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Carcass size, not source or taxon, dictates breeding performance and carcass use in burying beetle

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Author-supplied statements

Relevant information will appear here if provided.

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Ethics

Does your article include research that required ethical approval or permits?:
This article does not present research with ethical considerations

Statement (if applicable):
CUST_IF_YES_ETHICS :No data available.

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Data

It is a condition of publication that data, code and materials supporting your paper are made publicly available. Does your paper present new data?:
Yes

Statement (if applicable):
Data and code used in this manuscript are publicly available on Zenodo (DOI:
<https://doi.org/10.5281/zenodo.12747445>).

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Conflict of interest

I/We declare we have no competing interests

Statement (if applicable):
CUST_STATE_CONFLICT :No data available.

Response to Reviewers' Comments

Reference number: RSOS-241265

Title of article: Carcass size, not source or taxon, dictates breeding performance and carcass use in burying beetle

Dear Dr. Bart Pannebakker,

Thank you for inviting us to submit a revised version of the manuscript. We greatly appreciate the valuable comments and feedback from you and the reviewers. We have carefully considered each comment and incorporated the suggestions. In particular, we have made the following major changes:

- Expanded the discussion on carcass source and taxon as suggested by Reviewer 1.
- Addressed the reviewers' concerns about the statistical analyses (controlling the false discovery rates for multiple comparisons).

Please also see the following section for our detailed point-by-point responses. All line numbers refer to the changes we made in the revised manuscript (with tracked changes). We believe that the revisions have further improved the quality of this manuscript, and we hope that it is now suitable for publication in *Royal Society Open Science*.

Sincerely,

Gen-Chang Hsu (submitting author)

Department of Entomology, Cornell University

Editor's comments

Comment 1 > Thank you for submitting your work to Royal Society Open Science. Your work has now been reviewed by two reviewers. As you can see, both are positive and praise the quality of your science and of the readability of the manuscript, and I can only agree with them. Your work on the relation between carcass size, origin and taxon on the breeding success of *Nicrophorus nepalensis*, is a sound study which would fit Royal Society Open Science well. However, there a number of issues indicated by both reviewers that need to be revised before the paper can be accepted. Besides a list of minor issues, these concern some (small) concerns on the statistics, and a request by Reviewer 1 to extend the interpretation and discussion of the results. He provides some suggestions for papers which are a good starting point.

All in all, these are not big concerns, and I would like to encourage you to use the comments by the reviewers to further improve the paper. I am looking forward to seeing the revised version of your paper.

Response 1 > Thanks for the positive feedback on our manuscript. We greatly appreciate the comments and suggestions from the reviewers and have now revised the manuscript accordingly. We also enhanced our discission on the results of carcass source and taxon as suggested by Reviewer 1 and addressed the statistical concerns raised by Reviewer 2. Please see our point-by-point responses in the following section for more details.

Reviewer 1's comments

Comment 1 > I commend the authors for this interesting study. However, the authors have missed many studies in the scientific literature that test the same hypotheses and provide additional detail for other species of burying beetles. I suggest the authors dig deeper into the literature and revise their interpretation and discussion. Below are specific points that should be addressed.

Response 1 > Thanks for the feedback and suggestions. We have incorporated the relevant studies on carcass size and reproduction of burying beetles. We have also expanded the discussion on the results of carcass source and taxon. Please see our response to Comment 3 and Comment 4 for more details.

Comment 2 > Line 28 and 49 Rather than just using the word "carcasses", you should specify "small vertebrate carcasses".

Response 2 > Revised (Line 28 and 49).

Comment 3 > Lines 63-65 "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." This is not true, several papers have addressed this issue in burying beetles. These papers have used single bout and lifetime reproductive success to evaluate optimal carcass size. See:

Belk, Mark C.; Meyers, Peter J.; Creighton, J. Curtis. 2021. Bigger is better sometimes: the interaction between body size and carcass size determines fitness, reproductive strategies, and senescence in two species of burying beetles. *Diversity* 2021, 13, 662. <https://doi.org/10.3390/d13120662>.

Quinby, BM, Belk, MC, Creighton, JC. Behavioral constraints on local adaptation and counter-gradient variation: Implications for climate change. *EcolEvol.* 2020; 10: 6688– 6701. <https://doi.org/10.1002/ece3.6399>

Creighton, J.C., N.D. Heflin, and M.C. Belk. 2009. Cost of reproduction, resource quality, and terminal investment in a burying beetle. *American Naturalist* 174:673-684.

Response 3 > Thanks for pointing out this mistake. We have corrected the original statement and included the relevant references for it (Line 65–67).

Comment 4 > Lines 66-78. This is a really important point and a novel part of the study. I suggest the authors enhance the discussion of this point, and I commend them for including it in the study.

Response 4 > Thanks for the positive comment. We have modified the relevant paragraphs in the introduction section (Line 68–82) and the discussion section (Line 319–337) to strengthen our point on carcass source and taxa.

Comment 5 > I notice that all of your experiments and analyses are based on single bouts of reproduction (presumably the first bout, although this should be explained). There is a difference between single bout and lifetime reproductive output. For example, in another species of burying beetle there is a clear difference between single bout reproductive success on 20 and 30 gram carcasses. However, lifetime reproductive output is equivalent on these two sizes. At the very least you should consider this difference in the discussion.

Response 5 > Thanks for the suggestions. The study species *N. nepalensis* does reproduce multiple time throughout the life, and in our study, we recorded only the breeding outcomes during the first reproductive bout. We have now mentioned this in the methods section (Line 148–149). As suggested, we also brought up the difference between single vs. lifetime reproductive output in the discussion section (Line 364–367).

Comment 6 > Line 231. Please provide an explanation for using a type II sums of squares approach. Usually, Type III sums of squares provide a more complete test of the hypothesis with multiple predictors.

Response 6 > We used type II sums of squares in the ANOVA because it respects the principle of hierarchy (marginality). For example, in a model with two predictors A and B as well as the interaction $A \times B$, it first tests the interaction term $A \times B$ ($Y \sim A + B$ vs. $Y \sim A + B + A \times B$) and drops $A \times B$ to test the main effect of A ($Y \sim B$ vs. $Y \sim A + B$) and B ($Y \sim A$ vs. $Y \sim A + B$).

On the other hand, type III sums of squares tests the main effect when all other terms are kept in the model. For example, for the same model above, it first tests the interaction term $A \times B$ ($Y \sim A + B$ vs. $Y \sim A + B + A \times B$), which is the same as type II sums of squares. However, it tests the main effect of A ($Y \sim B + A \times B$ vs. $Y \sim A + B + A \times B$) and B ($Y \sim A + A \times B$ vs. $Y \sim A + B + A \times B$) in the presence of $A \times B$. The models $Y \sim B + A \times B$ and $Y \sim A + A \times B$ are meaningless because they contain the higher order term $A \times B$ without the lower-order terms A and B.

Besides the issue of marginality, studies have also shown that type II sums of squares generally has higher statistical power than type III sums of squares and therefore is more appropriate for testing main effects (Langsrud 2003, Smith & Cribbie 2014).

References:

Langsrud, Ø. (2003). ANOVA for unbalanced data: Use Type II instead of Type III sums of squares. *Statistics and computing*, 13(2), 163-167.

Smith, C. E., & Cribbie, R. (2014). Factorial ANOVA with unbalanced data: a fresh look at the types of sums of squares. *Journal of Data Science*, 12(3), 385-403.

Comment 7 > Lines 341-342. I don't think this is an accurate statement. The citation is old, and the level of involvement of both parents varies among species. Do you have information from field data about *N. nepalensis* specifically?

Response 7 > Based on the field data collected in a previous study, a group size of 2 is common in *N. nepalensis* (Fig. 2 in Liu et al. 2020). However, the group size can vary substantially across the elevations and with population densities. To avoid confusion, we have now removed the statement (Line 353–354).

Reference:

Liu, M., Chan, S. F., Rubenstein, D. R., Sun, S. J., Chen, B. F., & Shen, S. F. (2020). Ecological transitions in grouping benefits explain the paradox of environmental quality and sociality. *The American Naturalist*, 195(5), 818-832.

Comment 8 > Line 352. See above comment. This is not the first time this has been shown.

Response 8 > Revised (Line 369).

Comment 9 > Line 359-360. Not quite an accurate statement. The balance between size and number of offspring is more involved than simply carcass size. Many studies not cited here have addressed this relationship in burying beetles.

Response 9 > Thanks for pointing this out. Yes, we agree that there are many factors besides carcass size that can influence the offspring quality-quantity relationship in burying beetles. In fact, we did not intend to conclude that carcass size is the “only” or the “most important” factor. Instead, our study adds to the understanding that carcass size, among many other factors addressed in previous studies, can shape this quality-quantity balance. We have modified our statement to avoid potential misunderstandings (Line 375–378).

Reviewer 2's comments

Comment 1 > The authors examine the impacts of carcass size, origin (lab-reared vs wild), and taxon (mammal, bird, or reptile) on various breeding success metrics in captive experiments using a burying beetle (*Nicrophorus nepalensis*). The authors further test for nutritional differences among carrion, estimate optimal carcass size, and test for a trade-off between larval size and number in the context of carcass size, origin, and taxon. The authors find strong evidence for an optimal size of carcass under laboratory conditions and find little difference in reproductive success between carcass origin and taxon, the latter of which did not differ substantially in protein/fat content. Overall, the study provides important validation of other work that uses lab-reared carcasses in experiments with *Nicrophorus*, as well as interesting evidence for optimal carcass size and an influence of carcass size on life history strategies.

Overall, the manuscript was very clear and well written. The experiments were well-designed and carefully executed with clear and appropriate statistics, plots, and well-balanced and reasonable interpretations. It was just a really nice, refreshing manuscript to read. I had a few comments, suggestions, and corrections, listed below.

Response 1 > We greatly appreciate the positive feedback on this study and we have revised the manuscript based on the comments below.

Comment 2 > The authors conduct many statistical tests using non-independent data, which can lead to some p-values that fall below 0.05 simply due to chance. Have you considered controlling for false discovery rates (e.g., Pike 2011, *Methods in Ecology and Evolution*)? Controlling for false discovery rates wouldn't change the main results of the paper, but would put some of the borderline p-values/results into better context.

