**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, 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, and their breeding success depends critically on the carcass attributes

[Background and knowledge gap 1]

* Carcass size (weight) can affect breeding outcomes
* The relationship can be positive, negative, or non-linear

[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

[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 in lab and wild carcasses. Clutch size, number of larvae, and average larval mass exhibited a hump-shaped relationship with carcass weight, whereas larval density and larval carcass use efficiency decreased with carcass weight. Moreover, we found that the nutritional composition of the intestine and muscle tissues differed among the carcass sources, and larvae feeding on XXX had higher growth rates. Finally, we found a negative relationship between larval density and average larval mass in both lab and wild carcasses, suggesting a trade-off between larval quality and quantity. Taken together, our results indicate that the overall breeding outcomes of burying beetles are strongly dependent on carcass weight but not carcass source, and that higher nutritional contents of carcass tissues can enhance individual larval performance.

[Main finding 1 and discussion]

The breeding outcomes of burying beetles are dependent on resources

Consistent the previous studies,

The clutch size, number of larvae, and average larval mass all showed a humped-shaped patterns. The mid-sized carcasses are optimal for breeding

This can be due to XXX

On the other hand, larval density decreased with carcass weight. This is unexpected as there are more resources available. This could also explain the decrease in the larval carcass use efficiency with carcass weight.

We suspect that there is a carcass weight threshold beyond which the parent beetles are not able to process additional carcass tissues.

[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).

[Limitations and potential caveats]

[Conclusions]

* The medium-sized carcass is optimal for breeding outcomes
* Various sources of wild carcasses can provide sources for burying beetles
* 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.

**References**

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M. , & Bolker, B. M. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal,* *9*(2), 378-400.

Fox, J. , & Weisberg, S. (2019) *An R Companion to Applied Regression.* Sage, Thousand Oaks CA.

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

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

**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.