N. investigator* did have similar brood success to *N. defodiens* in the aspen, but there may be consequences later in their development.

Introduction

When similar species overlap in space, niche partitioning creates an area of specialization in which characteristics like reproduction, diet, and physiological tolerances can enable coexistence (Gilbert et al. 2008). Environmental aspects can influence coexistence when physiological needs vary among organisms. The difference in habitat, (i.e. habitat heterogeneity) is linked with spatial heterogeneity, as the landscape is made up of a network of patches based on ecological processes (Vinatier et al. 2011). The increase in habitat types increases the amount of niche space for different species to coexist together where factors such as light or soil moisture are utilized differently by species (Valladares et al. 2015).

Temperature and soil moisture have an important effect on insect development and survival. Both temperature and soil moisture can aid in predicting the change in developmental rate, phenological development, or diapause in insects (Buckley 2022, Prather et al. 2023, and Ma et al. 2017). Studies have shown that just slight changes in moisture levels can impact larvae or adults of species (Bedick et al. 2006 and Hulthen and Clark 2006). Temperature also influences survival rates in larvae and adult insects where too low temperatures and high temperatures are fatal. Varying temperatures change developmental rates from slower in cool temperatures to faster in warmer temperatures (Keller et al. 2021 and Ludwig 1928). Thus, if environments differ in temperature and moisture levels then this may provide sufficient habitat heterogeneity to support the physiological niches of many insect species.

Two habitats of interest are aspen forests and montane meadows as they vary in soil types, temperatures, and moisture levels. Meadows generally have lower soil moisture content, but compared to coniferous and mixed forests, meadows and aspen forests have higher soil moisture and temperatures (Buck et al. 2012 and Ortega et al. 2012). Aspen forest soils are typically associated with cooler temperatures while montane meadows have higher temperatures. Within Gothic, Colorado, there are many patches of aspens and meadows throughout the landscape which provides the heterogeneity to promote multiple species. In this study, the insects of interest between these two habitats are burying beetles, as they reproduce and overwinter in the soil.

With their distinctive orange and black exoskeleton, burying beetles (Order Coleoptera, Family Silphidae, Genus *Nicrophorus*) are known for their life histories that include the burial of small mammals for both nutrition and reproductive needs (Eggert et al. 1998, Milne and Milne 1976, and Smith and Heese 1995). Through their olfactory system, burying beetles can detect the scent of carrion and can arrive relatively quickly after a carcass is placed in an area. The carcass is then used by the beetles for either reproductive purposes or food based on its weight and size (Smith and Heese 1995). If the carcass is suitable for reproduction, it is buried into the soil, stripped of its fur, and is turned into a ball in preparation for offspring. After mating the female lays eggs in the soil surrounding the prepared carcass. Once the larvae hatch and move to the carcass, regurgitation is then used by the male and female to feed their offspring in their early instars before they pupate (Eggert et al. 1998 and Milne and Milne 1976). Once the parents leave, larvae remain in the soil until they eclose as adults.

There are three species that occur near the Rocky Mountain Biological Laboratory in Gothic, Colorado at 2,900 meters. These are *Nicrophorus investigator*, *Nicrophorus defodiens*, and *Nicrophorus guttula* (Winkler 2008, Oliver and Smith 2013, and Rawinski 2021). Each species has a habitat preference from open meadows (*N. investigator*) to aspen forests (*N. defodiens*) and warmer sage-brush climates (*N. guttula*) based on temperature, soil types, or shade (Oliver and Smith 2013 and Anderson and Peck 1985), but this association is derived only from captures of adults feeding or entering traps (Garfinkel and McCain 2023). There are also different life histories between the beetles: *N. investigator* overwinters as a pre-pupa and emerges as an adult in late June while *N. defodiens* and *N. guttula* eclose in the fall and overwinter as adults. Both species are reproductive in late spring and early summer, with *N. defodiens* more abundant in early summer (Buehner 2001 and Anderson and Peck 1985). It is possible that both their habitat preference for reproduction and overwintering may differ. This study examines the natural variation in temperature and soil moisture of the landscape and how this may affect the reproductive success of burying beetles.

Studies of habitat preference with *N. investigator* show a preference for open meadows rather than forested, showed no adults bury carcasses under shaded conditions, and larvae reared under shade structures experienced smaller sizes (Smith and Heese 1995). However, there is little information on the differences in development and reproductive success between aspen forests and open meadows for *N. investigator* and *N. defodiens* in a natural setting. Differential

success could indicate a niche dimension supporting a potential mechanism for coexistence. I have three hypotheses. My null hypothesis is that both species will have similar reproductive success in both habitats. My first alternative hypothesis is that each species will have better reproductive success in different habitats (this would test the mechanism for how burying beetles are able to coexist). The second alternative hypothesis is that both species will have similar reproductive success in one habitat over the other (this does not support a mechanism of coexistence). Regarding my second alternative hypothesis, I predict that *N. investigator* will have a faster development and higher overall brood success in open meadows versus aspen forests, and that *N. defodiens* will have a faster development and higher overall brood success in aspen forests versus open meadows.

Methods

Setting

My experiment took place in Gothic, Colorado at the Rocky Mountain Biological Laboratory. The experiment was split into two locations: Trial 1: in the Research Meadow (Figure 1) and Trial 2: locations within the Gothic townsite (Figure 2). Within Gothic, the meadow plot was behind the Gothic Research Center and the aspen plot was behind Weekley Cabin. These sites are optimal as there was access to both an aspen forest and an open, montane meadow within 200 meters of each test plot.



Figure 1: Research Meadow plots for Trial 1

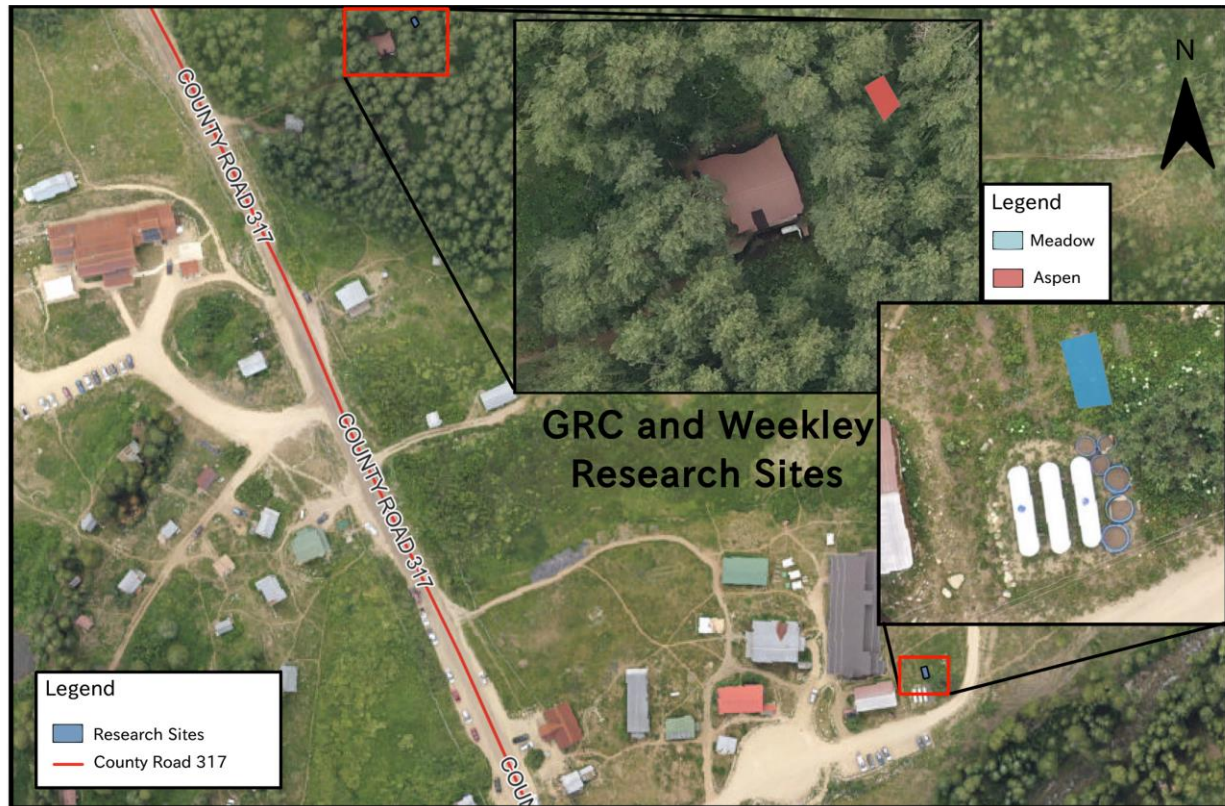


Figure 2: Research plots behind the Gothic Research Center (GRC) and Weekley Cabin.

