**Title**

Breeding outcomes and carcass use efficiency of a burying beetle (*Nicrophorus nepalensis*) depend on carcass weight but not carcass source

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**Abstract**

**Keywords**

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

**Introduction**

[General opening]

* Carcasses represent a critical resource for various organisms including microbes, invertebrates, and vertebrates
* In particular, burying beetles use carcasses as their breeding sites, the parents provide parent car and prepare carcass for their offspring
* Therefore, it is crucial to understanding how their breeding success depends critically on the carcass attribute to better understand the reproduction performance 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. The beetles breed on a wide range of carcass sizes (Belk et al. 2021), and parents can adjust their reproductive investment accordingly (Hopwood et al. 2016). For example, females lay more eggs on larger carcasses within a certain carcass size range (Müller et al. 1990). Moreover, parents can regulate the brood size via filial cannibalism (Bartlett 1987). As larger carcasses provide more resource for larvae, 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). However, larger carcasses may be more difficult to utilize (Trumbo 1992), and the energetic costs of processing carcass tissue may 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 are fed with a fixed standard diet in a controlled environment, whereas wild carcasses are feeding on various diet and in an variable environment.
* More specifically, the nutritional compositions and other carcass attributes may differ, thus affecting the outcomes
* It is important to understand whether the outcomes differ between lab and wild carcasses

[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 lab and wild carcasses 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 in growth chambers using beetles in the lab colony collected from XX. We half-filled each breeding container (XX cm in diameter) with commercial potting mix (XX brand) and placed a carcass on the soil surface. A male and a female beetle were then introduced to the carcass as breeding parents. 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 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 kept at XX°C under a relative humidity of XX% and a X:X h light:dark cycle. Five rounds of breeding experiments were conducted, with 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 beetles. At day XX, we examined the carcass and recorded the number of larvae as well as the total larval weight. We calculated the average larval mass as the total larval weight divided by the number of larvae. Larval density was computed as the number of larvae divided by the carcass weight. We also measured the total weight of the breeding container at the beginning and at the end of the breeding experiment (when larvae were removed from the carcasses for measurement). Carcass use efficiency was calculated as the proportion of carcass tissues consumed by larvae.

*Nutritional analysis of carcasses and larval feeding experiment*

* Nutritional analysis of the intestine and muscle tissues of lab and wild carcasses
* Larval feeding experiment

*Data analyses*

To examine how beetle breeding outcomes (clutch size, number of larvae, average larval mass, larval density) and larval carcass use efficiency (proportion of carcass consumed) varied with carcass weight in lab and wild carcasses, we fit generalized linear mixed effects models (GLMMs) with each of the aforementioned variables as the response, carcass weight and carcass source as the fixed effects, and breeding 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 number of larvae, we used a negative binomial error distribution with a log link function to account for data overdispersion; for average larval mass and larval density, we used a Gaussian error distribution; for proportion of carcass consumed, we used a beta error distribution with a logit link function 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). The GLMMs were fitted via the glmmtmb() function in the R “glmmTMB” package (Brooks et al. 2017).

To compare the larval growth between the lab and wild carcass diet treatment, we XXX

To examine the trade-off between larval quality and quantity, we tested for the relationship between larval density and average larval mass using a linear model. For all models in the study, we checked the model assumptions using quantile residuals generated from the function “simulateResiduals()” in the R “DHARMa” package (Hartig 2022), and used the likelihood ratio test to assess predictor significance using 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 outcomes and carcass use efficiency*

The clutch size, number of larvae, and average larval mass showed a hump-shaped relationship with carcass weight (clutch size: *P* < 0.001; number of larvae: *P* < 0.001; average larval mass: *P* < 0.001; Table 1) and peaked in medium-sized carcasses (Fig. 1a–c). However, these breeding outcomes did not differ between lab and wild carcasses (clutch size: *P* = 0.40; number of larvae: *P* = 0.78; average larval mass: *P* = 0.39) (Table 1; Fig. 1a–c). The larval density decreased with carcass weight (*P* < 0.001) but did not differ between lab and wild carcasses (*P* = 0.80; Table 1; Fig. 1d).

