

The Fate of Burying Beetles and their Carcasses: Hardships, Competition and Environmental Factors

Abstract:

Burying Beetles face both intraspecific and interspecific competition throughout the whole process of rearing a brood. *Nicrophorus* (burying beetles) are a well-studied genus that provides bi-parental care on the carcass resource that they bury for rearing their brood. Because carcasses are often rare, there is a threat of intruders that may take over the resource. Lab studies have shown a significant cost of intruders, and the roles that the adults play in defense. However, few studies have been done on intraspecific intrusion in the wild, or how these might be affected by developmental stage, carcass size, and habitat type. This research at RMBL can help provide further information on the factors that influence the reproductive success of *N. investigator* and *N. defodiens*. Overall, no successful intrusion happened in any of the experiments with 72 mice placed out across 2 experiments. Aspen habitat had the most successful reproduction with 7 total successful broods of larvae, five *N. defodiens*, and two *N. investigator*. Meadow overall had more beetles finding the carcasses but had almost every single carcass abandoned due to factors such as competition, soil moisture, and high temperatures. The meadow had a single successful brood of larvae with *N. investigator*. Intrusion might not happen as frequently as previously thought in the wild.

Intro:

Parental care is common in many vertebrate species, as it increases the young's survival (Gross, 2005). Biparental care is rarer, found primarily in birds, fish, and some mammals (Royle et al., 2016). The parents work together as bi-parental care ensures the offsprings fitness is greater than the fitness loss of the parents from the lack of multiple reproduction events (Pilakouta et al., 2018). Biparental care is even more rare in insect orders with only 3 orders of insects that have bi-parental care with the father of the offspring helping the young (Suzuki, 2013). Burying Beetles (*Nicrophorus spp.*) are unique in that they are one of the few insect species to exhibit this behavior. Naturally, Burying Beetles have been of interest for scientists to

understand their ecology. Burying beetles are important detritivores that help consume carrion and nutrients into the environment that increase soil fertility (Hoback & Peterson, 2013). These beetles will use a wide variety of small carrion to lay their eggs and rear their broods, including small mammals such as mice, gophers, and voles (Smith & Heese, 1995). The availability of these resources is low, which results in intrasexual competition between multiple males and females attempting to claim the carcass for their future offspring (Scott, 1998). After being victorious the new future beetle parents will begin to bury the carcass to begin the process of reproduction (Scott, 1998). Burying Beetles also use oral and anal secretions to prevent bacterial growth (Trumbo 1994). They maintain the carcass and feed the young, leaving only when the larvae have nearly consumed the carcass.

However not much is known to why male burying beetles stay in the brood chamber. Males tend to leave first, up to 7 days before the female (Scott, 1990). Females then continue to take care of young for about an extra week before they take off and let the larvae pupate (Scott, 1990). One hypothesis is that male burying beetles stay around because of intra or inter-specific competition (Scott, 1990). Males often are specialized in defense in certain *Nicrophorus* species, this allows them to protect the female from other potential males. (Trumbo 2022). A pair of *N. defodiens* are more likely to fight off intruders and prevent infanticidal takeovers (Trumbo 2022). There have been several lab and field studies that measure carcass discovery and burial. For example, Wilson et al. 1984 in the field measured time to discover carcasses, burial depth, number of parents; then in the lab they investigated how carcass size affects brood success. Another field experiment determined the intrusion rates in *N. defodiens*, by placing a pair on the mice and then measuring intrusion (Trumbo 1990). In this study, I investigated carcass discovery and burial, and measured intrusion rates in the wild. Three species at RMBL (Rocky Mountain Biological Laboratory) (*Nicrophorus defodiens*, *Nicrophorus guttula*, and *Nicrophorus investigator*) co-occur both temporally and spatially, however only *Nicrophorus investigator* and *Nicrophorus defodiens* were studied. In this field experiment I placed carcasses of native rodents in two habitats and asked the question: What factors influence reproductive success in wild populations of burying beetles?

Methods:

Old Deer Creek Trail is the study area at the Rocky Mountain Biological Laboratory in Gothic Colorado see map below.