Experimental Design

The adult beetles were collected from baited traps at Research Meadow and other collecting sites within Gothic. Adults were separated by sex and held in containers with water and food (raw chicken) until the start of the trials. In Trial 1, each plot had ten pairs of *N. investigator* and *N. defodiens*, while in Trial 2, I used fifteen pairs of *N. investigator*. The plots at the Research Meadow were used first to include both *N. investigator* and *N. defodiens* because *N. defodiens* reproduce during late June and early July. The Gothic Townsite was used to test *N. investigator* beetles to look at the habitat differences while *N. investigator* reproduces during mid to late July.

There were four plots, two in the aspen and two in the meadow. For Trial 1, within each treatment plot there were ten blocks, where each block consisted of two cans placed one meter apart: one can with a pair of *N. defodiens* and the other with a pair of *N. investigator*. The blocks were placed randomly within the two habitat types and were dug into the dirt with 2-3 cm of the can above ground. Trial 2 only used pairs of *N. investigator* as *N. defodiens* were not caught in mid-July. One additional can at each site was used for measuring temperature and soil moisture as a “dummy can” within each plot. At the start of each trial, I placed randomly selected male-female pairs of *N. investigator* and *N. defodiens* beetles in a can with a *Peromyscus maniculatus*

carcass. I did not use *N. guttula* in this experiment because I couldn't collect more than four pairs.

Table 1: Trial 1 - Research Meadow

Species	Aspen	Meadow
<i>N. investigator</i>	10	10
<i>N. defodiens</i>	10	10

Table 2: Trial 2 - Gothic Townsite

Species	Aspen	Meadow
<i>N. investigator</i>	15	15

Each can in Trial 1 was supplied with one (19-21 g) dead mouse and Trial 2 was supplied with one (16-20g) dead mouse (*Peromyscus maniculatus*) previously salvaged from the cabins in Gothic (Salvage Permit: Colorado Division of Parks and Wildlife #3036077078). These mice were placed in the freezer in the Gothic Research Center. This kept the weight of the carcasses constant throughout this experiment. Screen lids with no holes were secured by a large rubber band or wire on top of the cans once a mouse and a pair of burying beetles were in the can to prevent anything from coming in or leaving. To keep the soil in the cans from becoming too wet, 15 holes were punched at the bottom of them for drainage.

Variables

I measured the stages and timing of brood development. I checked the cans in the aspen and meadow plot two times a day during the morning (9:00am-10:00am), and in the afternoon after lunch (2:00-3:00pm). This continued for a week until the beetles buried the carcasses or did not.

Table 3: Variables Measured

Variable	Units	Description
Number Buried	Count	Number of mouse carcasses buried.
Burial Speed	Day (ex: ½ day)	Days until the mouse is completely buried.
Beetle Death	Count (out of 2)	Number of beetles that died.
Adult Parent Emergence	Day (date from burial)	Day of parent emergence after burial.
Number of Larvae	Count	Number of larvae counted after parent emergence.

Mass of Larvae	Grams	Mass of larvae weighed to 0.01 grams.
Offspring Adult Emergence (<i>Nicrophorus defodiens</i>)*	Day (date from burial)	Day of offspring adult eclosion (emergence).
Number of Offspring Adults (<i>Nicrophorus defodiens</i>)*	Count	Number of adults counted after offspring eclosion.
Pronotum Size (<i>Nicrophorus defodiens</i>)*	Millimeters	Pronotum measured with digital calipers after offspring emergence.
Temperature	Degrees Celsius	Temperature measured from HOBO temperature loggers.
Soil Moisture	Categories/Range	Moisture measured with a range from 0-10.

*(Note that the measurements on *N. defodiens* will be repeated with *N. investigator*. Also, this will be carried out into the late summer and into next year.)

My first count was the number of carcasses buried by the beetles in both habitat types. The second measurement was the days until burial, noting when the mouse was completely buried by the beetles. I then waited 2 weeks after carcass burial and checked daily, noting when the adult parents were at the surface of the soil within the cans. This indicated that they had completed their parental care of larvae. The adults on the surface were then released alive. When the majority of the adults had emerged, each can was emptied into a large tub and scanned for larvae or pupae. If there were pupae, the brood was returned immediately to the can and reburied in the ground. If there were larvae, we collected all the larva and weighed (field balance: Ohaus Portable scale, Scout II) them individually as well as together for a total brood mass. The larvae were then placed back into their original can with the soil replaced. If an adult was found, we released it alive. We also noted dead adults. This was repeated for each can in the meadow and the aspen. Each successful brood/can was given a metal tag for identification for later this summer (*N. defodiens*) and into next year (*N. investigator*).

With *N. defodiens*, as they overwinter as adults and will emerge this summer as adults, I will count the days until offspring adult emergence (estimated ~45 days), the number that survive, and measure pronotum width (mm) with digital calipers. *N. investigator* beetles overwinter as larvae, so I will do the same measurements next summer (estimated 10 months) when they emerge.

I also measured the temperature and soil moisture of both the aspen plots and the montane meadow plots. I measured temperature with eight HOBO Temperature Data Loggers where there were two per plot in a dummy can with soil. One of the HOBO loggers was placed at the bottom of the can at carcass burial depth while the other was placed at the surface with a shade for accurate temperature reads. I also measured soil moisture using a Lincoln Irrigation Soil Moisture Meter every one to three days throughout half of the experiment, both in the “dummy” cans and in cans with beetles.

Statistical Methods

My experiment in Trial 1 was a randomized block design with two main fixed effects – habitat and species. The statistical analysis was a two-way ANOVA that compared the means of the main effects (species type and habitat type) and their interactions. The interaction effect provides information for alternative hypothesis 1, where a significant interaction effect supports a mechanism of coexistence. When the data was not normal through the Shapiro-Wilks test, I used the Scheirer Ray Hare test. Contingency tests (chi-square or G-test) were used to examine number buried and number of successful broods (counts).

In Trial 2, I observed the differences between habitat types between one species. The statistical analysis was an Independent Samples Student t-test that compared the mean of a variable of interest between two habitats. If the data was not normal, I used the Mann-Whitney test.

Results

Temperature

In Trial 1, the temperature was recorded for both surface and soil temperatures. The highest surface temperature was 118.2 degrees Fahrenheit with the lowest temperature being 29.9 degrees Fahrenheit in the meadow. At 10 cm depth in the meadow's soil, the highest temperature was 72.7 degrees Fahrenheit and the lowest was 47.2 degrees Fahrenheit. In the aspen, the highest surface temperature was 98.5 degrees Fahrenheit and the lowest was 43.2 degrees Fahrenheit. Within the soil of the aspen, the highest temperature was 67 degrees Fahrenheit and the lowest was 49.4 degrees Fahrenheit. The surface temperature of the meadow was much higher than the aspen (Mann-Whitney Test, $W = 266287$, $p = < 0.001$), while the temperature 10 cm below the surface in the soil did not differ (Mann-Whitney Test, $W = 222584$, $p = 0.068$).

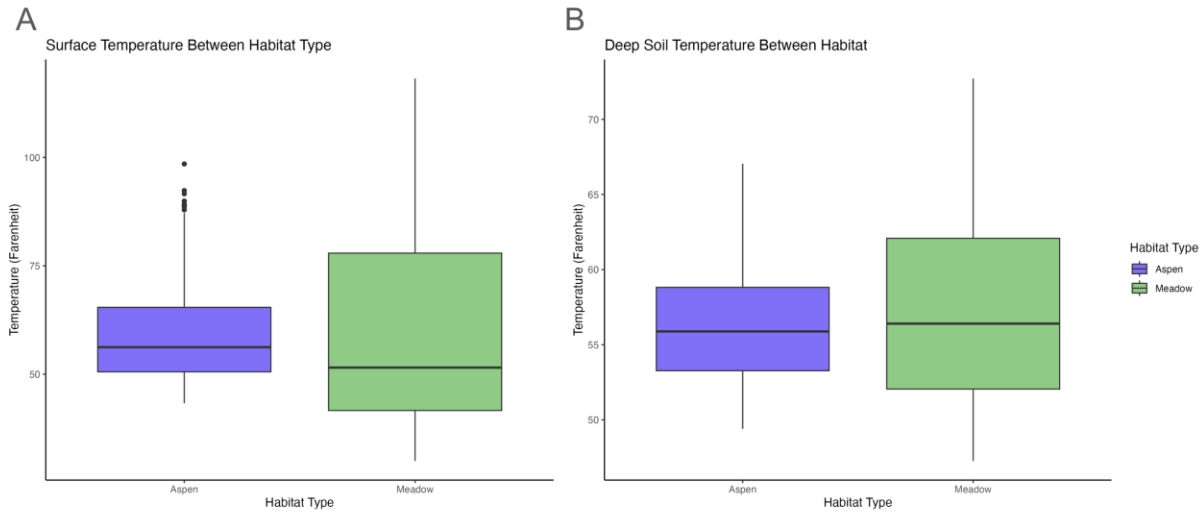


Figure 3: A) The surface of the meadow had a greater variation compared to the aspen. **B)** The temperature in the soil was comparable between sites, where the variation was greater in the meadow compared to the aspen.