Response 2 > Thanks for the nice suggestion. Yes, we did conduct multiple tests on various breeding outcomes and carcass use efficiency, each with multiple predictors (Table 1), which could potentially lead to significant results by chance. As suggested, we therefore performed the Benjamini–Hochberg multiplicity adjustment for the *p*-values in Table 1 to control for the false discovery rates at $\alpha = 0.05$:

Model response	<i>n</i>	Predictor		
		Carcass weight	Carcass source	Weight × Source
Clutch size	210	Original: <i>P</i> < 0.001 Adjusted: <i>P</i> < 0.001	Original: <i>P</i> = 0.39 Adjusted: <i>P</i> = 0.59	Original: <i>P</i> = 0.24 Adjusted: <i>P</i> = 0.45
Hatching success	176	Original: <i>P</i> < 0.001 Adjusted: <i>P</i> < 0.001	Original: <i>P</i> = 0.37 Adjusted: <i>P</i> = 0.59	Original: <i>P</i> = 0.88 Adjusted: <i>P</i> = 0.96
Brood size	238	Original: <i>P</i> < 0.001 Adjusted: <i>P</i> < 0.001	Original: <i>P</i> = 0.93 Adjusted: <i>P</i> = 0.96	Original: <i>P</i> = 0.17 Adjusted: <i>P</i> = 0.36
Brood mass	129	Original: <i>P</i> < 0.001 Adjusted: <i>P</i> < 0.001	Original: <i>P</i> = 0.99 Adjusted: <i>P</i> = 0.96	Original: <i>P</i> = 0.004 Adjusted: <i>P</i> = 0.01
Carcass use efficiency	95	Original: <i>P</i> < 0.001 Adjusted: <i>P</i> < 0.001	Original: <i>P</i> = 0.96 Adjusted: <i>P</i> = 0.96	Original: <i>P</i> = 0.57 Adjusted: <i>P</i> = 0.77

In fact, we did not have borderline *p*-values in the original results, so there is less concern about this issue. Since the adjustment did not alter the overall conclusions, we decided to keep the original results.

For the pairwise comparisons between the three wild carcass taxa in Fig. 3 and Fig. 4, we did perform the Tukey multiplicity adjustment in the original analysis to control for the family-wise error rates.

Comment 3 > lines 61-65: What about additional costs of competitive interactions that vary with carcass size? For example, are large carcasses more likely to be found and eaten by vertebrates, thereby reducing fitness? How does microbial competition vary with carcass size and with different environmental temperatures? [I see this discussed on lines 344-350 - great!]

Response 3 > Thanks for bringing these points up. As mentioned, we did talk about how competition from vertebrate scavengers and microbes may influence the optimal carcass size for burying beetles in nature in the discussion section. We have now added a brief explanation to the

original sentence in the introduction by saying that larger carcasses might be more difficult to utilize because of greater competition from other carcass-feeding organisms (Line 62–63). Regarding the effect of temperature on optimal carcass size, we have included this in the discussion section. Please see our response to Comment 13 for more details.

Comment 4 > lines 117-120: Were carcasses from the lab versus wild at similar levels of decomposition?

Response 4 > The wild carcasses used in the study were roadkill animals immediately transferred to the –20°C freezers upon discovery. Even though these wild carcasses might not be as fresh as the lab carcasses (which were killed via euthanasia and frozen right away), we selected wild carcasses with decomposition levels as similar to those of the lab carcasses to minimize the potential confounding effects of carcass freshness on breeding outcomes (degraded wild carcasses would not be used in the experiments).

Comment 5 > lines 126-128: I really liked the controlled and paired design.

Response 5 > Thanks for the positive feedback.

Comment 6 > lines 151-153: Isolating viscera and muscle seems to miss independent fat deposits (e.g., often well-developed between the skin and muscle on migratory birds). Why not estimate fat/protein composition of the entire carcass (after removing external hair/feathers)? Wouldn't the entire carcass (excluding bones) be a better representation of what the beetles use?

Response 6 > Thanks for the comment. First, there were not many fat deposits under the skin (subcutaneous fat) for the wild bird carcasses we dissected (we did not have migratory birds in our study). Second, we did include the visible subcutaneous fat as well as fat deposits around the internal organs (visceral fat) in the viscera tissue samples, and the criterion for the inclusion of fat tissues was consistent across the carcasses. Since the muscle and viscera tissues are the main

parts of the carcasses that the beetles use, our sampling should fairly characterize the carcass resources for them.

Comment 7 > line 208: What is carcass ID? If it relates to only one row of data per analysis, then why include it as a random effect?

Response 7 > In the analysis of carcass tissue nutritional composition, we collected three muscle and three viscera tissue samples from each carcass (a total of seven lab carcasses, seven wild mammal carcasses, six wild bird carcasses, and six wild reptile carcasses were dissected and analyzed), and each row in the dataset represents one tissue sample. Since the tissue samples from the same carcass were not independent, we included carcass ID as a random effect to account for this.

Comment 8 > lines 219-220: "Dead larvae were excluded from the analysis." How often did larvae die in the experiment?

Response 8 > Thanks for the comment. The larval survival rates in the feeding experiments were low (22.3%); only 42 out of 188 larvae survived after the five-day feeding period. We have provided this information in the methods section (Line 224–225). In fact, this indicates the importance of parental care in the reproduction of burying beetles: without parents, larval survival is low, presumably because of competition from microbes or a lack of food provisioning.

Comment 9 > lines 262-263 vs Figure 4f: "... although larvae feeding on wild bird carcasses tended to gain more weight compared to those feeding on wild mammals and reptiles (Fig. 4f)." Larval growth was highest for mammal carcasses, according to figure 4f.

Response 9 > Thanks for pointing this out. We double checked the raw data and larval growth indeed tended to be higher on wild bird carcasses (although not statistically significant). We also checked the R script for the figure and found a mistake in the axis labels. We have corrected this and updated the figure.

Comment 10 > lines 297-299: Was this evident in your study as well? Or would this only be evident if you use natural soil or conduct the experiments in nature?

Response 10 > We do not have direct evidence for this as we did not analyze the microbial communities on the carcasses, but we proposed it as a potential explanation for why breeding performance did not simply increase with carcass size as one would predict based solely on resource availability. This speculation can be tested via the correlation between carcass size and skin/gut microbial communities (abundance, composition, etc.) either in the field to allow for natural variation in background soil microbes or in the lab settings to control for the background microbes.

Comment 11 > lines 304-307: An interesting idea, and consistent with some suggestions that Yarrowia (in *Nicrophorus secretions*) 'pre-digest' carrion for the young.

Response 11 > Yes, regurgitation is an important part of parent care in burying beetles.

Comment 12 > lines 321-322: Again, I don't see this result in figure 4f.

Response 12 > Please see our response to Comment 9 for more details.

Comment 13 > lines 346-347: I would think that temperature may further interact with carcass size and competitors to shift optimal carcass size in nature.

Response 13 > Thanks for the suggestion. This is a great point and we have now brought it up in the discussion section (Line 359–361).

Comment 14 > lines 344-350: I really appreciated the discussion section acknowledging the potential for different optimal carcass sizes in nature, where other selective pressures (e.g., vertebrate scavengers) could play important roles.

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Response 14 > Thanks for the positive feedback.

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Abstract

Small vertebrate carcasses represent critical resources for many terrestrial organisms, including burying beetles, which rely on carcasses for survival and breeding. Carcass attributes can influence the reproduction of burying beetles, yet most studies on their breeding ecology have used laboratory-reared carcasses of limited sizes. We conducted breeding and feeding experiments using a wide size range of lab (laboratory mice) and wild carcasses (wild mammals, birds, and reptiles) to investigate how carcass size, source, and taxon affect various breeding outcomes (e.g., clutch size, brood size, and brood mass) of the burying beetle *Nicrophorus nepalensis*. Our results reveal a hump-shaped relationship between carcass size and breeding performance, with optimal breeding outcomes occurring on medium-sized carcasses. Furthermore, despite the variation in carcass tissue nutritional composition, breeding outcomes and larval growth did not differ between the two carcass sources or among the three wild carcass taxa. Finally, we found a larval quality-quantity trade-off across the range of carcasses examined, with carcass size shaping the larval life history traits. Overall, these results elucidate how carcass resources may influence the breeding performance of burying beetles. Importantly, our study provides solid evidence validating decades of research using lab carcasses to study the reproductive ecology of burying beetles.

Keywords

breeding performance, burying beetle, carcass, nutritional composition, offspring quality-quantity trade-off, resource use efficiency

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Introduction

Small vertebrate cCarcasses represent a rich resource for a wide variety of terrestrial organisms, including vertebrate scavengers, saprophagous invertebrates, and microbial decomposers [1-3]. These carcass-feeding organisms facilitate the recycling of carcass nutrients and make the resource available to other species [4]. 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 [5]. 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 [5-9]. Moreover, parents can adjust their reproductive investment based on carcass size [10]. For example, females lay more eggs on larger carcasses within a certain carcass size range [11], and parents regulate the brood size via filial cannibalism when carcass resource is limited [12]. However, despite the resource benefits, large carcasses can be more difficult to utilize because of greater competition from other carcass-feeding organisms-[9], 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 [13]. A few studies have empirically examined the relationship between optimal carcass size and reproductive output in burying beetles [e.g., 14], yet a general understanding is still lacking.

While most studies on the reproduction of burying beetles have focused on Besides carcass size, the source of carcass may also influence their breeding outcomes 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 [15], and these differences can alter larval survival and growth [1, 16]. Therefore, 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, burying beetles have been documented to breed on carcasses from a variety of taxonomic groups [5, 17]. Different carcass taxa can vary not only in their abundance but also in their tissue nutritional composition [18], which ~~may~~ influence the overall reproductive performance of parents as well as individual larval growth and development [19]. However, it remains unknown how breeding outcomes and larval performance may vary among different groups of wild carcasses.