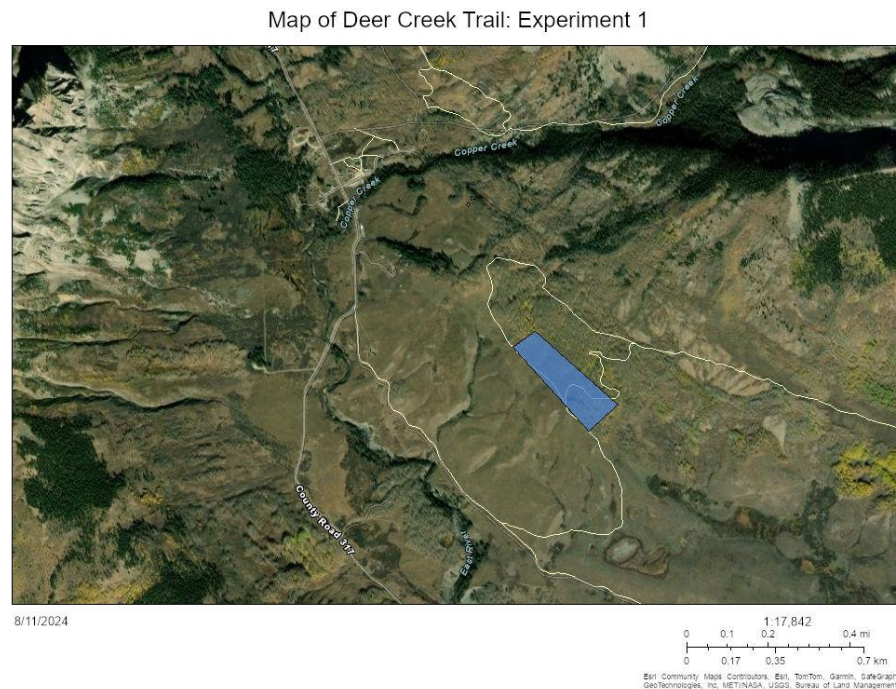


Figure 1. Map of Deer Creek Trail in Gothic, Colorado apart of Experiment 1 in this study

The Deer Creek study area has a mix of aspen and meadow important for the experimental design. For phase 1, I set up two transects in a U shape to better accommodate checking each carcass. One transect was in the meadow habitat while the other was in the aspen forest. Deer mice (*Peromyscus maniculatus*) carcasses were used. The Deer mice were salvaged from the cabins on RMBL property (Salvage permit, Scientific Collection License #3036077078). Carcasses were split into 3 size categories: small (14-15 g; N=20), medium (22-24g; N=20), and large (29-31 grams, N=12). The two trials started 7 days apart (July 8 and July 15, 2024). A total of 52 mice were placed on the transects in a random order, 40m apart. The leg of the mouse had a string and aluminum tag attached so it could be located visually by the string or with a metal detector (Garrett Pro-Pointer All terrain). The mice were placed onto the ground.

Once the mice were placed, they were checked daily. At each check the number and species of burying beetle was noted. I continued to check daily until a clear dominant male and female won the carcass. This was determined once the beetles were observed to bury the carcass, or when there were only 2 beetles left. I then marked these beetles with a unique (to the pair) needle tattoo into the elytra. Once they buried, I waited approximately 7-10 days of development

(the window for intrusion noted in Trumbo 2022). I then dug up the carcass and tried to locate the parents. If they were present, I noted whether or not they were the marked individuals or whether there was a new individual present (potentially an intruder). I also noted the presence of larvae.

For phase 2 of my experiment, I ran a semi-wild experiment with the beetles in the Research Meadow of Rocky Mountain Biological Laboratory.