As in Trial 1, Trial 2 recorded temperature for both surface and soil temperatures. At the meadow, the highest surface temperature was 117.2 degrees Fahrenheit with the lowest temperature being 32.6 degrees Fahrenheit. Within the soil of the meadow, the highest temperature was 76.7 degrees Fahrenheit and the lowest was 51.6 degrees Fahrenheit. In the aspen, the highest surface temperature was 88.6 degrees Fahrenheit and the lowest was 46.3 degrees Fahrenheit. Within the soil of the aspen, the highest temperature was 68.9 degrees Fahrenheit and the lowest was 52.4 degrees Fahrenheit. The surface temperature of the meadow was significantly higher than the aspen (Mann-Whitney Test, $W = 82488$, $p < 0.001$), while the temperature in the soil did not differ (Mann-Whitney Test, $W = 105839$, $p = 0.156$).

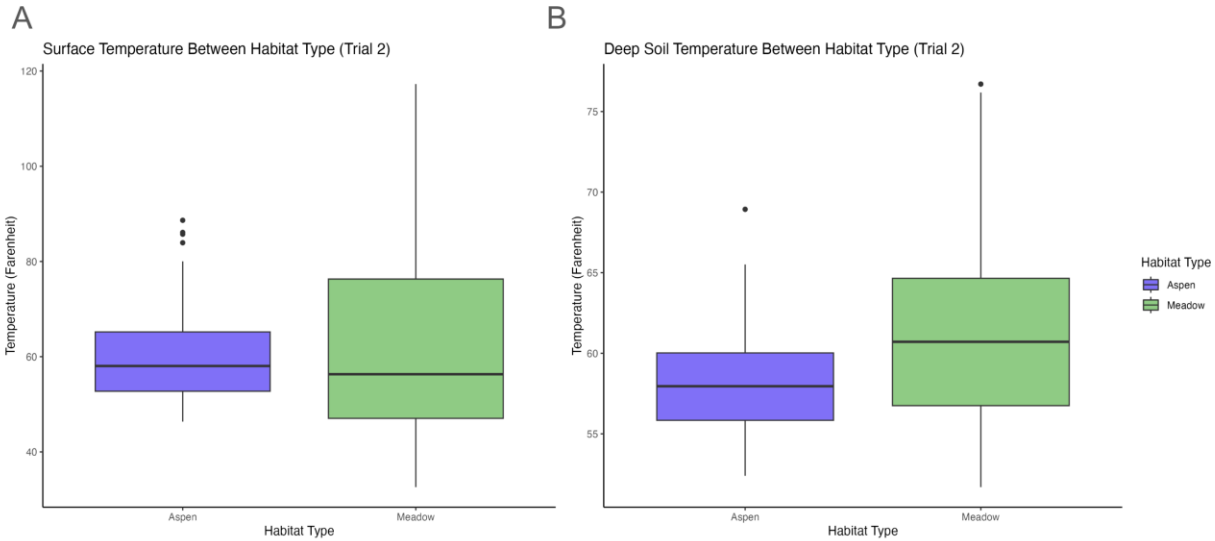


Figure 4: **A)** As with Trial 1, the surface of the meadow had a greater variation compared to the aspen. **B)** As with Trial 1, 10 cm below the surface of the soil, the temperature was comparable between sites, where the variation was greater in the meadow compared to the aspen.

Soil Moisture

As with temperature, soil moisture was measured at the surface and at the bottom of the can. In Trial 1, the average surface moisture in the meadow was 0.333 and in the aspen the average was 1.43. Within the can, the average soil moisture in the meadow was 2.2 and 3.9 in the aspen. The meadow was significantly drier than the aspen both at the surface (Mann-Whitney Test, $W = 161.5$, $p = 0.001$) and within the cans (Mann-Whitney Test, $W = 164.5$, $p = 0.001$).

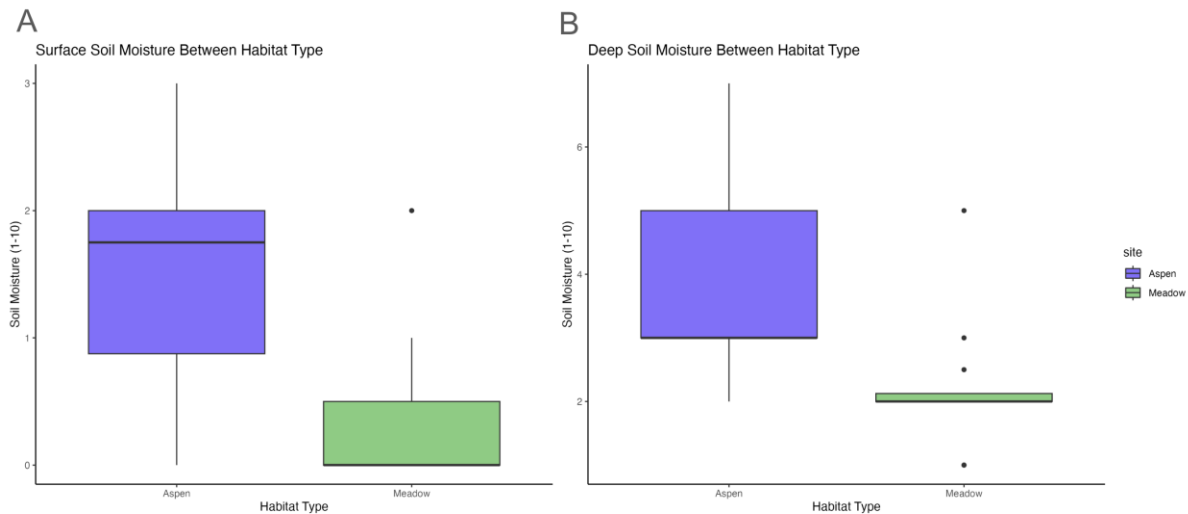


Figure 5: **A)** The aspen had a higher soil moisture on the surface of the soil compared to the meadow. **B)** The aspen had a higher soil moisture within the cans compared to the meadow.

For Trial 2, the average surface moisture in the meadow was 1.35 and in the aspen the average was 1. Within the can, the average soil moisture in the meadow was 2.14 and 1.76 in the aspen. The meadow and aspen did not differ in moisture at the surface (Mann-Whitney Test, $W = 170$, $p = 0.383$) or within the soil (Mann-Whitney Test, $W = 172$, $p = 0.186$).

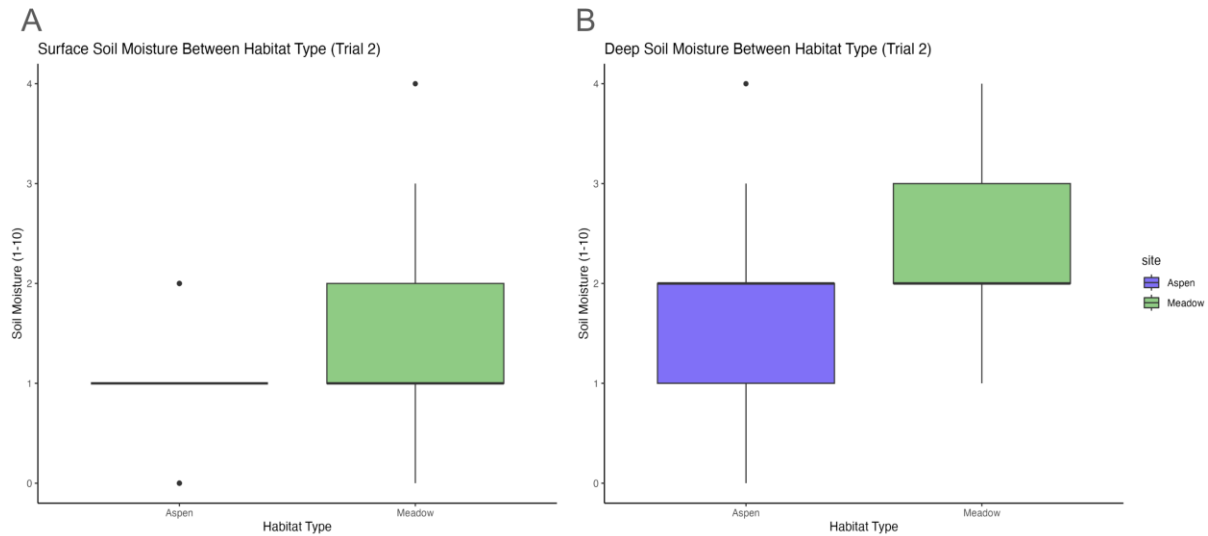


Figure 6: A) The surface soil moisture between sites is comparable. There is a greater distribution of moisture in the meadow. **B)** The soil moisture in the cans is also comparable, where aspen is slightly drier than the meadow.