Studies have shown that brood size and larval mass of burying beetles are often negatively correlated with each other [6, 12, 20]. Such a trade-off between larval quality and quantity may vary with carcass size [8, 21] because resource quantity can shape the life history traits of organisms [22-24]. 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 [8]. 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 25]. 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 taxon) influence the breeding outcomes, larval performance, and offspring quality-quantity trade-off in burying beetles. We conducted breeding experiments on the species *Nicrophorus*

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3 97 *nepalensis*, which has been shown to provide extensive parental care for offspring. First, we
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5 98 examined how breeding outcomes (clutch size, brood size, brood mass, etc.) and carcass use
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10 100 this study) on lab (laboratory mice) and wild carcasses (wild mammals, birds, and reptiles).
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12 101 We further focused on the wild carcasses and compared the larval breeding outcomes and
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14 102 carcass use efficiency of *N. nepalensis* on the three wild carcass taxa. We expected that there
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17 103 would be an optimal carcass size for breeding, and the breeding outcomes may differ between
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19 104 lab and wild carcasses as well as among different wild carcass taxa. We next quantified the
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21 105 tissue nutritional composition of lab and wild carcasses and conducted a larval feeding
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23 106 experiment using carcass tissues from different sources and taxa. We expected that the larvae
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26 107 would perform better when feeding on diets with higher nutritional quality. Finally, we
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28 108 examined the larval quality-quantity trade-off on lab and wild carcasses. We expected a trade-
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30 109 off across a broad range of carcass sizes, and the trade-off pattern would differ between lab and
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112 **Materials and Methods**

113 *Breeding experiments*

114 We conducted breeding experiments on *N. nepalensis* from the lab colony established in 2023.
115 Adult beetles were collected from Taipei and New Taipei City, Taiwan and reared in growth
116 chambers under a relative humidity of 70% and a 10:14 h light:dark cycle. The temperature
117 was set to mimic diurnal temperature fluctuation (mean: 17.8°C; range: 16–20°C). This
118 represents the natural temperature conditions during the breeding season (November–April) of
119 *N. nepalensis* in northern Taiwan. A male and a female were placed in a plastic breeding
120 container (14.2 cm in diameter and 6.3 cm in height) half-filled with moist commercial potting
121 mix (2 cm in depth, equivalent to 300 mL), and a defrosted carcass was then placed on the soil
122 surface. Frozen dead laboratory mice/rats were used as lab carcasses. Wild carcasses were
123 obtained from the Taiwan Roadkill Observation Network (<https://roadkill.tw/eng/home>) and
124 the Wild Bird Society of Taipei. These wild carcasses weighed from 1.6 to 99.5 grams and
125 consisted of small mammals, birds, and reptiles. The carcasses used for breeding experiments
126 were animals that had died within the past four months due to traffic collisions and other
127 accidental causes but not poisoning. Upon discovery, these carcasses were immediately
128 transferred to –20°C freezers for preservation. We paired each wild carcass with a lab carcass
129 of a similar weight (measured to the nearest 0.1 g using an electronic analytical balance
130 ATX224R, Shimadzu, Japan) and applied a sibship design where the two males and the two
131 females used in each lab-wild carcass were from the same family line, respectively, to control
132 for parental genotypes (the males and females came from genetically unrelated families). The
133 breeding containers were maintained under the same environmental conditions as those of the
134 lab colony. Five rounds of breeding experiments were conducted from May 2023 to March
135 2024 (each with a different beetle parent generation), consisting of a total of 121 lab-wild
136 carcass pairs (14, 76, and 31 wild mammal, bird, and reptile carcasses).

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We recorded the clutch size of each breeding container at day 4 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 while providing an accurate estimate of the exact clutch size ($r = 0.94$, $P < 0.001$, $n = 70$ broods) [26]. Eleven days after beetle pairing, we inspected the carcass to record the brood size (number of larvae) and brood mass (total larval weight; measured to the nearest 0.0001 g). 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 parents and larvae during the breeding process (larvae were removed from the carcasses). Carcass use efficiency was calculated as the amount of carcass tissue used divided by the initial carcass weight. All the aforementioned breeding outcomes and carcass use were recorded during the first reproductive bout of the breeding pairs.

Nutritional analysis of carcass tissue

To quantify the nutritional composition of lab and wild carcasses, which is essential for understanding how burying beetles use different types of carcasses, we estimated the protein and fat content of carcass tissue by adopting a proximate analysis approach as described by Al Shareefi and Cotter [27]. We dissected the carcasses by first skinning the animals and retaining the trunks. Trunk tissue was then separated from the bones with a pair of fine tweezers and a scalpel and divided into viscera (all organs inside the peritoneum) and muscles (all visible muscle parts). We next used a meat tenderizer to pound the viscera and muscles evenly and sampled three pieces of visceral and muscle tissue for each carcass for the analysis of

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3 160 nutritional composition. A total of seven lab mice, seven wild mammals, ~~six~~even wild birds,
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5 161 and ~~six~~even wild reptiles were dissected and analyzed.
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9 162 For each tissue sample, we dried approximately 100 mg (106 ± 18 mg) of wet tissue in
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11 163 a 40°C oven for 5 days until all water was removed. To determine the fat content, the dried
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13 164 tissue was thoroughly mixed with 100 µl of -20°C acetone and vortexed for one minute. The
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15 165 mixture was then placed in a -20°C fridge for a 30-minute reaction period [28]. After the
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17 166 extraction, the mixture was centrifuged to separate the components, and the acetone was
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19 167 carefully removed. If the acetone appeared turbid after centrifugation, the solvent was
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21 168 discarded and replaced with fresh acetone for further extraction. The process was repeated until
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23 169 the solvent became clear. The residual solvent was then allowed to evaporate at room
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25 170 temperature for 12 hours. After the fat removal process, the final product was weighed to
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27 171 determine the protein content, and the fat content was determined by subtracting the protein
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36 37 38 174 *Larval feeding experiments*

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41 175 We conducted larval feeding experiments using the remaining dissected carcass tissue from the
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43 176 nutritional composition analysis. We placed *ca.* 400 mg (401 ± 21 mg) of carcass tissue into
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45 177 individual plastic containers filled with moist commercial potting mix (soil volume 3.2×3.2
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47 178 $\times 2.7$ cm). Newly hatched larvae (five days after female oviposition) were obtained from pairs
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49 179 of breeding beetles (25 families) from the lab colony and one larva was introduced to each
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51 180 container ($n = 188$). After five days of feeding, the larval mass at dispersal was recorded and
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53 181 larval growth was measured as the larval weight gain during the experimental period.
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183 *Data analyses*

184 1. Breeding outcomes and carcass use efficiency

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

200 To compare the brood size, brood mass, average larval mass, and carcass use efficiency
201 on wild mammal, bird, and reptile carcasses, we fit generalized linear models (GLMs) with
202 each of the aforementioned breeding outcomes as the response and wild carcass taxon as the
203 fixed effect. Carcass weight, pronotum widths of the parents, and parent generation were
204 included as the covariates in the models. The error distribution and link function for each of
205 the responses were the same as the GLMMs. Because the carcass range was considerably
206 smaller for reptiles (1.6–64.4 g) than for mammals (3.8–94.8 g) and birds (3.2–99.5 g), we

restricted the carcass weight range to that of reptiles (≤ 64.4 g) so that the results were more comparable among the three wild taxa.

2. Nutritional composition and larval growth

To compare the nutritional composition between the two carcass sources and the three wild carcass taxa, we fit GLMMs with the proportion of protein/fat as the responses, carcass source/taxon and tissue type (viscera vs. muscles) as the fix effects, and carcass ID as the random effect (a total of four GLMMs). We used a beta error distribution with a logit link function for model fitting in the GLMMs.

To compare the larval growth between the two carcass sources and the three wild carcass taxa, we fit GLMMs with larval weight gain as the response, carcass source/taxon and tissue type as the fix effects, and carcass ID and larval family as the random effects (a total of two GLMMs). Larval mass at hatching was included in the models as a covariate. We used a Gaussian error distribution for model fitting in the GLMMs. To further investigate the effect of nutrient content on larval growth on both carcass sources and on wild carcasses only, we fit GLMMs with larval weight gain as the response, proportion of protein, proportion of fat, and tissue type as the fixed effects, and larval family as the random effects (a total of two GLMMs). Larval mass at hatching was included as a covariate. Dead larvae ($n = 146$) were excluded from the analysis (larval survival rate was 22.3% in the five-day feeding experiments).

3. Larval quality-quantity trade-off

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,

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230 and their interaction as the predictors. A significant negative slope indicates a larval quality-

231 quantity trade-off.

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233 We fit all aforementioned models using the `glmmTMB()` function in the R “glmmTMB” package

234 [29]. Model assumptions were checked via the quantile residuals generated from the

235 `simulateResiduals()` function in the R “DHARMa” package [30]. Predictor significance was

236 assessed with the Wald chi-square test via the `Anova()` function (type II sums of squares) in

237 the R “car” package [31]. Post-hoc pairwise comparisons among carcass taxa with the Tukey

238 multiplicity adjustment were conducted via the `emmeans()` function in the R “emmeans”

239 package [32]. All analyses were performed in R version 4.3.3 [33].

240 Results

241 *Breeding outcomes and carcass use efficiency*

242 Clutch size, hatching success, brood size, and brood mass all showed a quadratic relationship
 243 with carcass weight (clutch size: $\chi^2_2 = 44.6$, $P < 0.001$; hatching success: $\chi^2_2 = 32.1$, $P < 0.001$;
 244 brood size: $\chi^2_2 = 63.3$, $P < 0.001$; brood mass: $\chi^2_2 = 91.9$, $P < 0.001$; Table 1) and peaked on
 245 medium-sized carcasses (Fig. 1). Moreover, these breeding outcomes did not differ between
 246 lab and wild carcasses (clutch size: $\chi^2_1 = 1.4$, $P = 0.39$; hatching success: $\chi^2_1 = 0.8$, $P = 0.37$;
 247 brood size: $\chi^2_1 = 0.009$, $P = 0.93$; brood mass: $\chi^2_1 = 0.001$, $P = 0.99$; Table 1; Fig. 1). Carcass
 248 use efficiency decreased with carcass weight ($\chi^2_2 = 64.5$, $P < 0.001$) but did not differ between
 249 lab and wild carcasses ($\chi^2_1 = 0.003$, $P = 0.96$; Table 1; Fig. 2).

