**Title**

Breeding performance and carcass use efficiency of a burying beetle (*Nicrophorus nepalensis*) depend on carcass size and carcass nutritional composition but not carcass source

**Author names and affiliations**

Gen-Chang Hsu1,Syuan-Jyun Sun2 (add other authors)

1Department of Entomology, Cornell University, Ithaca, New York, USA

2International Degree Program in Climate Change and Sustainable Development, National Taiwan University, Taipei, Taiwan

**ORCID iD**

Gen-Chang Hsu: 0000-0002-6607-4382

Syuan-Jyun Sun: 0000-0002-7859-9346

**Corresponding author**

Name: Syuan-Jyun Sun

Email: sjs243@ntu.edu.tw

**Abstract**

TBA

**Keywords**

burying beetle, carcass use efficiency, clutch size, brood mass, brood size, nutritional composition, offspring quality-quantity trade-off

**Introduction**

Carcasses represent a rich resource for a wide variety of terrestrial organisms, including vertebrate scavengers, saprophagous invertebrates, and microbial decomposers (Rozen et al. 2008, Barton et al. 2013, Stiegler et al. 2020). These carcass-feeding organisms facilitate the recycling of carcass nutrients and make the resource available to other species (Tomberlin et al. 2017). For some species such as burying beetles (*Nicrophorus* spp.), carcasses are particularly important because they serve as not only food resource but also breeding sites where the offspring grow and develop under parental care (Scott 1998). Carcass attributes, therefore, can strongly influence the reproduction of burying beetles.

Carcass size is a key factor for the reproductive success of burying beetles because it determines the amount of resource available for breeding. Brood size and brood mass are generally greater on larger (heavier) carcasses (Scott and Traniello 1990, Trumbo 1992, Scott 1998, Creighton 2005, Smiseth et al. 2014). Moreover, parents can adjust their reproductive investment based on carcass size (Hopwood et al. 2016). For example, females lay more eggs on larger carcasses within a certain carcass size range (Müller et al. 1990), and parents regulate the brood size via filial cannibalism when carcass resource is limited (Bartlett 1987). However, despite the resource benefits, large carcasses can be more difficult to utilize (Trumbo 1992), and the energetic costs of processing carcass tissue also increase with carcass size. Such cost-benefit trade-offs suggest that reproductive performance might not necessarily be greater on larger carcasses, yet no study has empirically examined whether there is an optimal carcass size for breeding.

Besides carcass size, the source of carcass can also influence the reproduction of burying beetles. Carcasses in the wild come from animals feeding on diverse diets in various environments. However, most breeding experiments use laboratory mice and chicks, which are usually fed fixed diets and reared in a controlled environment. Consequently, lab and wild carcasses may have considerably different body compositions as well as skin and gut microbiomes (Weldon et al. 2015), and these differences can alter larval survival and growth (Rozen et al. 2008, Shukla et al. 2018). Experiments comparing the breeding outcomes of burying beetles on lab versus wild carcasses are essential for evaluating whether the results of past studies are representative of natural patterns. Furthermore, carcasses from different taxonomic groups (e.g., birds, mammals, and reptiles) can vary in their tissue nutritional compositions (May and El‐Sabaawi 2022), and larval growth and development are critically dependent on diet quality (Scriber and Slansky Jr 1981). As burying beetles encounter a wide variety of carcasses as potential breeding sites, examining how breeding outcomes and larval performance vary among different groups of wild carcasses will provide useful insights into carcass selection and utilization by beetles in the wild.

Studies have shown that brood size and larval mass of burying beetles are often negatively correlated with each other (Bartlett 1987, Creighton 2005, Monteith et al. 2012). Such a trade-off between larval quality and quantity may vary with carcass size (Bartlett and Ashworth 1988, Smiseth et al. 2014) because resource quantity can shape the life history traits of organisms (Tessier and Consolatti 1991, Boggs 2009, Richardson and Smiseth 2020). However, most studies on the offspring trade-off in burying beetles were conducted under a limited range of carcass sizes, and the results have been mixed because of the differential responses of brood size and larval mass to carcass size (Smiseth et al. 2014). Moreover, carcass sources with different quality can influence larval performance and thereby alter the trade-off patterns, yet few studies have examined this (but see Woelber et al. 2018). Therefore, examining breeding outcomes across a wide range of carcass sizes from different carcass sources (e.g., lab and wild carcasses) will help better understand how resource variation affects the offspring life history trade-off in burying beetles.