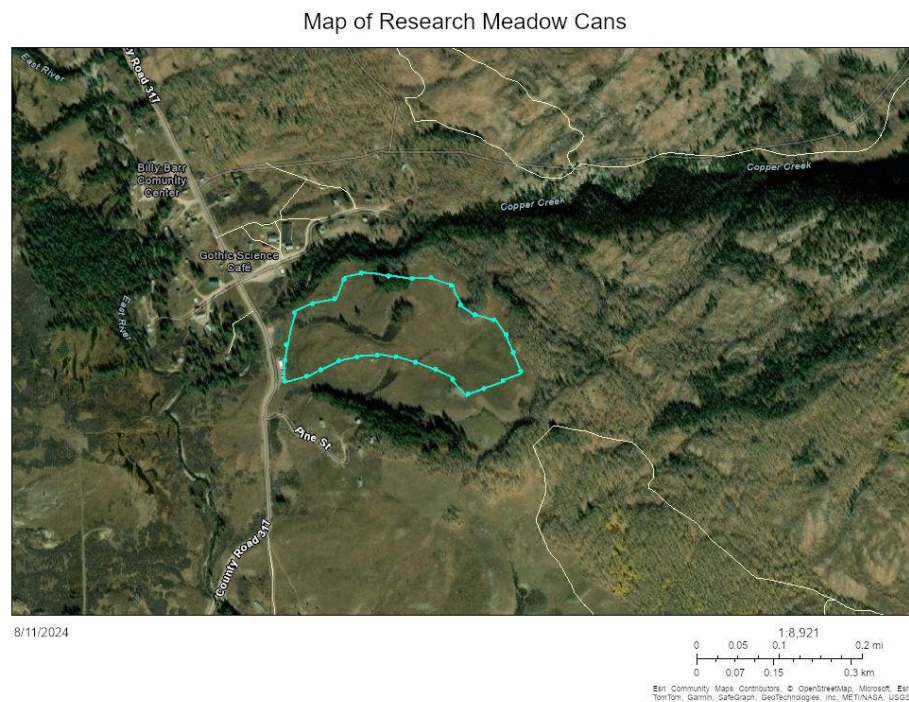


Figure 2. Map of the Rocky Mountain Biology Laboratory Research Meadow Cans in Gothic, Colorado used for Experiment 2.

I used captive wild caught *N. investigator* and randomly paired the males and females together. I made unique elytra tattoos for each pair. I used coffee cans buried in the ground to contain soil, mice and beetles. These cans were placed around 40- 50 meters apart. I placed the beetle pairs on a random sized mouse either small (15-16g) or medium (22-26g), covered the can with a screen lid, and then allowed them to bury. The medium carcass range had to be modified for this experiment as we used up much of the medium sized ranged carcasses in the previous experiment. I then put water on the surface to make the soil a little moist for beetles' survival. I checked the carcasses once a day until the pair buried the carcass. Once the pair buried, I

removed the lid allowing other beetles to intrude into the cans from the wild. I then waited 7-8 days to check on the beetles and unbury them to determine if larvae were present and if intrusion had happened or not. Pairs were determined to have no intrusion if larvae were present with no clear indication of infanticide, and the adults were the ones that were marked at time of burial. In some cases, after multiple checks, only 1 parent was ever seen on the carcass with the potential of a single parent raising the brood. This applied to both experiment 1 and experiment 2.

Data and Statistical Analyses: I ran χ^2 as my statistical analysis to determine if the results are considered expected results. I expected the variables to not be different, so the expected would be 50% of one variable and 50% of the other. I made tables showing the comparisons between each experiment as well as some graphs to showcase my results.

Results:

Carcass Discovery. *N. investigator* and *N. defodiens* were counted each time I observed both the meadow and the aspen habitat and a comparison between the number of individuals of a species and habitat preference was compared using a chi square test and graphed below.