250 Brood size, brood mass, average larval mass, and carcass use efficiency did not differ
 251 among wild mammal, bird, and reptile carcasses (brood size: $\chi^2_2 = 0.6$, $P = 0.75$; brood mass:
 252 $\chi^2_2 = 3.6$, $P = 0.17$; average larval mass: $\chi^2_2 = 3.3$, $P = 0.19$; carcass use efficiency: $\chi^2_2 = 0.4$,
 253 $P = 0.81$; Fig. 3).

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255 *Nutritional composition of carcasses*

256 Protein content was similar between lab and wild carcasses (mean proportion: lab = 25.5%,
 257 wild = 27.9%; $\chi^2_1 = 3.5$, $P = 0.06$; Fig. 4a) but differed among wild carcass taxa (mean
 258 proportion: mammal = 28.7%, bird = 30.6%, reptile = 24.3%; $\chi^2_2 = 26.6$, $P < 0.001$; Fig. 4b).
 259 Specifically, reptile carcasses had significantly lower protein content than mammal and bird
 260 carcasses (Fig. 4b). Fat content was similar between lab and wild carcasses (mean proportion:
 261 lab = 4.0%, wild = 3.7%; $\chi^2_1 = 1.1$, $P = 0.29$; Fig. 4c) and among wild carcass taxa (mean
 262 proportion: mammal = 4.4%, bird = 4.4%, reptile = 2.1%; $\chi^2_2 = 3.5$, $P = 0.18$; Fig. 4d).

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Larval growth

Growth was similar for larvae feeding on tissue from lab and wild carcasses ($\chi^2_1 = 0.1$, $P = 0.74$; Fig. 4e). Similarly, larval growth did not differ significantly among the three wild carcass taxa ($\chi^2_2 = 5.2$, $P = 0.07$; Fig. 4f), although larvae feeding on wild bird carcasses tended to gain more weight compared to those feeding on wild mammals and reptiles (Fig. 4f). When lab and wild carcasses were combined, larval growth was not associated with either tissue protein content ($\chi^2_1 = 0.9$, $P = 0.34$) or fat content ($\chi^2_1 = 0.05$, $P = 0.83$) (Fig. S2a and b). On the other hand, larvae feeding on wild carcass tissue with higher fat content ($\chi^2_1 = 5.2$, $P = 0.02$), but not protein content ($\chi^2_1 = 0.01$, $P = 0.92$), did grow better (Fig. S2c and d).

Larval quality-quantity trade-off

Average larval mass decreased with larval density on both lab and wild carcasses ($\beta = -0.096$, $\chi^2_1 = 74.7$, $P < 0.001$; Fig. 5). The interaction between larval density and carcass source was not significant ($\chi^2_1 = 1.2$, $P = 0.28$), 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 the burying beetle *N. nepalensis* varied with carcass size on lab and wild carcasses. Clutch size, hatching success, brood size, and brood mass all exhibited a quadratic relationship with carcass size, whereas carcass use efficiency decreased with carcass size. Furthermore, these breeding outcomes and carcass use efficiency did not differ between lab and wild carcasses. Despite the variation in tissue nutritional composition (protein content) among wild mammal, bird, and reptile carcasses, larval traits (brood size, brood mass, and average larval mass), carcass use efficiency, and larval growth did not differ among the three wild carcass taxa. 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 carcass size, but not carcass source or carcass taxon, is the main determinant for the breeding performance and carcass resource use of burying beetles.

As expected, clutch size, hatching success, brood size, and brood mass all showed a quadratic relationship with carcass size, with optimal breeding outcomes occurring on medium-sized carcasses. The increase in breeding performance from small to medium carcasses is consistent with previous studies on other burying beetle species [6, 10, 34]. Interestingly, when the parents bred on large carcasses, their breeding performance decreased, along with a reduction in 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 [11] found that clutch size levels off beyond a certain carcass weight threshold, suggesting an energetic or physiological constraint on beetles breeding on larger carcasses. Parents breeding on large carcasses also face stronger competition with microbes, which can reduce the usable resource for breeding [5] or produce compounds harmful to eggs and larvae [1].

Contrary to our prediction, we found no major difference in the breeding outcomes and carcass use efficiency of *N. nepalensis* on lab versus wild carcasses. A potential explanation is that the parents manipulated the carcasses (e.g., by secreting antimicrobial compounds) such that the eggs and larvae experienced similar growing environments regardless of carcass source. Studies have shown that parental care is crucial for larval performance in burying beetles [1, 35], and we speculate that parental food preparation and regurgitation may offset the difference between the two carcass sources. Further experiments comparing breeding outcomes on lab and wild carcasses with versus without parents will help verify our speculation. The analyses did reveal an interaction between carcass size and carcass source for brood mass. In fact, the patterns were mostly similar between lab and wild carcasses on small and medium carcasses, whereas the difference on large carcasses was mainly driven by two observations on large wild carcasses (the interaction became non-significant when these two observations were removed; $P = 0.38$, Fig. S3). Overall, our results support the validity of research using lab-reared organisms as breeding carcasses to study the reproductive biology of burying beetles.

Our tissue nutritional analysis showed that protein content was higher in wild mammal and bird carcasses than in wild reptile carcasses, whereas fat content was similar among these taxa. Yet, despite the variation in tissue protein content, larval traits and carcass use efficiency in the breeding experiments as well as larval growth in the feeding experiments did not vary significantly among the three wild carcass taxa. In fact, ~~we found~~ our feeding experiments showed that it was fat content, not protein content, that affected larval growth on wild carcasses. Since fat content did not vary among the three wild carcass taxa, we did not observe major difference in larval growth. This may also partially explain why larval traits and carcass use efficiency were similar among the three wild carcass taxa in ~~the~~ our breeding experiments. These results suggest that parents can utilize carcass resources from different vertebrate taxa that vary in their nutritional content and potentially carcass abundance in the wild while having

~~similar breeding outcomes. Without parents, larval survival can be quite low (22.3% in the feeding experiments), and carcass taxon may potentially influence individual larval performance, as larvae did tend to grow better on bird carcasses in the feeding experiments. Interestingly, larvae did tend to grow better on bird carcasses in the feeding experiments without parents. This suggests highlights the importance of~~ parental care in burying beetles (e.g., carcass preparation and food provisioning) ~~may help in maintaining~~ breeding performance on a variety of carcasses in the wild. ~~But without parental care, carcass taxon may potentially influence individual larval performance.~~

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. Similar trade-off patterns have been shown in previous studies [21, 36] and can arise from both larval competition and brood regulation by parents [36]. Stronger interspecific competition under a higher larval density may reduce individual larval growth, leading to lower average larval mass. On the other hand, parents may regulate brood size by culling excess larvae to reduce larval competition [37], thereby leading to greater larval growth and higher average biomass. Furthermore, 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 size for small and medium carcasses, whereas larval density decreased (Fig. S1). This suggests that the larval 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 size in the breeding outcomes of a single parent pair. ~~This is the most common breeding system in burying beetles on small and medium-sized carcasses.~~ However, multiple males and females may engage in cooperative breeding to better

utilize large carcasses in the wild [38], although past results for the reproductive benefits of cooperation are mixed [39-41]. Additionally, burying beetles in nature may face carcass competition not only from microbes but also from various vertebrate scavengers and invertebrate carcass feeders [42, 43], and such interspecific competition can interact with carcass size to influence breeding success [44]. Abiotic factors such as temperature may further affect the optimal carcass size by altering carcass decomposition rates, carcass handling time, and parents' activity levels [45]. Therefore, ~~F~~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. It is also noteworthy that burying beetles can reproduce multiple times throughout their lifespans, and the patterns of single reproductive output may differ from the lifetime reproductive output [14]. Research quantifying the lifetime reproductive output will help better understand how carcass resources affect the over fitness of breeding individuals.

Using a broad range of carcass sizes from both lab and wild sources, our study revealed ~~afor the first time the~~ quadratic relationship between breeding performance and carcass size in burying beetle, with optimal breeding outcomes occurring on medium-sized carcasses. Breeding outcomes did not differ between lab and wild carcasses. Furthermore, despite the variation in tissue nutritional composition (particularly protein content) among wild mammal, bird, and reptile carcasses, larval traits, carcass use efficiency, and larval growth were generally similar among these wild carcass taxa. Finally, the larval quality-quantity trade-off existed across the range of lab and wild carcass sizes, ~~and larval life history traits may shift depending on carcass size,~~ with higher larval quantity (larval density) but lower quality (average larval mass) on smaller carcasses ~~favoring larval quantity and~~ lower larval quantity but higher larval quality on larger carcasses ~~ss favoring larval quality~~. Taken together, our study confirms that previous results from lab carcasses are fairly representative of natural patterns and provides a

380 more complete picture of how carcass resources shape the breeding performance of burying
381 beetles.

382

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393

394 **Conflict of interest**

395 The authors declare no conflict of interest regarding this manuscript.

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397 **Author contributions**

398 Gen-Chang Hsu and Syuan-Jyun Sun conceived the ideas; Gen-Chang Hsu, Wei-Jiun Lin, Yue-
399 Jia Lee, and Syuan-Jyun Sun designed the experiments; Gen-Chang Hsu, Wei-Jiun Lin, Chi-
400 Heng Hsieh, Yue-Jia Lee, and Syuan-Jyun Sun collected the data; Gen-Chang Hsu and Syuan-

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Jyun Sun analyzed the data; Gen-Chang Hsu and Syuan-Jyun Sun wrote the first draft of the manuscript; all authors revised the manuscript and approved the final version for publication.

Data accessibility

Data and code used in this manuscript are publicly available on Zenodo [46].~~(DOI:~~
~~<https://doi.org/10.5281/zenodo.12747445>~~).