In this study, we aimed to understand how various carcass attributes (size, source, and nutritional quality) influence the reproductive success, larval performance, and offspring quality-quantity trade-off in burying beetles. We conducted breeding experiments on the species *N. nepalensis*, which has been shown to provide extensive parental care for offspring. First, we examined how breeding outcomes (clutch size, brood size, brood mass, etc.) and carcass use efficiency varied across a broad range of carcass weight (used as a proxy for size) on lab and wild carcasses. We expected that there would be an optimal carcass weight for breeding, and the patterns may differ between lab and wild carcasses. Next, we examined the breeding outcomes of *N. nepalensis* on different wild carcass taxa (mammal, bird, and reptile). We also quantified the tissue nutritional composition of these wild carcass taxa and conducted a larval feeding experiment using prepared diets. We expected that breeding outcomes and larval performance would be greater on carcass taxa with higher nutritional contents. Finally, we examined the larval quality-quantity trade-off on lab and wild carcasses. We expected a trade-off to occur across a broad range of carcass weight, and the trade-off pattern would differ between lab and wild carcasses.

**Materials and Methods**

*Breeding experiments*

We conducted breeding experiments on *N. nepalensis* from the lab colony established in 2023. Adult beetles were collected from XX and reared in growth chambers at XX°C under a relative humidity of XX% and a X:X h light:dark cycle. A male and a female were placed in a plastic breeding container (XX cm in diameter and XX cm in height) half-filled with moist commercial potting mix, and a defrosted carcass was then placed on the soil surface. Frozen dead laboratory mice were used as lab carcasses. Wild carcasses were obtained from the Taiwan Roadkill Observation Network (https://roadkill.tw/eng/home). These wild carcasses weighed from 2 to 100 grams and consisted of small mammals, birds, and reptiles. We paired each wild carcass with a lab carcass of a similar weight and used the males and females from the same family line, respectively, for each lab-wild carcass pair to control for parental genotypes. The breeding containers were maintained under the same environmental conditions as those of the lab colony. Five rounds of breeding experiments were conducted from May 2023 to March 2024 (each with a different beetle parent generation), consisting of a total of 123 lab-wild carcass pairs.

We recorded the clutch size of each breeding container at day XX by counting the number of eggs around the wall and at the bottom of the container from the outside. This minimized the disturbance to the carcass and parents. At day XX, we inspected the carcass to record the brood size (number of larvae) and brood mass (total larval weight). We calculated hatching success as brood size divided by clutch size, average larval mass as brood mass divided by brood size, and larval density as brood size divided by carcass weight. We also measured the total weight of breeding containers at the beginning and at the end of the experiments to estimate the amount of carcass tissue consumed by the larvae (larvae were removed from the carcasses). Carcass use efficiency was calculated as the amount of carcass tissue consumed divided by the initial carcass weight.

*Nutritional analysis of wild carcasses and larval feeding experiment*

* Nutritional analysis of different wild carcass taxa
* Larval feeding experiment

*Data analyses*

To examine how clutch size, hatching success, brood size, brood mass, and carcass use efficiency varied with carcass weight on lab and wild carcasses, we fit generalized linear mixed effects models (GLMMs) with each of the aforementioned breeding outcomes as the response, carcass weight and carcass source as well as their interaction as the fixed effects, and lab-wild carcass pair as the random effect. The pronotum widths of the parents and parent generation were included as the covariates in the models. For clutch size and brood size, we used a negative binomial error distribution with a log link function for model fitting to account for data overdispersion; for hatching success, we used a binomial error distribution with a logit link function; for brood mass, we used a Gaussian error distribution; for carcass use efficiency, we used a beta error distribution with a logit link function. Because clutch size and brood size contained many zero values, we additionally included a zero inflation structure in the models. We determined whether a quadratic curve better described the relationship between each response and carcass weight by comparing the GLMMs fitted with and without a quadratic term for carcass weight via the likelihood ratio test. Results from the quadratic model were reported if the test was significant (*α* = 0.05).