Larval 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).

*Carcass nutritional composition and larval feeding experiment*

Nutritional composition of carcasses and larval growth (Fig. 3)

*Larval quality-quantity trade-off*

The average larval mass decreased with larval density in both lab and wild carcasses (*P* < 0.001; Fig. 4).

**Discussion**

We examined how breeding outcomes and carcass use efficiency of a burying beetle varied with carcass weight on lab and wild carcasses. Clutch size, brood size, and average larval mass exhibited a hump-shaped relationship with carcass weight, whereas larval density and carcass use efficiency decreased with carcass weight. Moreover, we found that the nutritional composition differed between the intestine and muscle tissue, and larvae feeding on XXX had higher growth rates. Finally, we found a negative relationship 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 outcomes of burying beetles are strongly dependent on carcass weight but not carcass source, and that higher nutritional contents of carcass tissue can enhance larval performance.

The breeding of *N. nepalensis* are strongly dependent on carcass resource. Clutch size, brood size, and average larval mass all showed a humped-shaped relationship with carcass weight, with optimal breeding outcomes occurring on mid-sized carcasses. The increase in clutch size and brood size from small to medium-sized carcasses is consistent with previous studies on other burying beetle species (citation). However, when the parents bred on large carcasses, clutch size, brood size, and average larval mass indeed decreased with carcass weight. This may be because large carcasses are more energetically costly to process, resulting in females laying fewer eggs. Moreover, the parents may fail to regulate the microbial communities on large carcasses (Bacterial Infection Increases Reproductive Investment in Burying Beetles). In fact, some studies show that clutch size levels off as carcass size increases (Clutch size regulation in the burying beetleNecrophorus vespilloides Herbst (Coleoptera: Silphidae), Joint breeding in female burying beetles), suggesting a constraint on parents breeding on large-sized carcasses.

This is also partially supported by the low carcass use efficiency on large carcasses, low brood size on large carcass also explains the low larval density on large carcass.

Articles:

> Carcass Fungistasis of the Burying Beetle Nicrophorus nepalensis Hope (Coleoptera: Silphidae)

[Main finding 1 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 2 and discussion]

* Carcass nutritional composition and larval growth

[Main finding 3 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]

[Conclusions]

* A full range of carcass size instead of discrete size groups (e.g., small, medium, and large) will better capture the breeding patterns, a hump-shaped relationship for breeding outcomes and the medium-sized carcass is optimal for breeding outcomes
* Various sources of wild carcasses can provide sources for burying beetles, first evidence for breeding on reptiles
* No difference between lab and wild carcasses suggests that past studies using lab mice are fairly representative of the natural patterns
* 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 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 | 212 | < 0.001 | 0.40 | 0.22 |
| Number of larvae | 240 | < 0.001 | 0.78 | 0.12 |
| Average larval mass | 128\* | < 0.001 | 0.39 | 0.28 |
| Larval density | 139\* | < 0.001 | 0.80 | 0.47 |
| Proportion of carcass consumed | 95† | < 0.001 | 0.96 | 0.60 |

\*Observations without any larva were excluded from the analysis.

†Carcass use was not measured in the first two rounds of the breeding experiment. Observations without any larva were excluded from the analysis.



Figure 1. The relationship between carcass weight and clutch size (a), number of larvae (b), average larval mass (c), and larval density (d) in lab and wild carcasses.



Figure 2. The relationship between carcass weight and proportion of carcass consumed by the larvae in lab and wild carcasses. Note that the observations without any larva were excluded from the analysis.

Figure 3. Nutritional composition of lab and wild carcasses and larval growth.



Figure 4. The relationship between larval density and average larval mass in lab and wild carcasses.

Notes:

1. Change “number of larvae” to “brood size”
2. Change “in carcass” to “on carcass”
3. Provide brood mass and hatching rate figure in the supporting information