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Figures

Figure 1

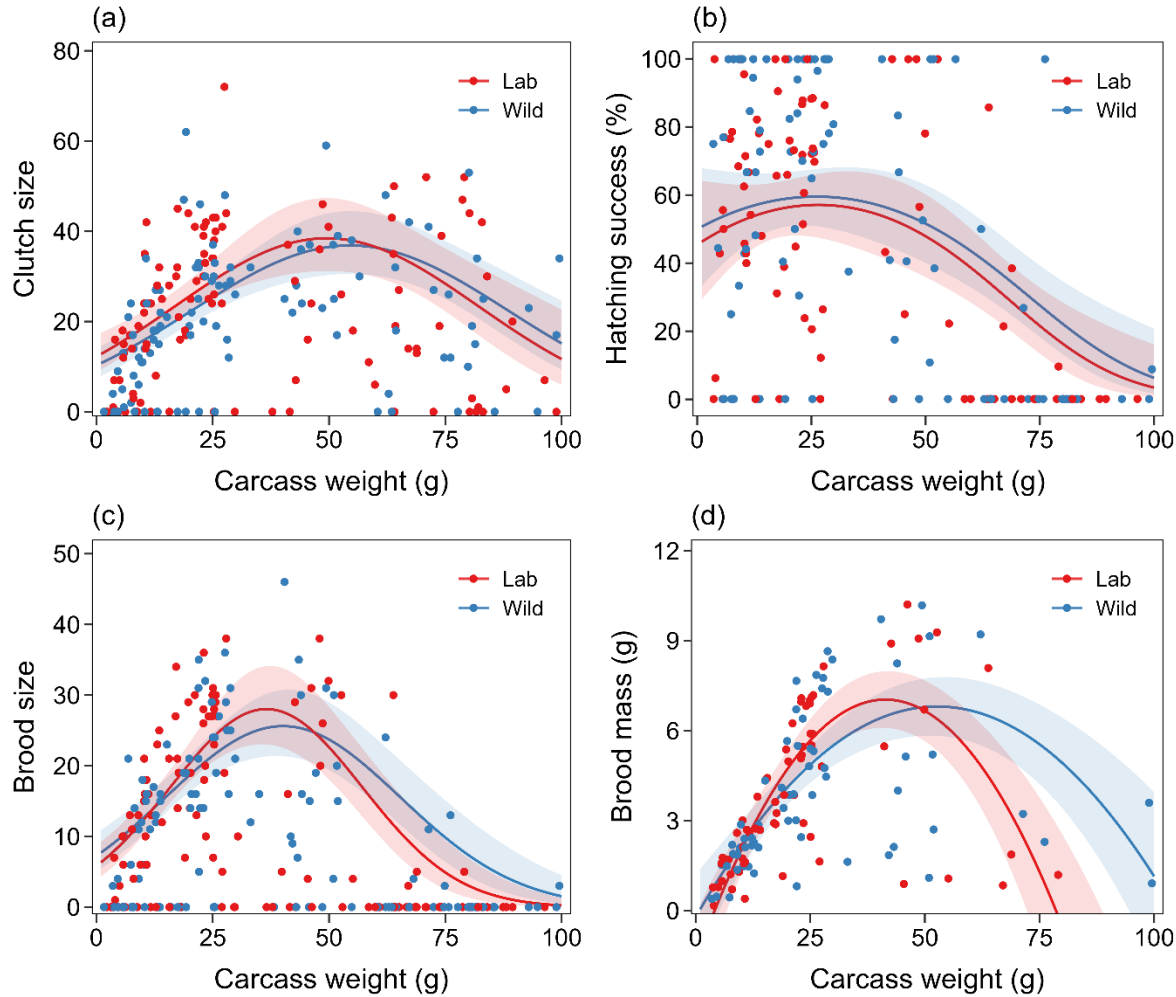
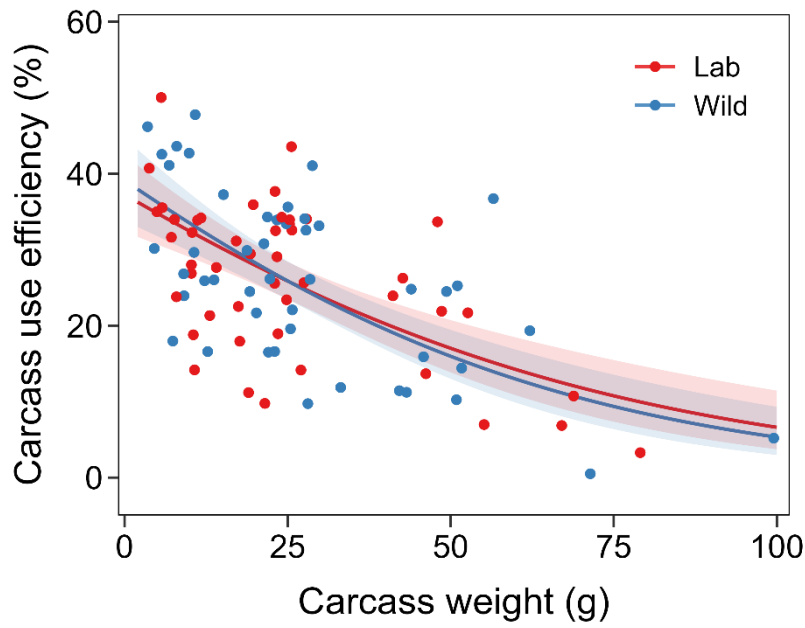


Figure 2



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Figure 3

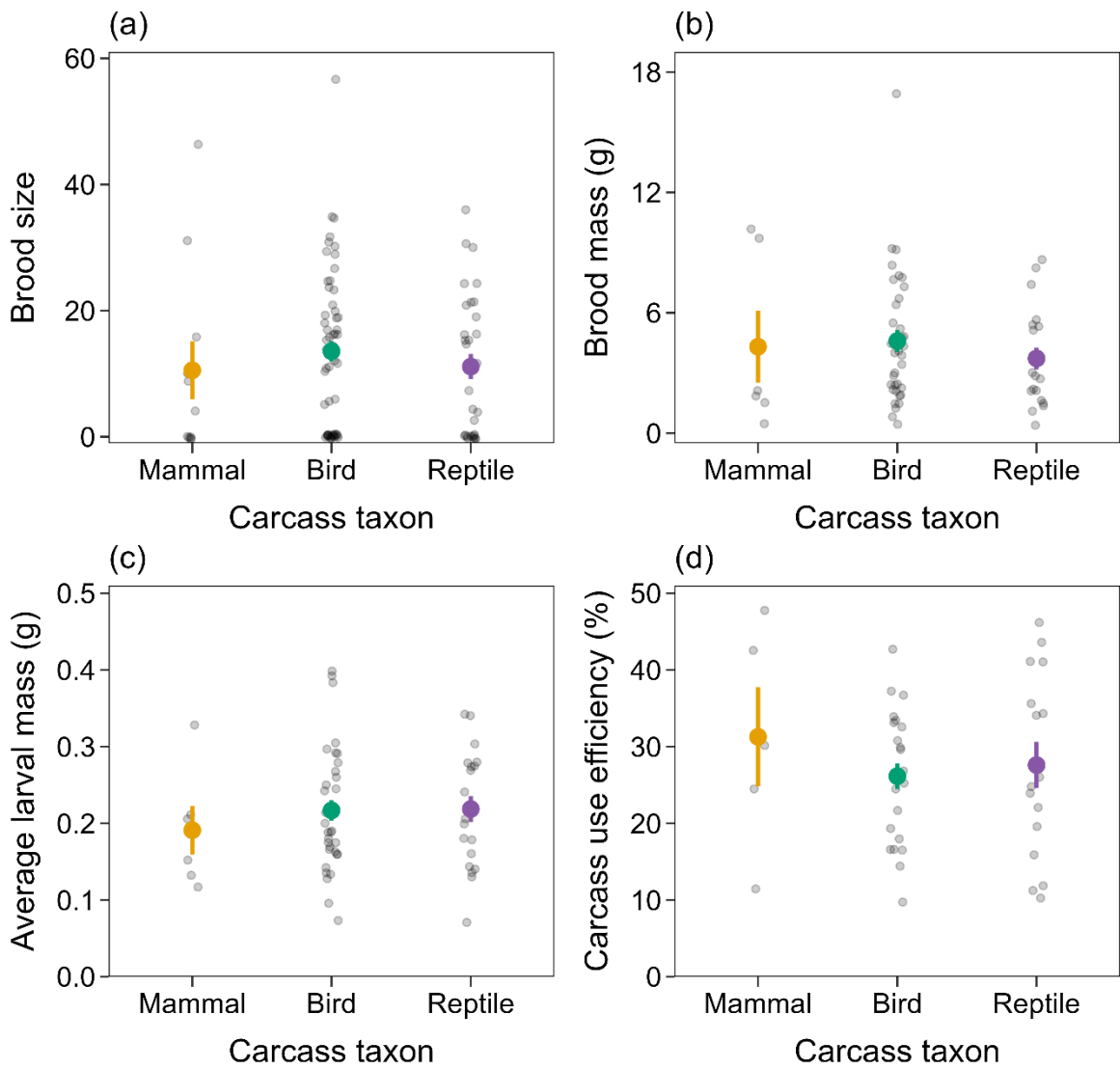
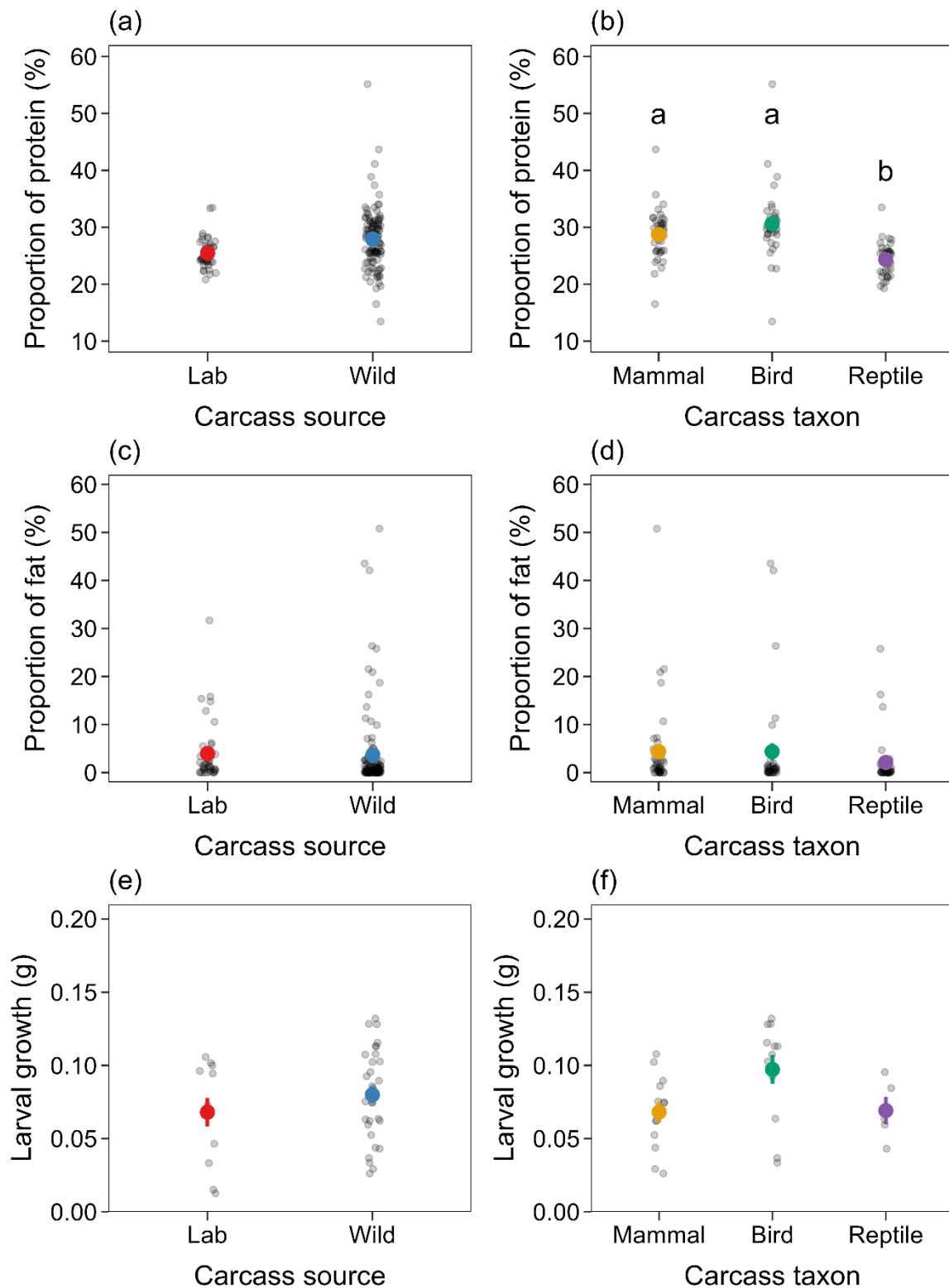