Number of Mice Buried

Within the aspen, both *N. investigator* and *N. defodiens* pairs had fully buried carcasses out of ten cans each (20 total). In the meadow, *N. investigator* had 6 burials while *N. defodiens* had only 1 burial, having a total of 7 burials in the meadow. The number of mice buried in the aspens at the Research Meadow for Trial 1 did not differ between the species (G-test, $G = 0$, $df = 1$, $p = 1$), while in the meadow *N. investigator* buried significantly more carcasses than *N. defodiens* (G-test, $G = 3.96$, $df = 1$, $p = 0.046$). In terms of species, *N. investigator* buried similarly in both habitats, (G-test, $G = 1.01$, $df = 1$, $p = 0.314$), whereas *N. defodiens* buried more in the aspen habitat (G-test, $G = 8.547$, $df = 1$, $p = 0.003$).

For Trial 2, *N. investigator* buried 15/15 mice in the aspen and 12/15 in the meadow. As in Trial 1, there was no significant difference between aspen and meadow in the total number of burials (Chi-square, $\chi^2 = 0.33333$, $df = 1$, $p = 0.564$).

Table 4: A) Both *N. investigator* and *N. defodiens* buried all 10 carcasses in the Aspen, while in the Meadow there was a decrease in burial number. *N. defodiens* did not have as many burials in

the Meadow compared to *N. investigator*. **B)** As in Trial 1, Trial 2 had *N. investigator* buried a similar proportion of mice in the aspen and meadow habitats.

A

Number of Mice Buried (Trial 1)		
Species	Aspen	Meadow
<i>N. investigator</i>	10	6
<i>N. defodiens</i>	10	1

*Number of
Dead
Beetles*

B

Number of Mice Buried (Trial 2)		
Species	Aspen	Meadow
<i>N. investigator</i>	15	12

The
number of
deaths did
not vary
between the
aspen and
meadow

between the two species. In Trial 1, the total number of deaths was calculated based on the day the larvae were measured (August 1 and August 2, 2024). In the aspen, *N. investigator* had 3 deaths and *N. defodiens* had 7 deaths. In the meadow, *N. investigator* had 7 deaths and *N. defodiens* had 11 deaths. The number of deaths between aspens and meadows for *N. investigator* did not differ (G-test, $G = 1.645$, $df = 1$, $p = 0.199$), and neither was the number of deaths for *N. defodiens* (G-test, $G = 0.896$, $df = 1$, $p = 0.343$). The number of deaths between *N. investigator* and *N. defodiens* did not differ in the aspen (G-test, $G = 1.645$, $df = 1$, $p = 0.199$) or the meadow (G-test, $G = 0.896$, $df = 1$, $p = 0.343$).

In Trial 2, there were 22 deaths in the meadow and 10 deaths in the aspen for *N. investigator*. Between the two sites there were more deaths in the meadow than the aspen (Chi-square, $\chi^2 = 4.5$, $df = 1$, $p = 0.033$).

Table 5: A) *N. investigator* had less deaths overall compared to *N. defodiens*. The number of deaths is greater in the meadow than in aspen, but there is no significant difference in deaths between both habitat and species. **B)** As in Trial 1, the number of deaths in the meadow is greater than that of the aspen for *N. investigator*.

A

Number of Dead Beetles (Trial 1)		
Species	Aspen	Meadow
<i>N. investigator</i>	3	7
<i>N. defodiens</i>	7	11

B

Number of Dead Beetles (Trial 2)		
Species	Aspen	Meadow
<i>N. investigator</i>	10	22

Days Until Mouse Burial

In Trial 1, the longest time for burial for *N. investigator* in the aspen was 7 days and the shortest time was 1 day, while in the meadow, the longest time was 3 days and the shortest was 1 day. For *N. defodiens*, the longest time for burial in the aspen was 3 days and the shortest was 1 day, while in the meadow the longest and shortest day to burial was 2 days. The number of days until mouse carcass burial did not differ between the aspen and meadow within the Research Meadow (Scheirer Ray Hare, $df = 1$, $H = 0.298$, $p = 0.584$). However, while the relationship between species and days to burial was not significant, it seems to be biologically important (Figure 11). *N. defodiens* buries faster in the aspen compared to the meadow, and *N. investigator* buries faster in the meadow compared to the aspen (Scheirer Ray Hare, $df = 1$, $H = 3.616$, $p = 0.057$). There is no interaction between site, species, and the time until burial (Scheirer Ray Hare, $df = 1$, $H = 3.125$, $p = 0.077$).

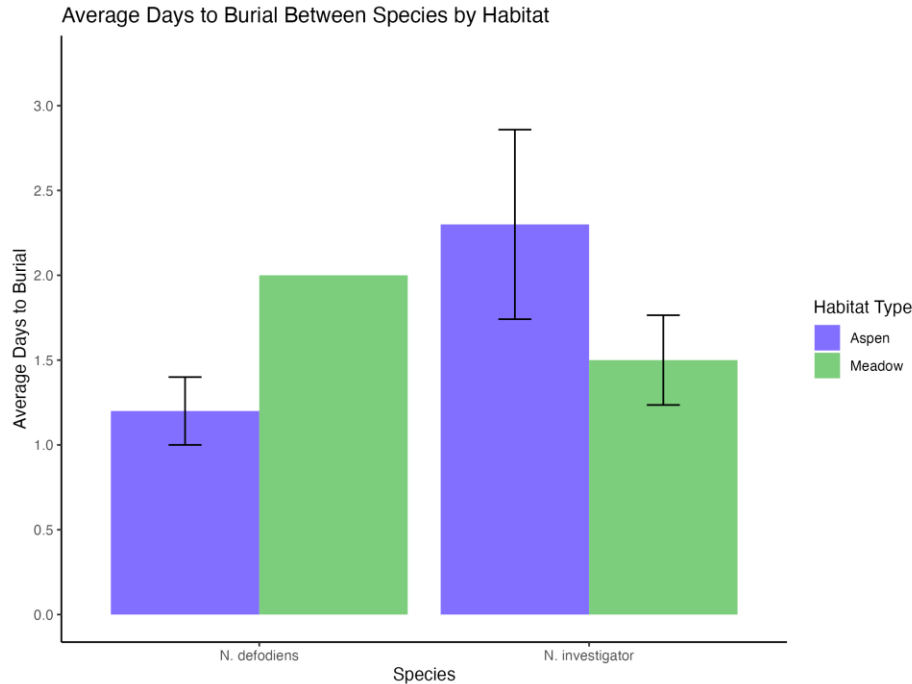


Figure 7: The time to burial has a clear distinction between species and habitat type. *N. defodiens* only had one burial, hence no error bar.

For Trial 2, the longest time for burial for *N. investigator* in the meadow was 7 days while the shortest was 1.5 days. In the aspen, the longest time

for burial was 3 days while the shortest amount of time was 1 day. There is no difference between aspen and meadows for *N. investigator* the amount of time until mouse burial (Mann-Whitney Test, $W = 69$, $p = 0.974$).

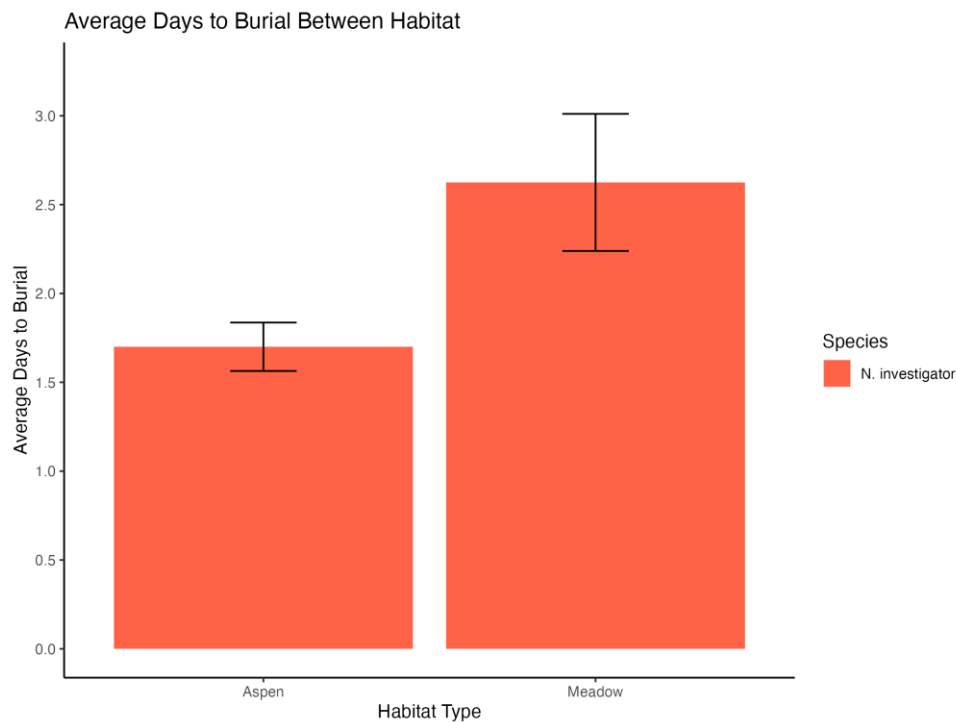


Figure 8: The average time to burial for *N. investigator* in the aspen was about 1 day shorter than the meadow.

