**Title**

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

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**Abstract**

**Keywords**

burying beetle, breeding outcomes, carcass use

**Introduction**

[General opening]

[Background and knowledge gap 1]

[Background and knowledge gap 2]

[Background and knowledge gap 3]

[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

* Compare the breeding outcomes between lab vs. wild carcasses
* Examine the carcass use patterns between lab vs. wild carcasses
* Examine the offspring quality vs. quantity trade-off between lab vs. wild carcasses

**Materials and Methods**

*Study organism*

* A brief introduction of *Nicrophorus nepalensis*
* The beetle source in the study

*Experimental design*

* The details of the breeding experiments: carcass sources and taxa and weight range, pairing each wild carcass with a lab carcass of similar weight and parents from the same family lines, growth chamber settings, duration of each experiment (five rounds of experiments with a total of 123 lab-wild carcass pairs)
* Measurement: parent pronotum width, clutch size, number of larvae, larval mass, carcass use, etc.

*Nutritional analysis of carcasses and larval feeding experiment*

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

*Statistical analyses*

To examine XXX, we fit a generalized linear mixed effects model (GLMM) with XXX as the response, YYY as the fixed effects, and ZZZ as the random effects. We use a XXX distribution with a YYY link function because the response is XXX.

The GLMM is fitted via the glmmtmb() function in the R “glmmTMB” package (citation).

We check the model assumptions using quantile residuals generated from the function “simulateResiduals()” in the R “DHARMa” package (citation).

We use the likelihood ratio test to assess predictor significance using the “Anova()” function in the R “car” package (citation).

We also perform pairwise comparisons of the estimated marginal means between treatment levels with XXX multiplicity adjustments using the R “emmeans” package (citation).

All analyses are performed in R version XXX (citation).

**Results**

*Breeding outcomes and carcass use*

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).

The proportion of carcass used by the larvae 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 feeding experiment*

Nutritional composition of lab vs. wild 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**

[Summary of the main findings]

* Breeding outcomes
* Carcass use
* Nutritional composition and larval growth
* Larval quality-quantity trade-off

[Main finding 1 and discussion]

* Explanations for hump-shaped relationship between breeding outcome and carcass weight
* Why larval density and carcass use decreased with carcass weight
* No difference between lab and wild sources

[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
* We showed that various sources of wild carcasses can provide sources for burying beetle
* No difference between lab and wild carcasses suggests that past studies using lab mice are fairly representative of the patterns in nature
* Differential 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**

**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 male and female parent 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 used | 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 used by the beetle 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.