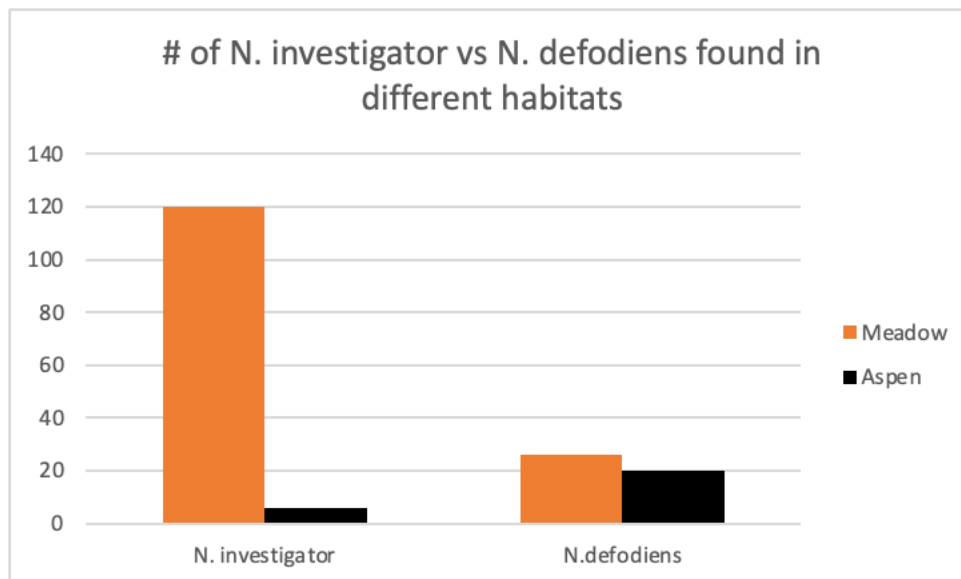


Figure 3. Shows the number of *N. investigator*, and *N. defodiens* in 2 different habitats. *N. investigator* are significantly more in the Meadow, and *N. defodiens* are significantly more in the aspen.

Significantly more individuals of *Nicrophorus investigator* (120) were seen in the meadow across 2 weeks than individuals of *Nicrophorus defodiens* (26) ($\chi^2 = 1$; p-value < 0.0001).

Overall fewer individuals were found on carcasses in the aspen forest (146:26). In the aspen, there were significantly more individuals of *N. defodiens* (20) compared to *N. investigator* (6) ($\chi^2=1$, $p=0.05$).

Comparing Habitat type and week of experiment

Results from observations:

In this experiment in the meadow habitat, I saw up to 4 beetles on one carcass for 2 days trying to claim a carcass.

Nicrophorus investigator were more likely to be both seen and successfully bury in the meadow compared to *defodiens*. But *defodiens* still buried in the meadow but just less frequently than the *Nicrophorus investigator*.

Habitat type differed in the number of mice found, had larvae, and confirmations of no intrusion.

Table 1: A) Number of mice that were buried by beetles, found but not buried, had larvae on them, or had confirmations of no intrusion in both meadow habitat and aspen habitat. B) Results of mouse carcass offerings in the first versus second week of the experiment. Here, the number of mice buried significantly differed.

A	Habitat	
Mouse Status	Meadow (N= 27)	Aspen (N= 25)
Found	26	15
Burial	13	13
Avg # of Beetles	2.13	1.4
No Intrusion	no data abandoned	8
Larvae	0	7