Figure 4



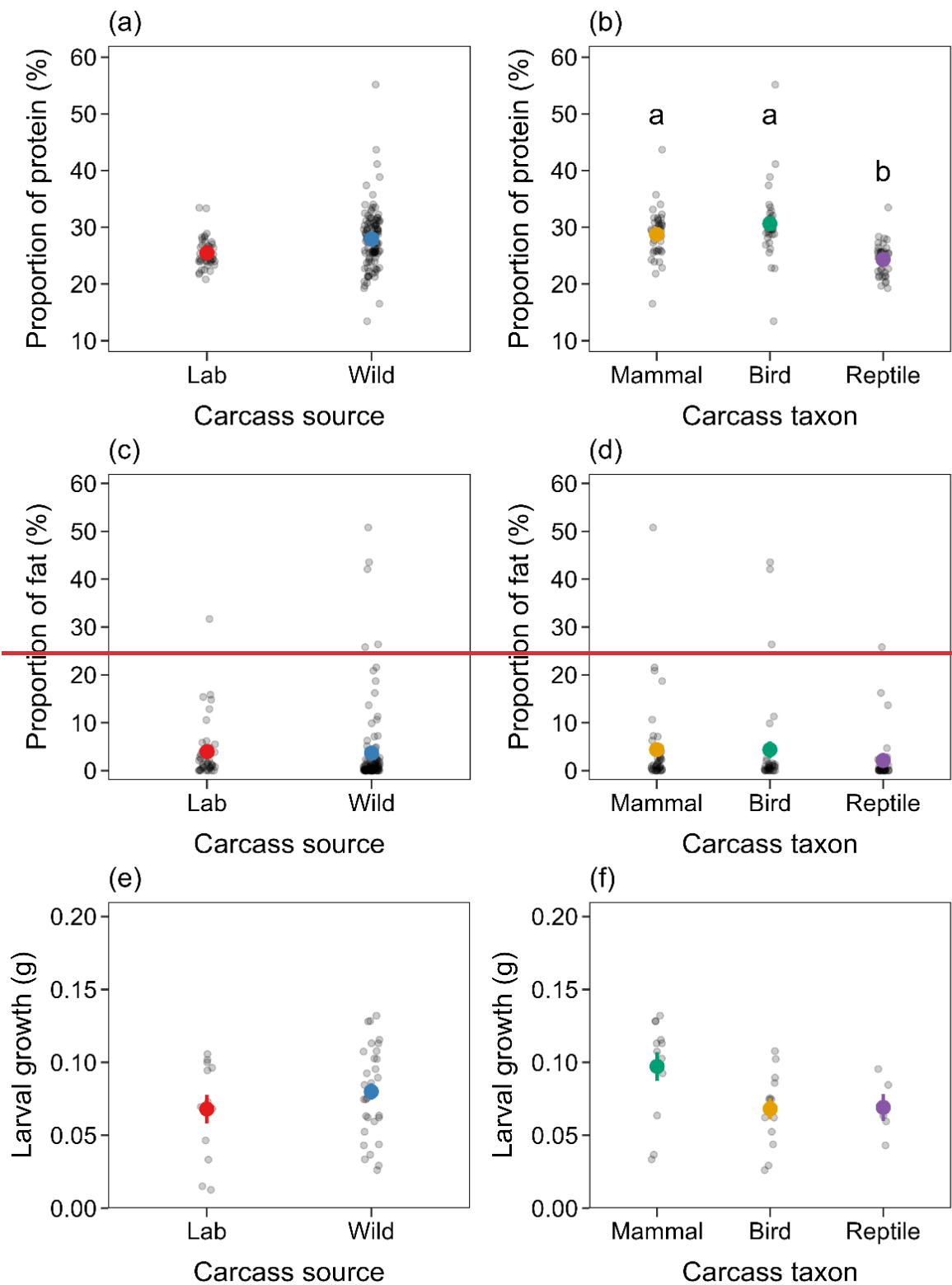
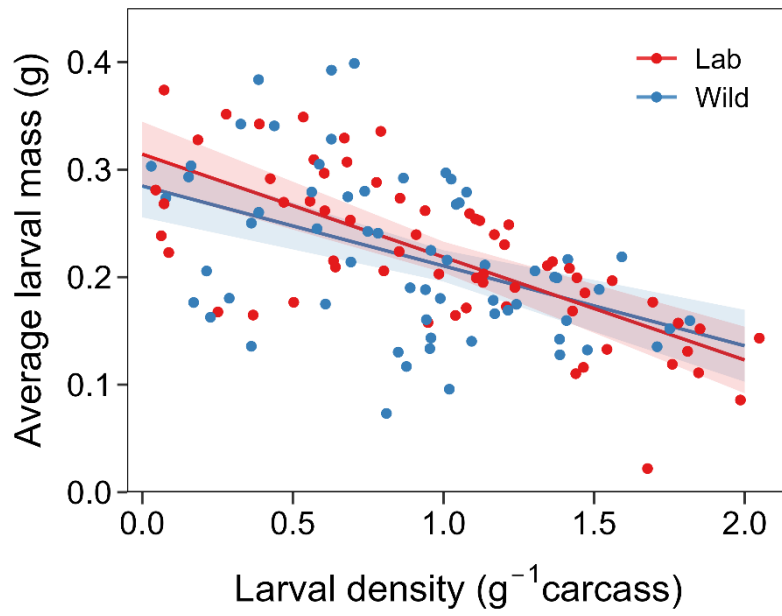


Figure 5



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Figure legends

Figure 1. The relationship between carcass weight and clutch size (a), hatching success (b), brood size (c), and brood mass (d) on lab and wild carcasses. Note that the observations without any larva were excluded from the brood mass analysis. Lines represent the statistically significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded areas represent the 95% confidence intervals.

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. Lines represent the statistically significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded areas represent the 95% confidence intervals.

Figure 3. Brood size (a), brood mass (b), average larval mass (c), and carcass use efficiency (d) on wild mammal, bird, and reptile carcasses. Points represent the means and error bars represent the standard errors. Note that the observations without any larva were excluded from the brood mass analysis.

Figure 4. Tissue protein and fat content (a–d) and larval growth (e and f) on lab and wild carcasses as well as on wild mammal, bird, and reptile carcasses. Points represent the means and error bars represent the standard errors. Letters denote significant difference with Tukey multiplicity adjustment ($\alpha = 0.05$).

Figure 5. The relationship between larval density and average larval mass on lab and wild carcasses. Lines represent the statistically significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded areas represent the 95% confidence intervals.

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Tables

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

Model response	<i>n</i>	Predictor		
		Carcass weight	Carcass source	Weight × Source
Clutch size	210*	$\chi^2_2 = 44.6,$ $P < 0.001$	$\chi^2_1 = 1.4,$ $P = 0.39$	$\chi^2_2 = 1.9,$ $P = 0.24$
Hatching success	176†	$\chi^2_2 = 32.1,$ $P < 0.001$	$\chi^2_1 = 0.8,$ $P = 0.37$	$\chi^2_2 = 0.3,$ $P = 0.88$
Brood size	238	$\chi^2_2 = 63.3,$ $P < 0.001$	$\chi^2_1 = 0.009,$ $P = 0.93$	$\chi^2_2 = 3.5,$ $P = 0.17$
Brood mass	129‡	$\chi^2_2 = 91.9,$ $P < 0.001$	$\chi^2_1 = 0.001,$ $P = 0.99$	$\chi^2_2 = 11.0,$ $P = 0.004$
Carcass use efficiency	95§	$\chi^2_1 = 64.5,$ $P < 0.001$	$\chi^2_1 = 0.003,$ $P = 0.96$	$\chi^2_1 = 0.3,$ $P = 0.57$

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

†Observations with a zero clutch size were excluded from the analysis.

‡Observations with a zero brood size were excluded from the analysis.

§Carcass 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.