To compare the brood size, brood mass, average larval mass, and carcass use efficiency on wild mammal, bird, and reptile carcasses, we XXX. Because the carcass range was considerably smaller for reptiles (XX to XX) than for mammals (XX to XX) and birds (XX to XX), we restricted the carcass weight range to that of reptiles so that the results were more comparable for the three taxa.

To compare the larval growth on wild mammal, bird, and reptile carcasses, we XXX.

To evaluate the trade-off between offspring quality and quantity on lab and wild carcasses, we fit a linear model with average larval mass as the response and larval density, carcass source, and their interaction as the predictors. A significant negative slope indicates a larval quality-quantity trade-off.

We fit the GLMMs using the glmmtmb() function in the R “glmmTMB” package (Brooks et al. 2017). Assumptions were checked for all fitted models via the quantile residuals generated from the simulateResiduals() function in the R “DHARMa” package (Hartig 2022). Predictor significance was assessed with the likelihood ratio test via the Anova() function in the R “car” package (Fox and Weisberg 2019). All analyses were performed in R version 4.3.3 (R Core Team 2024).

**Results**

*Breeding performance and carcass use efficiency*

Clutch size, hatching success, brood size, and brood mass all showed a quadratic relationship with carcass weight (clutch size: *P* < 0.001; hatching success: *P* < 0.001; brood size: *P* < 0.001; brood mass: *P* < 0.001; Table 1) and peaked on medium-sized carcasses (Fig. 1). Moreover, these breeding outcomes did not differ between lab and wild carcasses (clutch size: *P* = 0.40; hatching success: *P* = 0.40; brood size: *P* = 0.78; brood mass: *P* = 0.96; Table 1; Fig. 1). Carcass use efficiency decreased with carcass weight (*P* < 0.001) but did not differ between lab and wild carcasses (*P* = 0.96; Table 1; Fig. 2).

Brood size, brood mass, average larval mass, and carcass use efficiency on wild mammal, bird, and reptile carcasses (Fig. 3).

*Nutritional composition of wild carcasses and larval growth*

Nutritional composition of wild carcasses and larval growth (Fig. 4).

*Larval quality-quantity trade-off*

Average larval mass decreased with larval density on both lab and wild carcasses (*β* = −0.096; *P* < 0.001; Fig. 5). The interaction between larval density and carcass source was not significant (*P* = 0.28), indicating that the trade-off did not differ between lab and wild carcasses (Fig. 5).

**Discussion**

We examined how breeding outcomes and carcass use efficiency of a burying beetle *N. nepalensis* varied with carcass weight on lab and wild carcasses. Clutch size, hatching success, brood size, and brood mass all exhibited a hump-shaped relationship with carcass weight, whereas carcass use efficiency decreased with carcass weight. Moreover, the breeding outcomes differed among wild carcass taxa, and the nutritional compositions of wild carcasses differed among taxa, and larvae feeding on XXX had higher growth rates. Finally, a negative relationship existed between larval density and average larval mass on both lab and wild carcasses, suggesting a trade-off between offspring quality and quantity. Taken together, our results indicate that the breeding performance and carcass resource use of burying beetles are strongly dependent on carcass weight and but not carcass source, and that higher nutritional contents of carcass tissue can enhance individual larval performance as well as overall breeding outcomes.

As expected, the breeding performance of *N. nepalensis* depended on carcass resource. The clutch size, hatching success, brood size, and brood mass all showed a humped-shaped relationship with carcass weight, with optimal breeding outcomes occurring on medium carcasses. The increase in breeding performance from small to medium carcasses is consistent with previous studies on other burying beetle species (Eggert and Müller 1992, Creighton 2005, Hopwood et al. 2016). However, when the parents bred on large carcasses, their performance decreased with carcass weight, in turn leading to reduced carcass use efficiency. This may be because large carcasses are more energetically costly to process and females may lay fewer eggs as a result of lower energy storage. In fact, Müller et al. (1990) found that clutch size levels off beyond a certain carcass weight threshold, suggesting a constraint on beetles breeding on larger carcasses. Moreover, parents breeding on large carcasses face stronger competition with microbes, which can reduce the usable resource for breeding (Scott 1998) or produce compounds harmful to eggs and larvae (Rozen et al. 2008).