B	Week		
Mouse Status	1 (N=28)	2 (N=24)	
Burial	19	7	***
Found	25	16	

Larvae	5	2	
No Intrusion	5	3	

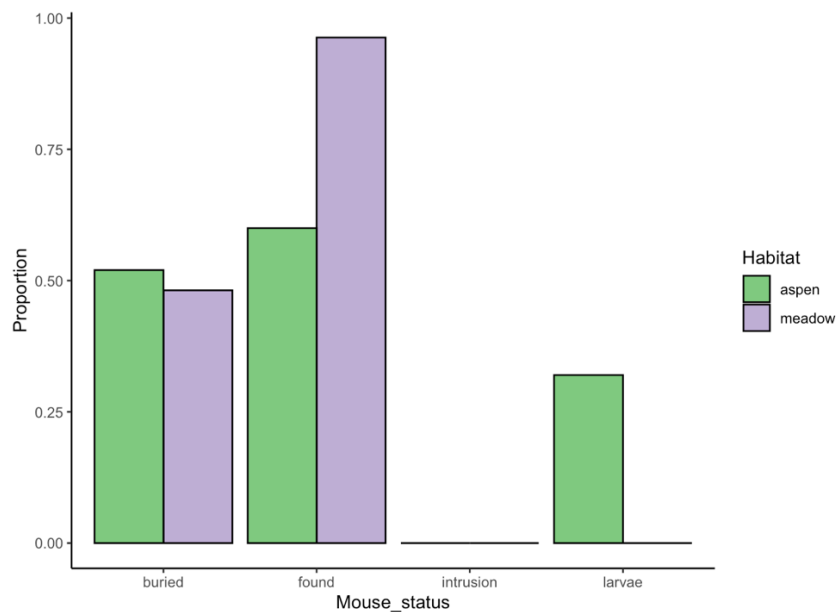


Figure 4. Shows proportion of buried carcasses, carcasses found, proportion of no intrusion, and larvae present on buried carcasses.

Table 1A shows that carcasses were found more often in the meadow than in the aspen ($X^2 = 1$; $p\text{-value} = 0.0858$), but only 50% of those found were buried. In the aspen, fewer were found, but they were more likely (87%) to be buried. When the buried carcasses were dug up, 8/13 carcasses in the aspen had adults present, but none of the carcasses in the meadow had adults present. Beetles were only reproductively successful in the aspen with 7/8 buried carcasses with larvae. No intrusion was found in any of the aspen pairs. Of the successful beetles in the aspen, 5 pairs were *Nicrophorus defodiens* and 2 pairs were *Nicrophorus investigator*.

In Table 1B combines the aspen and meadow habitats and shows the comparisons of the carcasses buried, found, larvae present, and confirmed no intrusion between the two phases/weeks. Overall, beetles were more successful in finding, burying, and rearing larvae in week 1 than week 2. Week 1 (July 8-14) was cooler than week 2 (July 15-21). The comparison of buried carcasses between weeks 1 and 2 is significant ($X^2 = 1$; $p\text{-value} = 0.0186$)

Does Carcass Size Affect Burial and Success of Brood?

Table 2: Small (15-16g), medium (22-24g), and large (29-31g) carcasses were used in this experiment. The number of carcasses of each size class buried by beetles did not differ.

	<u>Number of carcasses buried by size</u>	<u>Number with a brood</u>	<u>Success (brood/buried)</u>
<u>Small</u>	<u>8</u>	<u>3</u>	<u>.375</u>
<u>Medium</u>	<u>10</u>	<u>3</u>	<u>0.300</u>
<u>Large</u>	<u>7</u>	<u>1</u>	<u>0.143</u>

Nicrophorus beetles buried carcasses in all three size classes in the same proportion as was available. Both species had higher success on small and medium carcasses than on large ones.

Experiment 2

In this experiment I observed adult burying beetles on the screen lids of the cans, before the carcass was buried. I also noted that four of the marked parents died in the can before they could bury the carcass (potentially due to unforeseen hot weather).

Table 3: Shows how many carcasses out of 20 were successfully buried, had no intrusion and had larvae. 2 carcass sizes were used small(15-16g) and Medium (22-26g).

	Habitat
Mouse Status	Research Meadow n=20
Buried	12
No Intrusion	12
Larvae	1

In Table 3, of the 20 carcasses and pairs of beetles placed in cans, only 12 ended up successfully burying. The ones that didn't bury typically had a dead parent on them. When the buried carcasses were checked after 7-8 days only one of the carcasses had larvae still feeding on the carcass. An unmarked male was found in this can with a dead marked male, and no female was found. The larvae were too big, and no evidence of infanticide was seen in the can. As soon as I placed the unmarked male back into the can, the beetle flew off. Thus, this did not meet my definition of successful intrusion. In the remaining 11 cans without larvae, there were either one

or two marked parents (5 cans), no parents (4 cans) or unmarked beetles (2 cans). Most of the carcasses were dried out and not viable for larval development.