Days Until Adult Parent Emergence

The days until adult parent emergence is split into male and female emergence dates. The days until adult parent

emergence are based on the number of successful broods. In Trial 1, the average number of days

until female adult emergence in *N. investigator* in the aspen was 16.4 days and 17.33 days in the meadow. For male *N. investigator* emergence, the average number of days in the aspen was 16.25 days and 17.33 days in the meadow. The female adult emergence in *N. defodiens* was 18.75 days in the aspen and for male *N. defodiens* emergence, the average number of days in the aspen was 16.25 days. The female and male parent adult emergence days for *N. defodiens* within the meadow were not counted as they had no successful brood. Only *N. investigator* had larvae in the meadow so there is no comparison with either male or female *N. defodiens*. The number of days until female adult parent emergence does not differ between aspen or meadow within the Research Meadow (Two-way ANOVA, $df = 1$, $F = 0.004$, $p = 0.949$) nor does species between aspen (Two-way ANOVA, $df = 1$, $F = 1.884$, $p = 0.203$). Like the females, the number of days until male adult parent emergence does not differ by site (Scheirer Ray Hare, $df = 1$, $H = 0.005$, $p = 0.939$), species (Scheirer Ray Hare, $df = 1$, $H = 0.02$, $p = 0.886$), or an interaction between site or species (Scheirer Ray Hare, $df = 12$, $H = 13.979$, $p = 0.302$). Comparing female and male emergence in Trial 1, with no official test done, the number of days until emergence are similar as they range between 14-18 days (Figure 9A and 9B).

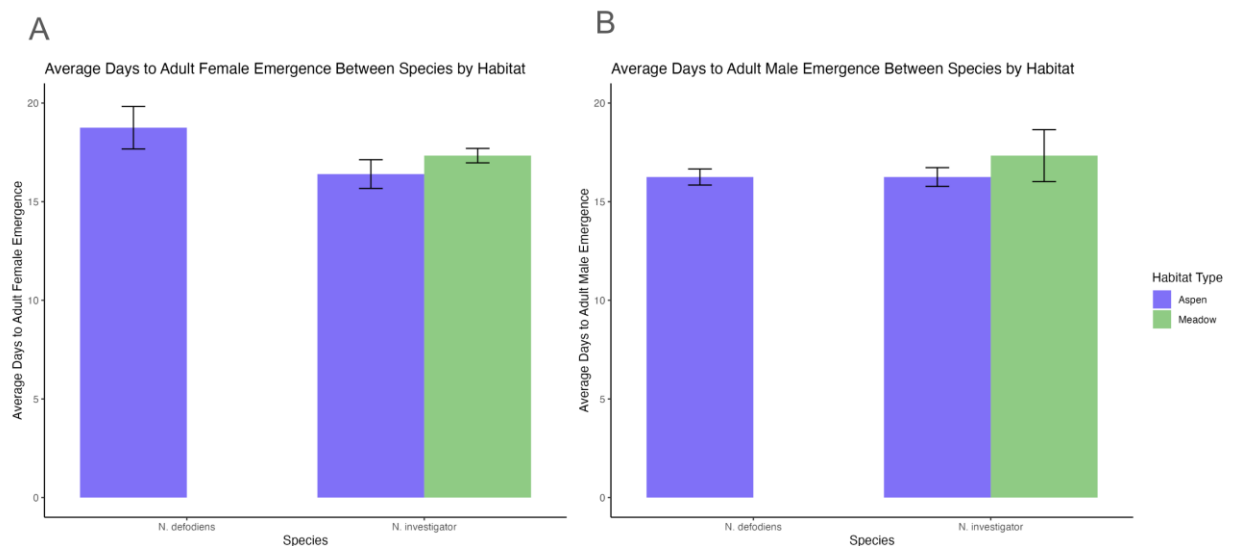


Figure 9: A) The average day for female adult emergence is longer in the aspens for *N. defodiens*, with no success in the meadows. *N. investigator* females have a comparable average day to emergence between both sites. **B)** The average day for male emergence is comparable between habitat and species, besides *N. defodiens* having no success in the meadow.

Like Trial 1, Trial 2 had the days to adult emergence split between male and females while only counting the pairs with successful broods. The average number of days in the meadow for female *N. investigator* was 13.75 days and 13.5 days in the aspen. For male *N. investigator*, the average number of days until emergence was 15 days in the meadow and 12.85 days in the aspen. The number of days until adult female emergence was no different between aspen and meadows (Mann-Whitney Test, $W = 20.5$, $p = 0.89$), but in male adult emergence, the

males left faster in the aspen than in the meadow (Independent Samples Student's t-test, $t = -2.37$, $df = 7$, $p = 0.049$). Between female and male emergence in Trial 2, with no official test done, the number of days until emergence is less in the males in the aspen than the females (Figure 10A and 10B).

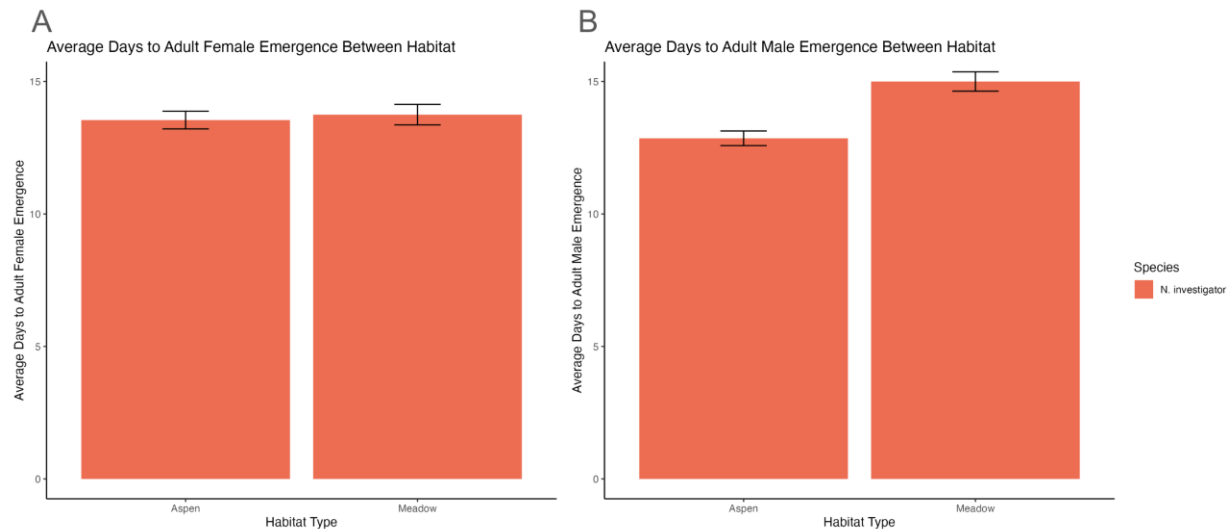


Figure 10: A) The average days until female emergence is comparable between habitats. **B)** The average days until male emergence is about 2 days faster in the aspen compared to the meadow.

Number of Broods and Number of Larvae

N. investigator was more successful (proportion of carcasses with broods) in the meadow than was *N. defodiens*. The total number of broods in Trial 1 in the meadow for *N. investigator* was 4, but for *N. defodiens* there were no successful broods. The aspen had 9 *N. investigator* broods, and 10 *N. defodiens* broods (Figure 13). There was no significant difference in the number of broods between species and site (Fisher's Exact test, $p = 1$).

For successful broods, the number of larvae/broods did not differ between the species (Two-way ANOVA, $df = 1$, $F = 0.068$, $p = 0.798$) or the sites (Two-way ANOVA, $df = 1$, $F = 2.779$, $p = 0.113$). *N. investigator* averaged 12.2 larvae and *N. defodiens* averaged 12.8 larvae in the aspen. In the meadow, *N. investigator* averaged 7.75 larvae while *N. defodiens* had no larvae.