We found no major difference in breeding outcomes and carcass use efficiency of beetles breeding on lab and wild carcasses. A potential explanation is that the parents manipulated the carcasses (e.g., by secreting antimicrobial compounds) such that the eggs and larvae on lab and wild carcasses experienced similar growing environments. Parental food preparation and regurgitation may also offset the difference between the two carcass sources. Experiments comparing breeding outcomes on lab and wild carcasses with versus without parents will help verify our speculation, as studies have shown that parental care is crucial for offspring performance (Eggert et al. 1998, Rozen et al. 2008). The analyses did reveal an interaction between carcass weight and carcass source for brood mass. In fact, the difference in brood mass between lab and wild carcasses occurred only on large carcasses, which may be largely due to the limited sample sizes, whereas the patterns on small and medium carcasses were mostly similar. Overall, our results support the validity of research using lab-reared organisms as breeding carcasses to study the reproductive biology of burying beetles.

[Main finding 3 and discussion]

* Breeding outcomes on different wild carcass taxa
* Carcass nutritional composition and larval growth

The negative relationship between average larval mass and larval density on both lab and wild carcasses indicates a trade-off between offspring quality and quantity regardless of carcass source, which has also been shown in previous studies (Bartlett and Ashworth 1988, Trumbo 1990). This trade-off can arise from both larval competition and brood regulation by parents (Trumbo 1990). Stronger interspecific competition under a higher larval density may reduce individual larval performance and biomass. On the other hand, parents may regulate brood size by culling excess larvae, thereby reducing the larval competition and allowing for greater individual larval growth. Moreover, the slope of the negative relationship between average larval mass and larval density did not depend on carcass source, agreeing with our findings that brood size and brood mass did not differ between lab and wild carcasses. Interestingly, we found that the average larval mass increased with carcass weight on small and medium carcasses, whereas larval density decreased (Fig. S1). This suggests that the reproductive life history traits of burying beetles can shift depending on breeding resource availability, with smaller carcasses favoring larval quantity (per capita carcass resource) and larger carcasses favoring larval quality.

Our results illustrate the role of carcass weight (size) in the breeding performance of a single parent pair. This is the most common breeding system in burying beetles on small- and medium-sized carcasses (Scott 1998). However, multiple males and females may engage in cooperative breeding to better utilize large carcasses in the wild (Scott et al. 2007), although results for the reproductive benefits of cooperation are mixed (Eggert and Sakaluk 2000, Müller et al. 2007, Komdeur et al. 2013). Additionally, burying beetles in nature may face carcass competition not only from microbes but also from various vertebrate scavengers and invertebrate carcass feeders (DeVault et al. 2003, Chen et al. 2020), and such interspecific competition can interact with carcass size to influence breeding success (Scott 1994). Field experiments using a wide range of carcass sizes will help elucidate how intraspecific and interspecific interactions as well as the interplay between biotic interactions and carcass size jointly shape the breeding performance of burying beetles.

[Conclusions]

* A full range of carcass size instead of discrete size groups (e.g., small, medium, and large) to better capture the breeding patterns > a hump-shaped relationship for breeding outcomes and the medium-sized carcass is optimal for breeding outcomes
* No difference between lab and wild carcasses suggests that past studies using lab mice and chicks are fairly representative of the natural patterns
* Various sources of wild carcasses can provide sources for burying beetles and the nutritional compositions of different wild carcass taxa can affect larval performance. Also the first evidence for breeding on reptiles
* A trade-off exists and the beetle seems to adopt different life history strategies depending on the carcass weight

**Acknowledgments**

We thank XXX for assisting with field sampling/experimental setup/data collection

This work was supported by XXX (grant number YYY)

**Conflict of interest**

The authors declare no conflict of interest regarding this manuscript.