Discussion

In my experiment, reproductive success was quite low under field conditions (8 broods on 72 carcasses), especially in the open meadow habitat when compared to the aspen forest. This low success in the meadow was likely due to higher competition from other *Nicrophorus* beetles, which delayed the burial time of the carcasses in the meadow. This left the carcass out in the sun causing the carcass to dry out more before burying. Even when a pair was successful at burying and they went through all the steps to ensure the carcass could be used for offspring, they ended up without larvae and abandoning. Abandonment was likely due to the dryness of the carcass. Even with quick burials after a day or 2 in some cases, the carcass still ended up either abandoned or eaten by carnivores. Meanwhile in the aspen habitat, the soil was much moister and cooler, allowing easier burials for the beetles. Beetles were less abundant, and carcasses were found by fewer individuals. This led to less competition in the aspen which resulted in higher success rates in burial and rearing a brood. For both species, the likely tradeoff for this lower competition is a potentially longer amount of time to rear a brood due to the cooler temperature in the aspen (Smith and Heese 1995).

Nicrophorus defodiens had higher success in the aspen habitat, both in terms of locating and burying and successful larvae. Five *N. defodiens* pairs successfully reared a brood, with a 6th likely to follow but didn't have larvae yet. The two *N. investigator* that were successful at rearing a brood in the aspen may have done so due to the short distance from the edge of the aspen to the meadow. Burying beetles in the meadow habitat were able to locate carcasses at a higher rate compared to the aspen forest. This could be due to the large number of trees blocking wind and scents or cooler temperatures delaying bacterial decomposition which are used for beetles to smell and locate the carcasses. Burying Beetles use the sulphur containing chemicals emitted from decomposing carcasses to locate the carcass (Kalinova et al. 2009). The meadow is open, and the smell of the carcass could reach the beetles at farther distances, allowing the beetles to locate the carcass easier. Carcasses were visited by burying beetles for feeding even after the carcass was considered mummified and unsuitable for breeding.

Week 1 and week 2 of the experiment had a significant difference in the number of burials experienced within the timeframe. This can be likely attributed to the dryness of soil as

well as temperature outside. Week 1 had a higher max temperature with more soil moisture, compared to the second week that had a little lower temperature but lower soil moisture. Burying beetles require moisture to function or risk drying out from the heat. This more than likely lowered the activity of the beetles during the day, especially in phase 2. In terms of carcass size, there was no major difference between the size types and burials. All size classes were acceptable to the burying beetles. The beetles are attracted to a carcass on smell and did not prefer a size when burying. This is surprising, as carcass size is well-known to affect both the size and the number of larvae able to be raised, and *N. investigator* was previously found to reject carcasses smaller than 16 g. (Smith and Heese 1995). It's possible that *N. defodiens* accepts a smaller carcass size than *N. investigator*.

When comparing number of *N. investigator* and *N. defodiens* across the different habitats it is clear that *N. investigator* favors the meadow. *N. investigator* had over 4.5 times more individual beetles counted on carcasses compared to *N. defodiens*. *N. defodiens* clearly showed that they are more common in the aspen habitat. Total burying beetles compared by habitat is 146 observed beetles in meadow to 26 in the aspen.

In previous studies captive *N. defodiens*, showed that around 58% single parents, and 40% pairs had lost to intrusion (Trumbo 2022). My results suggest that successful intrusion is not as common in the wild as previously theorized and this opens many new potential research questions. Intrusion has been regarded as one of the most important factors in Burying Beetle reproduction. In this study, if environmental conditions are not at optimal levels the parents cannot raise a brood, making intrusion less important. This also has implications for global warming. With increasing global temperatures, the summers in Colorado will be warmer and drier. What were optimal conditions, could not be going forward for the burying beetles.

Future studies could anticipate climate, and begin experiments earlier in the year. These experiments took place in July, in which it was the hottest, and driest portion of the summer. If the experiments took place in early July results might have shifted to more meadow success due to more soil moisture and lower temperatures. We could have seen successful intrusion as well since it would be less likely carcasses would be abandoned in the early summer. This study points to lower fecundity in the later portions of the year, conditions make it much more difficult to raise a brood later in the summer. More experiments on wild intrusion need to be conducted, as most intrusion experiments are run in captive environments/ scenarios. More can be learned

about the wild aspects of burying beetles as it is important to understand the ecology of these important beetles.