Including the unsuccessful broods (0 larvae), *N. investigator* averaged 11 larvae and *N. defodiens* averaged 10.3 larvae in the aspen. In the meadow, *N. investigator* averaged 3.1 larvae while *N. defodiens* had no larvae. In the aspen, *N. defodiens* had two broods already pupating, so they were not counted. The number of larvae was higher in the aspen than the meadow (Scheirer Ray Hare, $df = 1$, $H = 18.56$, $p < 0.001$). There was no difference between species in the number of larvae (Scheirer Ray Hare, $df = 1$, $H = 0.920$, $p = 0.337$) nor was there an interaction

between species and site with the number of larvae (Scheirer Ray Hare, $df = 1$, $H = 0.554$, $p = 0.456$).

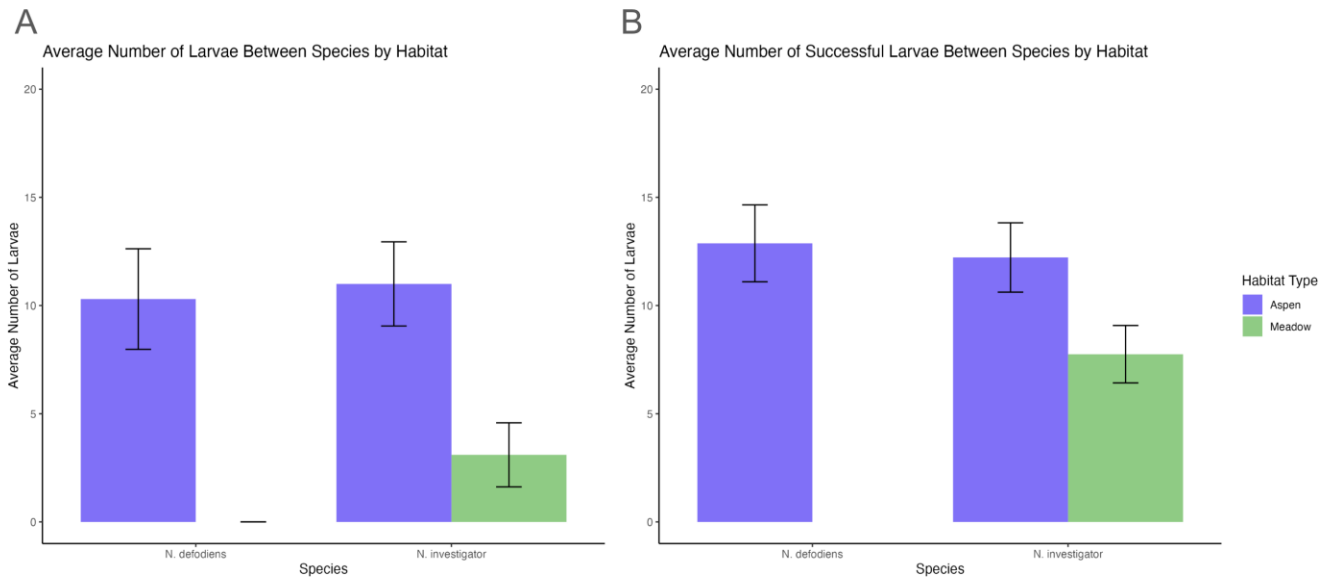


Figure 11: A) Including failed attempts (brood number = 0), the total reproductive success (# of larvae/brood) was significantly higher in the aspen than the meadow for both species. *N. investigator* had higher fitness in meadow than *N. defodiens*. **B)** When not including the broods, the number of larvae in the aspen is greater for *N. defodiens* and greater in the meadow for *N. investigator*.

In Trial 2, *N. investigator* had 9 broods in the meadow and 14 broods in the aspen, which is not significantly different. Within those successful broods, there are 2 broods still developing in the meadow, while in the aspen there are 3. The average number of successful larvae in the meadow was 8.14 larvae and 11.5 larvae in the aspen. Including the broods with zero larvae, the average number of larvae in the meadow was 4.38 and 10.58 in the aspen (including the broods with zero larvae) (Figure 17). There were significantly more larvae in the aspen than the meadow (Mann-Whitney Test, $W = 121.5$, $p = 0.017$). When looking at only the successful broods, there is no difference in the number of larvae between aspen and meadows (Independent Samples Student's t-test, $t = 1.505$, $df = 16$, $p = 0.151$).

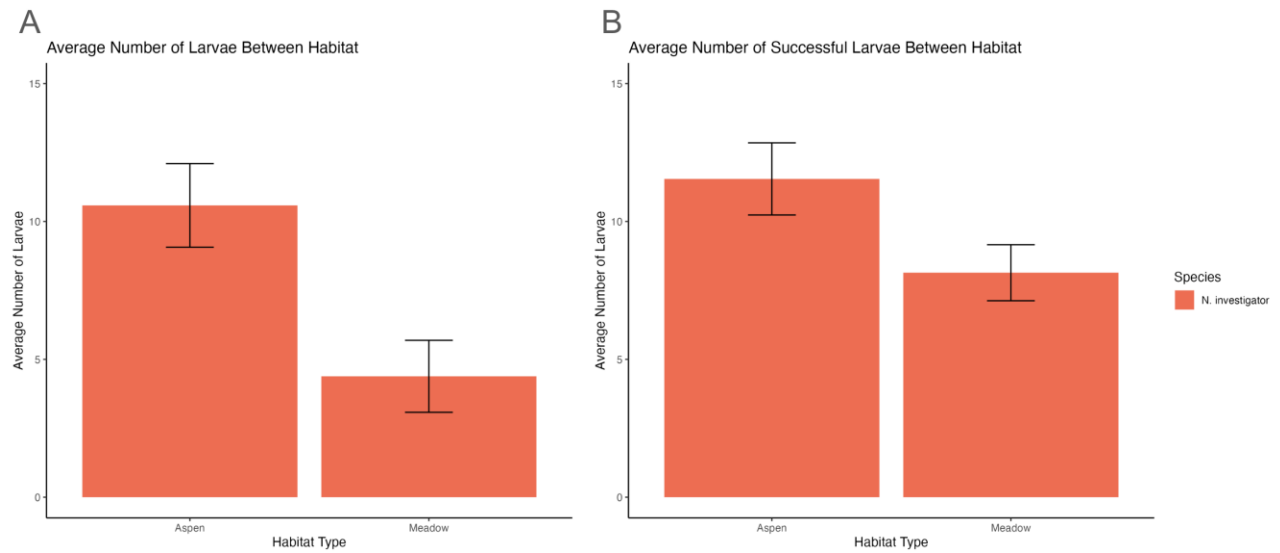


Figure 12: A) When including failed broods (number of larvae = 0), the average number of larvae between species in the aspen is greater in the aspen than the meadow. **B)** When not including failed broods, the number/successful brood is similar.

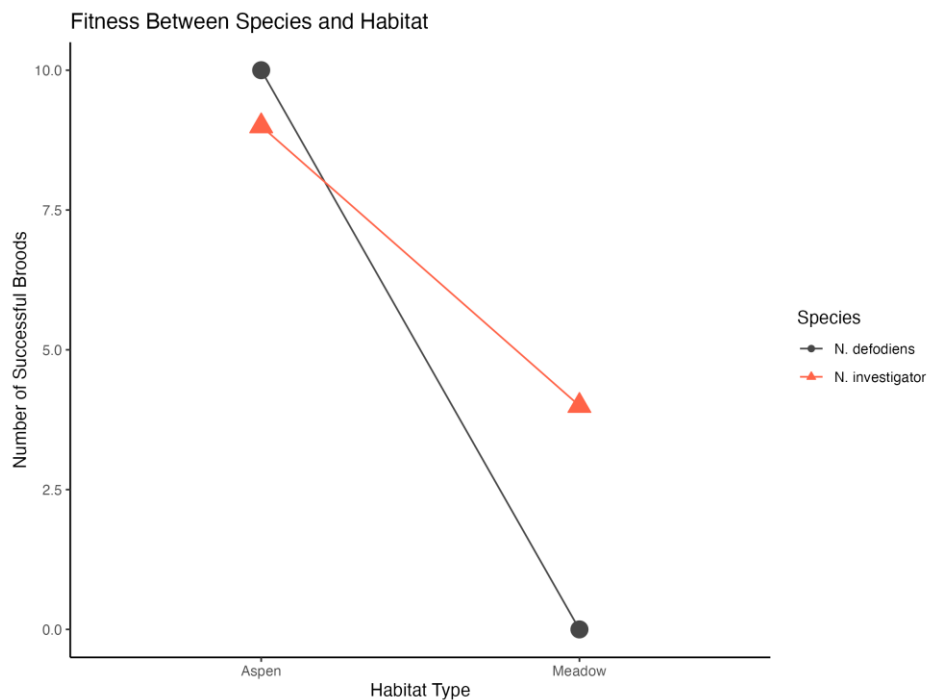


Figure 13: The number of successful broods in the aspen is higher in *N. defodiens* than *N. investigator*. In the meadow, the number of successful broods is higher in *N. investigator* than *N. defodiens*.