**Author contributions**

GCH and SJS conceived the ideas and designed the experiments; SJS, GCH, and XXX conducted the experiments and collected the data; GCH and SJS analyzed the data; GCH and SJS wrote the first draft of the manuscript. All authors revised the manuscript and approved the final version for publication.

**Data availability statement**

Data and code used in this manuscript will be publicly available on Zenodo if the manuscript is accepted for publication.

**References**

Bartlett, J. 1987. Filial cannibalism in burying beetles. Behavioral Ecology and Sociobiology **21**:179-183.

Bartlett, J., and C. Ashworth. 1988. Brood size and fitness in Nicrophorus vespilloides (Coleoptera: Silphidae). Behavioral Ecology and Sociobiology **22**:429-434.

Barton, P. S., S. A. Cunningham, D. B. Lindenmayer, and A. D. Manning. 2013. The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. Oecologia **171**:761-772.

Boggs, C. L. 2009. Understanding insect life histories and senescence through a resource allocation lens. Functional Ecology **23**:27-37.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal **9**:378-400.

Chen, B. F., M. Liu, D. R. Rubenstein, S. J. Sun, J. N. Liu, Y. H. Lin, and S. F. Shen. 2020. A chemically triggered transition from conflict to cooperation in burying beetles. Ecology Letters **23**:467-475.

Creighton, J. C. 2005. Population density, body size, and phenotypic plasticity of brood size in a burying beetle. Behavioral Ecology **16**:1031-1036.

DeVault, T. L., J. Rhodes, Olin E, and J. A. Shivik. 2003. Scavenging by vertebrates: behavioral, ecological, and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems. Oikos **102**:225-234.

Eggert, A.-K., and J. K. Müller. 1992. Joint breeding in female burying beetles. Behavioral Ecology and Sociobiology **31**:237-242.

Eggert, A.-K., M. Reinking, and J. K. Müller. 1998. Parental care improves offspring survival and growth in burying beetles. Animal Behaviour **55**:97-107.

Eggert, A. K., and S. K. Sakaluk. 2000. Benefits of communal breeding in burying beetles: a field experiment. Ecological Entomology **25**:262-266.

Fox, J., and S. Weisberg. 2019. An R Companion to Applied Regression. Third edition. Sage, Thousand Oaks CA.

Hartig, F. 2022. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.

Hopwood, P. E., A. J. Moore, T. Tregenza, and N. J. Royle. 2016. Niche variation and the maintenance of variation in body size in a burying beetle. Ecological Entomology **41**:96-104.

Komdeur, J., M. J. Schrama, K. Meijer, A. J. Moore, and L. W. Beukeboom. 2013. Cobreeding in the burying beetle, Nicrophorus vespilloides: tolerance rather than cooperation. Ethology **119**:1138-1148.

May, E. M., and R. W. El‐Sabaawi. 2022. Life stage and taxonomy the most important factors determining vertebrate stoichiometry: A meta‐analysis. Ecology and Evolution **12**:e9354.

Monteith, K. M., C. Andrews, and P. T. Smiseth. 2012. Post‐hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. Journal of evolutionary biology **25**:1815-1822.

Müller, J. K., V. Braunisch, W. Hwang, and A.-K. Eggert. 2007. Alternative tactics and individual reproductive success in natural associations of the burying beetle, Nicrophorus vespilloides. Behavioral Ecology **18**:196-203.

Müller, J. K., A.-K. Eggert, and E. Furlkröger. 1990. Clutch size regulation in the burying beetle Necrophorus vespilloides Herbst (Coleoptera: Silphidae). Journal of Insect Behavior **3**: 265–270.

R Core Team. 2024. R: A Language and Environment for Statistical Computing. Vienna, Austria.

Richardson, J., and P. T. Smiseth. 2020. Effects of variation in resource acquisition during different stages of the life cycle on life‐history traits and trade‐offs in a burying beetle. Journal of evolutionary biology **32**:19-30.

Rozen, D., D. Engelmoer, and P. T. Smiseth. 2008. Antimicrobial strategies in burying beetles breeding on carrion. Proceedings of the National Academy of Sciences **105**:17890-17895.