Conclusion:

This paper investigates the factors that influence reproductive success in wild populations of burying beetles (*Nicrophorus* spp.), focusing on different habitats at the Rocky Mountain Biological Laboratory (RMBL). Field experiments were conducted to observe carcass discovery, burial, and intrusion rates while comparing two habitats: meadow and aspen forest. This study found differences in beetle behavior and success between these habitats. *Nicrophorus investigator* was more common in the meadow, while *Nicrophorus defodiens* had higher reproductive success in the aspen forest. The results suggest that environmental factors such as soil moisture and temperature influence beetle success, with the aspen habitat providing more favorable conditions for raising broods. Additionally, this study highlights that intrusion rates in the wild are lower than previously thought, challenging the assumption that intrusion is the major factor influencing burying beetle reproduction. These results address the potential impact of climate change; warmer and drier conditions could reduce the beetles' reproductive success. Overall, this research contributes to understanding the ecological dynamics of burying beetles, particularly how habitat and environmental conditions affect their reproductive strategies.

References:

Gross, M. R. (2005). The evolution of parental care. *The Quarterly Review of Biology*, 80(1), 37–45. <https://doi.org/10.1086/431023>

Hoback, W., & Peterson, B. (2013). Burying the dead: The effects of carcass burial and brood development of *Nicrophorus* beetles on surrounding soil nutrients.

Kalinová, B., Podskalská, H., Růžicka, J. *et al.* Irresistible bouquet of death—how are burying beetles (Coleoptera: Silphidae: *Nicrophorus*) attracted by carcasses. *Naturwissenschaften* **96**, 889–899 (2009). <https://doi.org/10.1007/s00114-009-0545-6>

Pilakouta, N., Hanlon, E. J. H., & Smiseth, P. T. (2018). Biparental care is more than the sum of its parts: Experimental evidence for synergistic effects on offspring fitness. *Proceedings of the Royal Society B: Biological Sciences*, 285(1884), 20180875. <https://doi.org/10.1098/rspb.2018.0875>

Royle, N. J., Alonzo, S. H., & Moore, A. J. (2016). Co-evolution, conflict and complexity: What have we learned about the evolution of parental care behaviours? *Current Opinion in Behavioral Sciences*, 12, 30–36. <https://doi.org/10.1016/j.cobeha.2016.08.004>

Scott, M. P. (1990). Brood Guarding and the Evolution of Male Parental Care in Burying Beetles. *Behavioral Ecology and Sociobiology*, 26(1), 31–39.

Scott, M. P. (1998). THE ECOLOGY AND BEHAVIOR OF BURYING BEETLES. *Annual Review of Entomology*, 43(1), 595–618. <https://doi.org/10.1146/annurev.ento.43.1.595>

Smith, R. J., & Heese, B. (1995). Carcass Selection in a High Altitude Population of the Burying Beetle, *Nicrophorus investigator* (Silphidae). *The Southwestern Naturalist*, 40(1), 50–55.

Suzuki, S. (2013). Biparental Care in Insects: Paternal Care, Life History, and the Function of the Nest. *Journal of Insect Science*, 13, 131. <https://doi.org/10.1673/031.013.13101>

Trumbo, S. T. (1994). Interspecific Competition, Brood Parasitism, and the Evolution of Biparental Cooperation in Burying Beetles. *Oikos*, 69(2), 241–249. <https://doi.org/10.2307/3546144>

Trumbo, S. T. (2022). *Why do males stay in. Bi-parental burying beetles?* *Behaviour*, 159 (2022) 1301–1318

Trumbo, S. T. (1990). *Interference competition among burying beetles (Silphidae, Nicrophorus)*. *Ecological Entomology*, 15(3), 347–355. <https://doi.org/10.1111/j.1365-2311.1990.tb00816.x>

Wilson, D. S., & Fudge, J. (1984). Burying beetles: Intraspecific interactions and reproductive success in the field. *Ecological Entomology*, 9(2), 195–203. <https://doi.org/10.1111/j.1365-2311.1984.tb00715.x>