Size of Larvae

For Trial 1, the average mass of larvae in the meadow was 0.254 grams and 0.303 grams in the aspen for *N. investigator*. The average mass of larvae in the aspen for *N. defodiens* was 0.36 grams. There were no larvae in the meadow for *N. defodiens*, so there is no larval mass. By site, the average mass of larvae was greater in the aspen than the meadow (Two-way ANOVA, $df = 1$, $F = 31.64$, $p = < 0.001$). Only *N. investigator* had larvae in the meadow so there is no comparison with *N. defodiens*. In the aspen, the average larval mass was higher for *N. defodiens* than *N. investigator* (Two-way ANOVA, $df = 1$, $F = 37.42$, $p = < 0.001$). For total brood mass in Trial 1, the total mass was greater in the aspen than the meadow (Two-way ANOVA, $df = 1$, $F = 5.66$, $p = < 0.028$) (Figure 15). Only *N. investigator* had larvae in the meadow so there is no comparison for brood mass with *N. defodiens*.

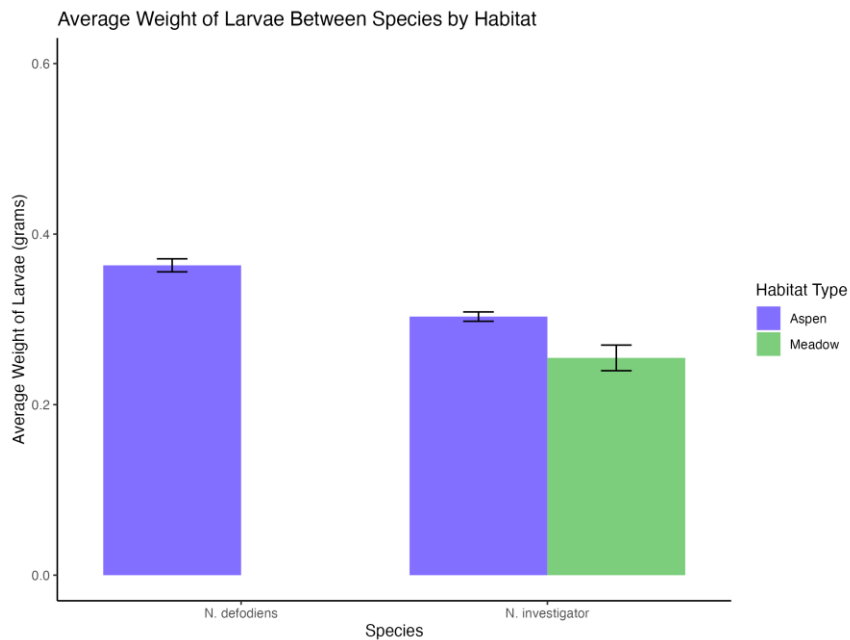


Figure 14: The average mass of larvae varied by site and species. *N. defodiens* larvae were larger than *N. investigator* in the aspen, but only *N. investigator* was successful in the meadow.

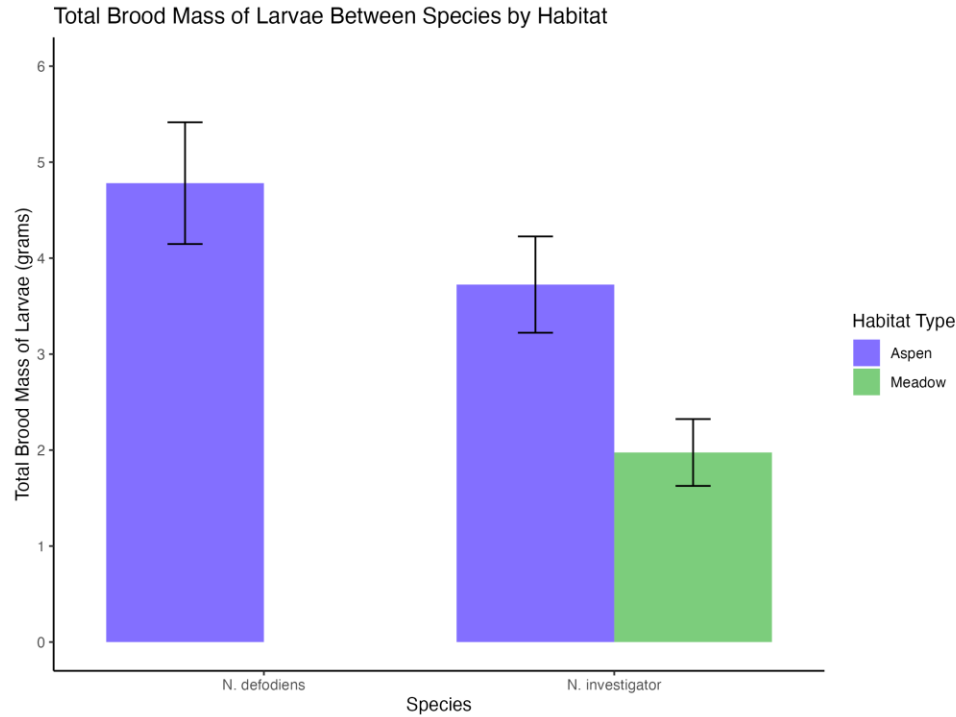


Figure 15: The total brood mass of larvae varied by species. As with the average larval mass, *N. defodiens* broods mass was larger than *N. investigator* in the aspen, but only *N. investigator* was successful in the meadow.

In Trial 2, (*N investigator* only, successful broods): the average mass of larvae in the meadow was significantly less than the aspen (Mann-Whitney Test, $W = 5418.5$, $p < 0.001$). For total mass, the larvae in the meadow were also significantly less than the aspen (Independent Samples Student's t-test, $t = 2.55$, $df = 16$, $p = 0.021$) (Figure 17).

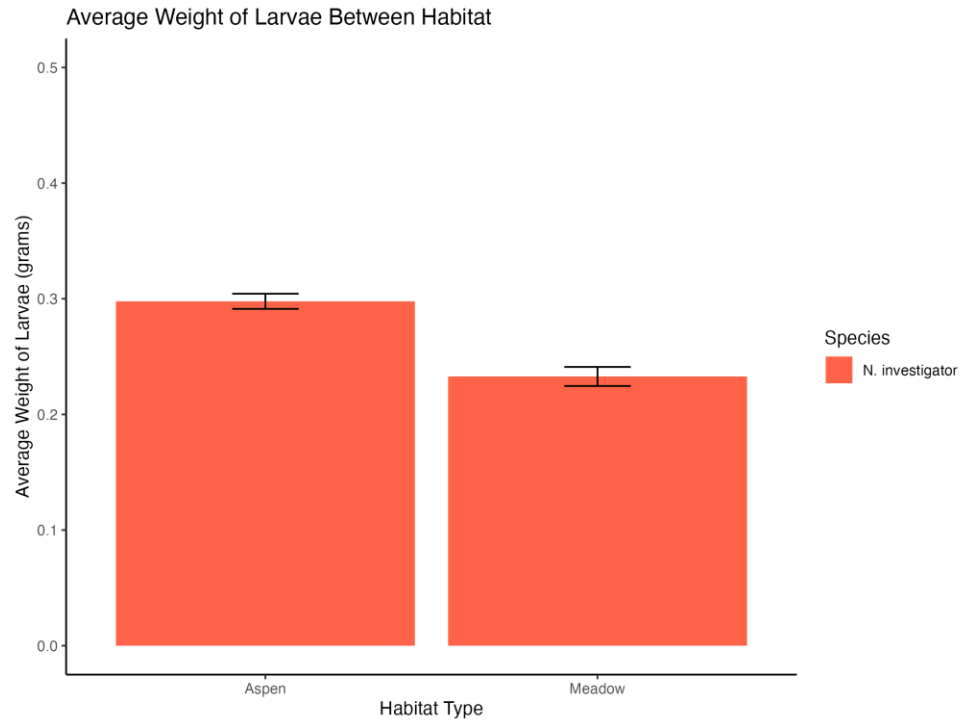


Figure 16: For *N. investigator*, the average mass (g) of larvae in the aspen was greater than the meadow.