1 *For submission to Royal Society Open Science*

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7 **2 Carcass size, not source or taxon, dictates breeding performance and carcass**
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Abstract

Small vertebrate carcasses represent critical resources for many terrestrial organisms, including burying beetles, which rely on carcasses for survival and breeding. Carcass attributes can influence the reproduction of burying beetles, yet most studies on their breeding ecology have used laboratory-reared carcasses of limited sizes. We conducted breeding and feeding experiments using a wide size range of lab (laboratory mice) and wild carcasses (wild mammals, birds, and reptiles) to investigate how carcass size, source, and taxon affect various breeding outcomes (e.g., clutch size, brood size, and brood mass) of the burying beetle *Nicrophorus nepalensis*. Our results reveal a hump-shaped relationship between carcass size and breeding performance, with optimal breeding outcomes occurring on medium-sized carcasses. Furthermore, despite the variation in carcass tissue nutritional composition, breeding outcomes and larval growth did not differ between the two carcass sources or among the three wild carcass taxa. Finally, we found a larval quality-quantity trade-off across the range of carcasses examined, with carcass size shaping the larval life history traits. Overall, these results elucidate how carcass resources may influence the breeding performance of burying beetles. Importantly, our study provides solid evidence validating decades of research using lab carcasses to study the reproductive ecology of burying beetles.

Keywords

breeding performance, burying beetle, carcass, nutritional composition, offspring quality-quantity trade-off, resource use efficiency

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Introduction

Small vertebrate carcasses represent a rich resource for a wide variety of terrestrial organisms, including vertebrate scavengers, saprophagous invertebrates, and microbial decomposers [1-3]. These carcass-feeding organisms facilitate the recycling of carcass nutrients and make the resource available to other species [4]. 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 [5]. 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 [5-9]. Moreover, parents can adjust their reproductive investment based on carcass size [10]. For example, females lay more eggs on larger carcasses within a certain carcass size range [11], and parents regulate the brood size via filial cannibalism when carcass resource is limited [12]. However, despite the resource benefits, large carcasses can be more difficult to utilize because of greater competition from other carcass-feeding organisms[9], 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 [13]. A few studies have empirically examined the relationship between optimal carcass size and reproductive output in burying beetles [e.g., 14], yet a general understanding is still lacking.

While most studies on the reproduction of burying beetles have focused on carcass size, the source of carcass may also influence their breeding outcomes. 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 [15], and these differences can alter larval survival and growth [1, 16]. Therefore, 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, burying beetles have been documented to breed on carcasses from a variety of taxonomic groups [5, 17]. Different carcass taxa can vary not only in their abundance but also in their tissue nutritional composition [18], which influence the overall reproductive performance of parents as well as individual larval growth and development [19]. However, it remains unknown how breeding outcomes and larval performance may vary among different groups of wild carcasses.

Studies have shown that brood size and larval mass of burying beetles are often negatively correlated with each other [6, 12, 20]. Such a trade-off between larval quality and quantity may vary with carcass size [8, 21] because resource quantity can shape the life history traits of organisms [22-24]. 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 [8]. 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 25]. 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 taxon) influence the breeding outcomes, larval performance, and offspring quality-quantity trade-off in burying beetles. We conducted breeding experiments on the species *Nicrophorus nepalensis*, which has been shown to provide extensive parental care for offspring. First, we

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97 examined how breeding outcomes (clutch size, brood size, brood mass, etc.) and carcass use
98 efficiency varied across a broad range of carcass size (weight was used as a proxy for size in
99 this study) on lab (laboratory mice) and wild carcasses (wild mammals, birds, and reptiles).
100 We further focused on the wild carcasses and compared the larval breeding outcomes and
101 carcass use efficiency of *N. nepalensis* on the three wild carcass taxa. We expected that there
102 would be an optimal carcass size for breeding, and the breeding outcomes may differ between
103 lab and wild carcasses as well as among different wild carcass taxa. We next quantified the
104 tissue nutritional composition of lab and wild carcasses and conducted a larval feeding
105 experiment using carcass tissues from different sources and taxa. We expected that the larvae
106 would perform better when feeding on diets with higher nutritional quality. Finally, we
107 examined the larval quality-quantity trade-off on lab and wild carcasses. We expected a trade-
108 off across a broad range of carcass sizes, and the trade-off pattern would differ between lab and
109 wild carcasses.

110

111 **Materials and Methods**

112 *Breeding experiments*

113 We conducted breeding experiments on *N. nepalensis* from the lab colony established in 2023.
 114 Adult beetles were collected from Taipei and New Taipei City, Taiwan and reared in growth
 115 chambers under a relative humidity of 70% and a 10:14 h light:dark cycle. The temperature
 116 was set to mimic diurnal temperature fluctuation (mean: 17.8°C; range: 16–20°C). This
 117 represents the natural temperature conditions during the breeding season (November–April) of
 118 *N. nepalensis* in northern Taiwan. A male and a female were placed in a plastic breeding
 119 container (14.2 cm in diameter and 6.3 cm in height) half-filled with moist commercial potting
 120 mix (2 cm in depth, equivalent to 300 mL), and a defrosted carcass was then placed on the soil
 121 surface. Frozen dead laboratory mice/rats were used as lab carcasses. Wild carcasses were
 122 obtained from the Taiwan Roadkill Observation Network (<https://roadkill.tw/eng/home>) and
 123 the Wild Bird Society of Taipei. These wild carcasses weighed from 1.6 to 99.5 grams and
 124 consisted of small mammals, birds, and reptiles. The carcasses used for breeding experiments
 125 were animals that had died within the past four months due to traffic collisions and other
 126 accidental causes but not poisoning. Upon discovery, these carcasses were immediately
 127 transferred to –20°C freezers for preservation. We paired each wild carcass with a lab carcass
 128 of a similar weight (measured to the nearest 0.1 g using an electronic analytical balance
 129 ATX224R, Shimadzu, Japan) and applied a sibship design where the two males and the two
 130 females used in each lab-wild carcass were from the same family line, respectively, to control
 131 for parental genotypes (the males and females came from genetically unrelated families). The
 132 breeding containers were maintained under the same environmental conditions as those of the
 133 lab colony. Five rounds of breeding experiments were conducted from May 2023 to March
 134 2024 (each with a different beetle parent generation), consisting of a total of 121 lab-wild
 135 carcass pairs (14, 76, and 31 wild mammal, bird, and reptile carcasses).

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We recorded the clutch size of each breeding container at day 4 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 while providing an accurate estimate of the exact clutch size ($r = 0.94$, $P < 0.001$, $n = 70$ broods) [26]. Eleven days after beetle pairing, we inspected the carcass to record the brood size (number of larvae) and brood mass (total larval weight; measured to the nearest 0.0001 g). 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 parents and larvae during the breeding process (larvae were removed from the carcasses). Carcass use efficiency was calculated as the amount of carcass tissue used divided by the initial carcass weight. All the aforementioned breeding outcomes and carcass use were recorded during the first reproductive bout of the breeding pairs.

Nutritional analysis of carcass tissue

To quantify the nutritional composition of lab and wild carcasses, which is essential for understanding how burying beetles use different types of carcasses, we estimated the protein and fat content of carcass tissue by adopting a proximate analysis approach as described by Al Shareefi and Cotter [27]. We dissected the carcasses by first skinning the animals and retaining the trunks. Trunk tissue was then separated from the bones with a pair of fine tweezers and a scalpel and divided into viscera (all organs inside the peritoneum) and muscles (all visible muscle parts). We next used a meat tenderizer to pound the viscera and muscles evenly and sampled three pieces of visceral and muscle tissue for each carcass for the analysis of

159 nutritional composition. A total of seven lab mice, seven wild mammals, six wild birds, and
160 six wild reptiles were dissected and analyzed.

161 For each tissue sample, we dried approximately 100 mg (106 ± 18 mg) of wet tissue in
162 a 40°C oven for 5 days until all water was removed. To determine the fat content, the dried
163 tissue was thoroughly mixed with 100 μ l of -20°C acetone and vortexed for one minute. The
164 mixture was then placed in a -20°C fridge for a 30-minute reaction period [28]. After the
165 extraction, the mixture was centrifuged to separate the components, and the acetone was
166 carefully removed. If the acetone appeared turbid after centrifugation, the solvent was
167 discarded and replaced with fresh acetone for further extraction. The process was repeated until
168 the solvent became clear. The residual solvent was then allowed to evaporate at room
169 temperature for 12 hours. After the fat removal process, the final product was weighed to
170 determine the protein content, and the fat content was determined by subtracting the protein
171 weight from the dry weight.

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173 *Larval feeding experiments*

174 We conducted larval feeding experiments using the remaining dissected carcass tissue from the
175 nutritional composition analysis. We placed *ca.* 400 mg (401 ± 21 mg) of carcass tissue into
176 individual plastic containers filled with moist commercial potting mix (soil volume 3.2×3.2
177 $\times 2.7$ cm). Newly hatched larvae (five days after female oviposition) were obtained from pairs
178 of breeding beetles (25 families) from the lab colony and one larva was introduced to each
179 container ($n = 188$). After five days of feeding, the larval mass at dispersal was recorded and
180 larval growth was measured as the larval weight gain during the experimental period.

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182 *Data analyses*

183 1. Breeding outcomes and carcass use efficiency

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

199 To compare the brood size, brood mass, average larval mass, and carcass use efficiency
200 on wild mammal, bird, and reptile carcasses, we fit generalized linear models (GLMs) with
201 each of the aforementioned breeding outcomes as the response and wild carcass taxon as the
202 fixed effect. Carcass weight, pronotum widths of the parents, and parent generation were
203 included as the covariates in the models. The error distribution and link function for each of
204 the responses were the same as the GLMMs. Because the carcass range was considerably
205 smaller for reptiles (1.6–64.4 g) than for mammals (3.8–94.8 g) and birds (3.2–99.5 g), we

restricted the carcass weight range to that of reptiles (≤ 64.4 g) so that the results were more comparable among the three wild taxa.

2. Nutritional composition and larval growth

To compare the nutritional composition between the two carcass sources and the three wild carcass taxa, we fit GLMMs with the proportion of protein/fat as the responses, carcass source/taxon and tissue type (viscera vs. muscles) as the fix effects, and carcass ID as the random effect (a total of four GLMMs). We used a beta error distribution with a logit link function for model fitting in the GLMMs.

