**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

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**Abstract**

**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 organisms from vertebrate scavengers to 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 burying beetles (*Nicrophorus* spp.), carcasses are particularly important because they serve as breeding sites where parents provide parental care and prepare food for their offspring (Scott 1998), and carcass attributes can strongly influence the breeding performance of 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). 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. Whether there is an optimal carcass size for breeding remains unclear.

[Background and knowledge gap 2]

* Carcass source can affect breeding outcomes as well: lab source is fed with a fixed standard diet in a controlled environment, whereas wild carcasses are feeding on various diet and in an variable environment.
* It is important to understand whether the outcomes differ between lab and wild carcasses because most studies use lab-reared carcass (mica and chicks) for breeding experiments, and whether this represents the patterns in nature remains unknown
* Moreover, different carcass taxa can have differential effects on breeding performance and larval growth performance because they differ in their nutritional composition
* More specifically, the nutritional compositions and other carcass attributes may differ, thus affecting the outcomes
* Understanding how different wild carcass taxa affect breeding performance, and how the nutritional composition of these carcasses differ and the effects on larval performance, will help us better understand how parent select and utilize the carcasses in the wild as they encounter a wide range of potential breeding sites

[Background and knowledge gap 3]

* The trade-off between larval number and larval mass may depend on the carcass source and weight as resource quality and quantity can shape the life history strategy of organisms

“Although nearly all studies show that larger carcasses support a larger number of offspring, the results for offspring mass are inconsistent. The aim of this study was to test for effects of phenotypic variation in resource acquisition (i.e. carcass size) on the number and mass of offspring and the trade-off between the two. Life-history theory for the trade-off between the number and mass of offspring predicts that variation in resource acquisition should have a strong effect on the number of offspring produced but that it should have no effect on offspring mass” (Phenotypic variation in resource acquisition influences trade-off between number and mass of offspring in a burying beetle)

““In all burying beetles studied to date, including N. orbicollis, there is a negative relationship between offspring body size and brood size on a given-sized carcass” (Population density, body size, and phenotypic plasticity of brood size in a burying beetle) (Effects of variation in resource acquisition during different stages of the life cycle on life‐history traits and trade‐offs in a burying beetle)

“For instance, the trade‐off between size and number of offspring is influenced by both carcass size (Smiseth et al., 2014) and female nutritional condition (Steiger et al., 2007).” (Effects of variation in resource acquisition during different stages of the life cycle on life‐history traits and trade‐offs in a burying beetle)

* Knowledge gap: most studies tested the trade-off on a single and a limited number of carcass size, but whether this pattern holds true across a range of carcass size remains unclear

Articles:

> Phenotypic variation in resource acquisition influences trade-off between number and mass of offspring in a burying beetle

> Population density, body size, and phenotypic plasticity of brood size in a burying beetle

> Effects of variation in resource acquisition during different stages of the life cycle on life‐history traits and trade‐offs in a burying beetle

[Study aims, questions, hypotheses, and predictions]

To XXX, we XXX

Specifically, we asked XXX

Specifically, we hypothesized that XXX

We predicted that XXX

Our aims are to XXX

* Examine the relationship between carcass weight (the carcass size measure in this study) and breeding outcomes as well as carcass use efficiency in lab and wild carcasses
* Quantify the nutritional composition of different wild carcass taxa and test the effects on larval growth
* Examine the offspring quality vs. quantity trade-off in lab and wild carcasses

**Materials and Methods**

*Breeding experiments*

We conducted breeding experiments on *N. nepalensis* using beetles from the lab colony established in 2023. 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. A defrosted carcass was provided on the soil surface. Frozen dead laboratory mice were used as the 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 ambient conditions as those in 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 examined the carcass and recorded the brood size (number of larvae) as well as the 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 used by the larvae (larvae were removed from the carcasses). Carcass use efficiency was calculated as the amount of carcass tissue used 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 variables 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 model. We determined whether a quadratic relationship existed between each response and carcass weight by comparing the model with versus 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 breeding performance on wild mammal, bird, and reptile carcasses, we XXX.

* Brood size, brood mass, average larval mass, and carcass use efficiency on wild mammal, bird, and reptile carcasses (restrict the carcass weight range to that of reptiles)

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 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 function “simulateResiduals()” 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*

The clutch size, hatching success, brood size, and brood mass all showed a hump-shaped 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). The 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)

*Offspring quality-quantity trade-off*

The average larval mass decreased with larval density on both lab and wild carcasses (*β* = −0.096; *P* < 0.001; Fig. 5). The trade-off did not depend on carcass source (larval density × carcass source: *P* = 0.28; 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 composition 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).

[Main finding 2 and discussion]

* No difference between lab and wild carcass sources: an explanation is that the parents manipulated the carcass microbial communities and thus the eggs and larvae were experiencing similar growing environments regardless of carcass source

[Main finding 3 and discussion]

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

[Main finding 4 and discussion]

* Larval quality-quantity trade-off

There was a negative relationship between the average larval mass and larval density for both lab and wild carcasses, indicating a larval quality-quantity trade-off for both carcass types. Moreover, the average larval mass increased with carcass weight (for small- and mid-sized carcasses), whereas the larval density decreased. This suggests that female beetles invest more in offspring quantity in smaller carcasses (higher larval density) and more in quality in larger carcasses (higher average larval mass).

Contrary to previous study finding no trade-off between the larval mass and density. They use total number of larvae and larval mass, but the amount of resource should be taken into account (Phenotypic variation in resource acquisition influences trade-off between number and mass of offspring in a burying beetle)

The pattern depends on the measure: if you use larval number, then you see positive, but if you account for the larval size, then the relationship becomes negative.

“Our finding that there was a significant negative correlation between the number and size of offspring at dispersal only when females bred on small carcasses confirms that variation in resource acquisition at the start of breeding masks the trade‐off between offspring size and number.” (Effects of variation in resource acquisition during different stages of the life cycle on life‐history traits and trade‐offs in a burying beetle)

[Limitations and potential caveats]

* In the wild, multiple parents can have communal brood, and this can better utilize large carcass and affect breeding outcomes
* Also in the wild, the beetles face other vertebrate and invertebrate competitors, and these can affect the breeding success.

[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 composition 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

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**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 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.

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**Tables and Figures**

Table 1. A summary of the GLMM results for the breeding outcomes and carcass use efficiency of the burying beetle. 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.