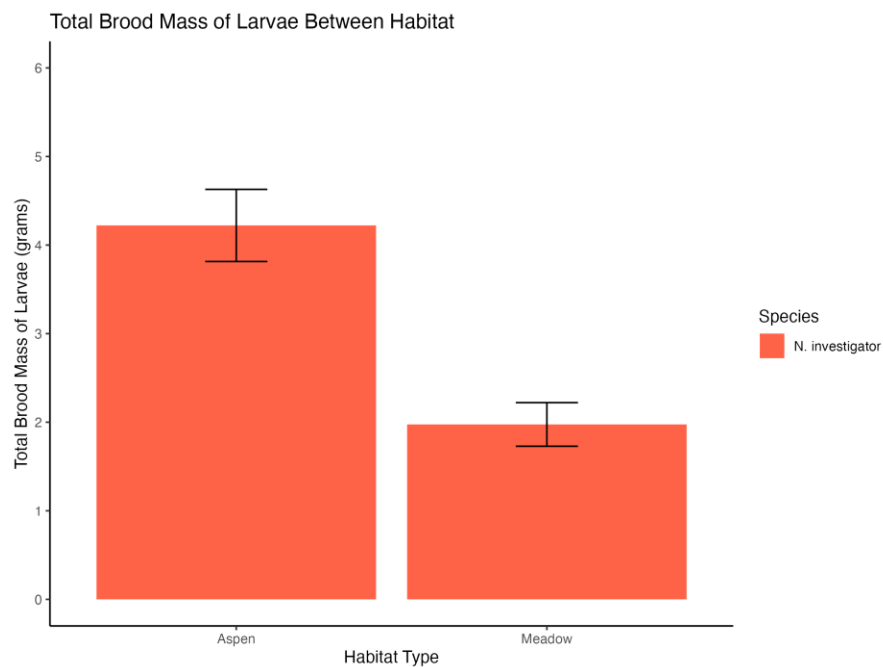


Figure 17: Like the average mass for *N. investigator*, the total brood mass (g) of larvae in the aspen was greater than the meadow.

Discussion

Results

For both Trial 1 and Trial 2, the soil temperature at the surface was extremely variable in the meadow compared to the aspen. The surface temperatures in both trials were significantly different by site which may have impacted the survival of the adults while burying the carcass. The soil surface temperatures exceeded 100 degrees Fahrenheit, which is lethal for adults (Merrick and Smith 2004). The temperatures 10 cm deep beneath the soil in the cans were more consistent and did not reach the extreme temperatures the surface temperatures reached. The consistency within the cans is similar between the two habitat types and may show that if the burying beetles can bury the carcass, the rate of development will be the same. The temperatures within the soil never reached over 80 degrees Fahrenheit, which has been shown to be lethal for developing larvae (Keller et al. 2021).

With soil moisture, the Research Meadow with Trial 1 had a separation between habitat types where aspens were more wet at both the surface and in the soil compared to meadows. This is consistent with another paper done at the RMBL, where the meadow was consistently dry in all temperature measurements (Ortega et al. 2012). However, soil moisture for both aspen and meadows can be similarly compared to other habitat types (Buck et al. 2012). The similarity can be seen in Trial 2, where the aspen and meadow were comparable, but the meadow demonstrated slightly higher soil moisture (NOTE: we also watered and shaded the meadow plot for the first week). The reasoning behind the dryness in the aspen could be due to the location, where the sun had more access to the plot. The dryness can also be associated with how the leaves placed on the top of the soil prevented it from becoming wet, especially when it rained. If the soil moisture is high, the beetles can withstand higher temperatures (Buck et al. 2012).

In Trial 1, both species buried well in the aspen, while in the meadow both suffered casualties. *N. investigator* had better success in the meadow than *N. defodiens*, which is consistent with knowledge between the two species (Buenher 2001, Oliver and Smith 2013, Anderson and Peck 1985). Both species suffered within the meadows and had fewer burials overall. A reason for the failed burials could be that the temperatures were too hot and that the soil was dry. Within the research meadow, both the surface temperature and soil moisture were not favorable for either species, but *N. investigator* was able to handle those conditions better. Another factor to *N. defodiens* having less burials is that they bury the mouse by placing vegetation over the carcass (Buenher 2001). At the meadow, there was no vegetation for *N. defodiens* to bury with. In Trial 2, the number of burials was not different between habitat types.

The number of dead beetles has a similar pattern in Trial 1 and in Trial 2. The aspen had less mortality than the meadows for both species. As with the number of burials, the conditions the beetles had to bury in play a major factor in whether they were successful. Many of the beetles that didn't end up burying died. This could be due to the meadow being too hot and too dry, which is consistent with previous studies and with the data collected (Bedick et al. 2006). *N.*

defodiens had the most deaths within the meadow, as the species is an early emerger in the summer and is found mostly in the aspen (Buehner 2001, Anderson and Peck 1985).

For Trial 1, the average number of days until burial demonstrates a possible mechanism for coexistence. *N. defodiens* buried faster in the aspen compared to *N. investigator*, while *N. investigator* buried faster in the meadow than *N. defodiens*. The slow burial in the meadow for *N. defodiens* can be due to previously mentioned factors of temperature, soil moisture, and burial type. For *N. investigator*, the aspen may be slow to bury as there may be other factors into choosing a site that plays into their fitness. *N. investigator* has been observed to prefer open habitats and have less burials in the shade, which can play parts in developmental rate and size (Smith and Heese 1995). For Trial 2, *N. investigator* did bury faster in the aspen compared to the meadow, but the difference is not significant. A factor that could play in the data seen in Trial 2 is that the aspen site was similar in condition to the meadow in terms of soil moisture, but cooler.

The days until adult emergence were not different between site and species between both species in Trial 1. Females did have a broader range of when they left in the aspen between both species, whereas male emergence dates were similar between the species in the aspen. For Trial 2, female emergence was comparable between habitats, while male adult emergence was sooner in the aspen compared to the meadow. Between males and females, the days until emergence were similar in Trial 1, while in Trial 2 males left earlier than females in the aspen. There were signs of earlier male emergence, which has been found in previous studies.

The average number of larvae, both Trial 1 and Trial 2 have higher amounts of larvae in the aspen and low numbers of larvae for both species. The differences in the aspen and the meadow do have temperature differences on the surface but have similar temperatures in the dirt. The meadow did have warmer soil, which may have effects on eclosure in the future (Keller et al. 2021). The temperature did not reach 80 degrees Fahrenheit within the soil in either Trial 1 or Trial 2 in either habitat, so the mortality of the larvae may be caused by other factors, such as the carcass drying out more. While there was high success in the aspen for *N. investigator*, there may be consequences that will occur later in development, such as slower development which impacts winter emergence dates (Smith and Heese 1995). While there was no official test comparing the number of larvae for *N. defodiens*, there is significance in having zero larvae. Based on the number of successful broods per species and between habitat, *N. defodiens* has a higher fitness than *N. investigator* in the aspen and *N. investigator* has a higher fitness in the meadow than *N. defodiens*, which has been previously seen (Oliver and Smith 2013). However, as the number of broods between site and species is not significant, *N. investigator* does pose as a generalist in terms of brood size.

The size of larvae was bigger in the aspen overall in both trials than the meadow. Like the number of larvae, *N. defodiens* also demonstrates higher fitness in the aspen as the average weight of the larvae is higher than *N. investigator*, and *N. investigator* has a higher fitness in the meadow than *N. defodiens*. Unlike the paper written by Smith and Heese, the weight of the larvae was larger in the aspen for both Trial 1 and Trial 2 rather than being smaller for *N. investigator* (1995). Temperature and soil moisture could have affected the size as the meadow

had higher mortalities and less parental care. While *N. investigator* has high success now, there may be consequences to development at the later stages.

Methodology

This experiment could be improved in a variety of ways that may make the data more accurate. The soil moisture readings started halfway through Trial 1, so we do not have data when the carcasses were being buried. For collecting the days until burial, these need to be checked every day to get an accurate count of how long it takes for the beetles to bury as they can bury quickly in a span of a day. In this experiment for Trial 1, July 16 was a day for observation while July 14 and 15 were skipped. In the future, having each day checked can improve the accuracy of the data collected. Another area of improvement is to allocate time for collecting the larval weight and the count as it took longer than expected due to the brood amounts. I also could go more in depth on the surface temperature on when they were burying, which is when the beetles were most impacted by the dry soil moisture and hot temperatures.

Future Directions

As this is an ongoing experiment, the next stages are to count the successful number of adult offspring that emerge later this summer and next summer, the days to adult offspring emergence, and measuring the size of the offspring. This is a critical part of the experiment as this is where I can see how the aspen and meadow affects the species in later development.

As for another experiment, I am interested in looking at the temperature niche in time rather than in space (as in early summer versus mid-summer than aspen versus meadow). In the summer, the early season is cooler and has more moisture, whereas mid-summer is hot and dry. I would like to do a similar experiment between species, to see how the temperature niche of reproduction is similar or different.

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