Scott, M. P. 1994. Competition with flies promotes communal breeding in the burying beetle, Nicrophorus tomentosus. Behavioral Ecology and Sociobiology **34**:367-373.

Scott, M. P. 1998. The ecology and behavior of burying beetles. Annual review of entomology **43**:595-618.

Scott, M. P., W. J. LEE, and E. Van Der Reijden. 2007. The frequency and fitness consequences of communal breeding in a natural population of burying beetles: a test of reproductive skew. Ecological Entomology **32**:651-661.

Scott, M. P., and J. F. Traniello. 1990. Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (Nicrophorus spp.). Animal Behaviour **39**:274-283.

Scriber, J., and F. Slansky Jr. 1981. The nutritional ecology of immature insects. Annual review of entomology **26**:183-211.

Shukla, S. P., C. Plata, M. Reichelt, S. Steiger, D. G. Heckel, M. Kaltenpoth, A. Vilcinskas, and H. Vogel. 2018. Microbiome-assisted carrion preservation aids larval development in a burying beetle. Proceedings of the National Academy of Sciences **115**:11274-11279.

Smiseth, P. T., C. P. Andrews, S. N. Mattey, and R. Mooney. 2014. Phenotypic variation in resource acquisition influences trade‐off between number and mass of offspring in a burying beetle. Journal of Zoology **293**:80-83.

Stiegler, J., C. Von Hoermann, J. Müller, M. E. Benbow, and M. Heurich. 2020. Carcass provisioning for scavenger conservation in a temperate forest ecosystem. Ecosphere **11**:e03063.

Tessier, A. J., and N. L. Consolatti. 1991. Resource quantity and offspring quality in Daphnia. Ecology **72**:468-478.

Tomberlin, J. K., B. T. Barton, M. A. Lashley, and H. R. Jordan. 2017. Mass mortality events and the role of necrophagous invertebrates. Current Opinion in Insect Science **23**:7-12.

Trumbo, S. T. 1990. Regulation of brood size in a burying beetle, Nicrophorus tomentosus (Silphidae). Journal of Insect Behavior **3**:491-500.

Trumbo, S. T. 1992. Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (Nicrophorus). Ecological Entomology **17**:289-298.

Weldon, L., S. Abolins, L. Lenzi, C. Bourne, E. M. Riley, and M. Viney. 2015. The gut microbiota of wild mice. PLoS One **10**:e0134643.

Woelber, B. K., C. L. Hall, and D. R. Howard. 2018. Environmental cues influence parental brood structure decisions in the burying beetle Nicrophorus marginatus. Journal of ethology **36**:55-64.

**Tables and Figures**

Table 1. A summary of the GLMM results for the breeding outcomes and carcass use efficiency of the burying beetle *N. nepalensis*. The pronotum widths of the parents and parent generation were included as the covariates in all models.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model response | *n* | *P* | | |
| Carcass weight | Carcass source | Weight × Source |
| Clutch size | 212a | < 0.001 | 0.40 | 0.22 |
| Hatching success | 178b | < 0.001 | 0.40 | 0.97 |
| Brood size | 240 | < 0.001 | 0.78 | 0.12 |
| Brood mass | 129c | < 0.001 | 0.82 | 0.005 |
| Carcass use efficiency | 95d | < 0.001 | 0.96 | 0.60 |

aClutch size was not recorded in the first round of breeding experiments.

bObservations with a zero clutch size were excluded from the analysis.

cObservations with a zero brood size were excluded from the analysis.

dCarcass use was not measured in the first and second round of the breeding experiments. Observations with a zero brood size were excluded from the analysis.



Figure 1. The relationship between carcass weight and (a) clutch size, (b) hatching success, (c) brood size, and (d) brood mass on lab and wild carcasses. Note that the observations without any larva were excluded from the brood mass analysis.



Figure 2. The relationship between carcass weight and carcass use efficiency on lab and wild carcasses. Note that the observations without any larva were excluded from the analysis.

Figure 3. Brood size, brood mass, average larval mass, and carcass use efficiency on different wild carcass taxa.

Figure 4. Nutritional composition of different wild carcass taxa and larval growth.



Figure 5. The relationship between larval density and average larval mass on lab and wild carcasses.