To compare the larval growth between the two carcass sources and the three wild carcass taxa, we fit GLMMs with larval weight gain as the response, carcass source/taxon and tissue type as the fix effects, and carcass ID and larval family as the random effects (a total of two GLMMs). Larval mass at hatching was included in the models as a covariate. We used a Gaussian error distribution for model fitting in the GLMMs. To further investigate the effect of nutrient content on larval growth on both carcass sources and on wild carcasses only, we fit GLMMs with larval weight gain as the response, proportion of protein, proportion of fat, and tissue type as the fixed effects, and larval family as the random effects (a total of two GLMMs). Larval mass at hatching was included as a covariate. Dead larvae ($n = 146$) were excluded from the analysis (larval survival rate was 22.3% in the five-day feeding experiments).

3. Larval quality-quantity trade-off

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,

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229 and their interaction as the predictors. A significant negative slope indicates a larval quality-
230 quantity trade-off.
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232 We fit all aforementioned models using the glmmTMB() function in the R “glmmTMB” package
233 [29]. Model assumptions were checked via the quantile residuals generated from the
234 simulateResiduals() function in the R “DHARMa” package [30]. Predictor significance was
235 assessed with the Wald chi-square test via the Anova() function (type II sums of squares) in
236 the R “car” package [31]. Post-hoc pairwise comparisons among carcass taxa with the Tukey
237 multiplicity adjustment were conducted via the emmeans() function in the R “emmeans”
238 package [32]. All analyses were performed in R version 4.3.3 [33].

Results

Breeding outcomes and carcass use efficiency

Clutch size, hatching success, brood size, and brood mass all showed a quadratic relationship with carcass weight (clutch size: $\chi^2_2 = 44.6$, $P < 0.001$; hatching success: $\chi^2_2 = 32.1$, $P < 0.001$; brood size: $\chi^2_2 = 63.3$, $P < 0.001$; brood mass: $\chi^2_2 = 91.9$, $P < 0.001$; Table 1) and peaked on medium-sized carcasses (Fig. 1). Moreover, these breeding outcomes did not differ between lab and wild carcasses (clutch size: $\chi^2_1 = 1.4$, $P = 0.39$; hatching success: $\chi^2_1 = 0.8$, $P = 0.37$; brood size: $\chi^2_1 = 0.009$, $P = 0.93$; brood mass: $\chi^2_1 = 0.001$, $P = 0.99$; Table 1; Fig. 1). Carcass use efficiency decreased with carcass weight ($\chi^2_2 = 64.5$, $P < 0.001$) but did not differ between lab and wild carcasses ($\chi^2_1 = 0.003$, $P = 0.96$; Table 1; Fig. 2).

Brood size, brood mass, average larval mass, and carcass use efficiency did not differ among wild mammal, bird, and reptile carcasses (brood size: $\chi^2_2 = 0.6$, $P = 0.75$; brood mass: $\chi^2_2 = 3.6$, $P = 0.17$; average larval mass: $\chi^2_2 = 3.3$, $P = 0.19$; carcass use efficiency: $\chi^2_2 = 0.4$, $P = 0.81$; Fig. 3).

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Nutritional composition of carcasses

Protein content was similar between lab and wild carcasses (mean proportion: lab = 25.5%, wild = 27.9%; $\chi^2_1 = 3.5$, $P = 0.06$; Fig. 4a) but differed among wild carcass taxa (mean proportion: mammal = 28.7%, bird = 30.6%, reptile = 24.3%; $\chi^2_2 = 26.6$, $P < 0.001$; Fig. 4b). Specifically, reptile carcasses had significantly lower protein content than mammal and bird carcasses (Fig. 4b). Fat content was similar between lab and wild carcasses (mean proportion: lab = 4.0%, wild = 3.7%; $\chi^2_1 = 1.1$, $P = 0.29$; Fig. 4c) and among wild carcass taxa (mean proportion: mammal = 4.4%, bird = 4.4%, reptile = 2.1%; $\chi^2_2 = 3.5$, $P = 0.18$; Fig. 4d).

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Larval growth

Growth was similar for larvae feeding on tissue from lab and wild carcasses ($\chi^2_1 = 0.1$, $P = 0.74$; Fig. 4e). Similarly, larval growth did not differ significantly among the three wild carcass taxa ($\chi^2_2 = 5.2$, $P = 0.07$; Fig. 4f), although larvae feeding on wild bird carcasses tended to gain more weight compared to those feeding on wild mammals and reptiles (Fig. 4f). When lab and wild carcasses were combined, larval growth was not associated with either tissue protein content ($\chi^2_1 = 0.9$, $P = 0.34$) or fat content ($\chi^2_1 = 0.05$, $P = 0.83$) (Fig. S2a and b). On the other hand, larvae feeding on wild carcass tissue with higher fat content ($\chi^2_1 = 5.2$, $P = 0.02$), but not protein content ($\chi^2_1 = 0.01$, $P = 0.92$), did grow better (Fig. S2c and d).

Larval quality-quantity trade-off

Average larval mass decreased with larval density on both lab and wild carcasses ($\beta = -0.096$, $\chi^2_1 = 74.7$, $P < 0.001$; Fig. 5). The interaction between larval density and carcass source was not significant ($\chi^2_1 = 1.2$, $P = 0.28$), 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 the burying beetle *N. nepalensis* varied with carcass size on lab and wild carcasses. Clutch size, hatching success, brood size, and brood mass all exhibited a quadratic relationship with carcass size, whereas carcass use efficiency decreased with carcass size. Furthermore, these breeding outcomes and carcass use efficiency did not differ between lab and wild carcasses. Despite the variation in tissue nutritional composition (protein content) among wild mammal, bird, and reptile carcasses, larval traits (brood size, brood mass, and average larval mass), carcass use efficiency, and larval growth did not differ among the three wild carcass taxa. 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 carcass size, but not carcass source or carcass taxon, is the main determinant for the breeding performance and carcass resource use of burying beetles.

As expected, clutch size, hatching success, brood size, and brood mass all showed a quadratic relationship with carcass size, with optimal breeding outcomes occurring on medium-sized carcasses. The increase in breeding performance from small to medium carcasses is consistent with previous studies on other burying beetle species [6, 10, 34]. Interestingly, when the parents bred on large carcasses, their breeding performance decreased, along with a reduction in 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 [11] found that clutch size levels off beyond a certain carcass weight threshold, suggesting an energetic or physiological constraint on beetles breeding on larger carcasses. Parents breeding on large carcasses also face stronger competition with microbes, which can reduce the usable resource for breeding [5] or produce compounds harmful to eggs and larvae [1].

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3 304 Contrary to our prediction, we found no major difference in the breeding outcomes and
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5 305 carcass use efficiency of *N. nepalensis* on lab versus wild carcasses. A potential explanation is
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8 306 that the parents manipulated the carcasses (e.g., by secreting antimicrobial compounds) such
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10 307 that the eggs and larvae experienced similar growing environments regardless of carcass source.
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12 308 Studies have shown that parental care is crucial for larval performance in burying beetles [1,
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14 309 35], and we speculate that parental food preparation and regurgitation may offset the difference
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17 310 between the two carcass sources. Further experiments comparing breeding outcomes on lab
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19 311 and wild carcasses with versus without parents will help verify our speculation. The analyses
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21 312 did reveal an interaction between carcass size and carcass source for brood mass. In fact, the
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23 313 patterns were mostly similar between lab and wild carcasses on small and medium carcasses,
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26 314 whereas the difference on large carcasses was mainly driven by two observations on large wild
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28 315 carcasses (the interaction became non-significant when these two observations were removed;
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30 316 $P = 0.38$, Fig. S3). Overall, our results support the validity of research using lab-reared
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33 317 organisms as breeding carcasses to study the reproductive biology of burying beetles.

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36 318 Our tissue nutritional analysis showed that protein content was higher in wild mammal
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38 319 and bird carcasses than in wild reptile carcasses, whereas fat content was similar among these
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40 320 taxa. Yet, despite the variation in tissue protein content, larval traits and carcass use efficiency
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42 321 in the breeding experiments as well as larval growth in the feeding experiments did not vary
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44 322 significantly among the three wild carcass taxa. In fact, our feeding experiments showed that
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46 323 it was fat content, not protein content, that affected larval growth on wild carcasses. Since fat
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48 324 content did not vary among the three wild carcass taxa, we did not observe major difference in
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50 325 larval growth. This may also partially explain why larval traits and carcass use efficiency were
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52 326 similar among the three wild carcass taxa in the breeding experiments. These results suggest
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54 327 that parents can utilize carcass resources from different vertebrate taxa that vary in their
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56 328 nutritional content and potentially carcass abundance in the wild while having similar breeding
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outcomes. Without parents, larval survival can be quite low (22.3% in the feeding experiments), and carcass taxon may potentially influence individual larval performance, as larvae did tend to grow better on bird carcasses in the feeding experiments. This highlights the importance of parental care in burying beetles (e.g., carcass preparation and food provisioning) in maintaining breeding performance on a variety of carcasses in the wild.

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. Similar trade-off patterns have been shown in previous studies [21, 36] and can arise from both larval competition and brood regulation by parents [36]. Stronger interspecific competition under a higher larval density may reduce individual larval growth, leading to lower average larval mass. On the other hand, parents may regulate brood size by culling excess larvae to reduce larval competition [37], thereby leading to greater larval growth and higher average biomass. Furthermore, 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 size for small and medium carcasses, whereas larval density decreased (Fig. S1). This suggests that the larval 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 size in the breeding outcomes of a single parent pair. However, multiple males and females may engage in cooperative breeding to better utilize large carcasses in the wild [38], although past results for the reproductive benefits of cooperation are mixed [39-41]. Additionally, burying beetles in nature may face carcass competition not only from microbes but also from various vertebrate scavengers and invertebrate carcass feeders [42, 43], and such interspecific competition can interact with

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3 354 carcass size to influence breeding success [44]. Abiotic factors such as temperature may further
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5 355 affect the optimal carcass size by altering carcass decomposition rates, carcass handling time,
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8 356 and parents' activity levels [45]. Therefore, field experiments using a wide range of carcass
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10 357 sizes will help elucidate how intraspecific and interspecific interactions as well as the interplay
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12 358 between biotic interactions and carcass size jointly shape the breeding performance of burying
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15 359 beetles. It is also noteworthy that burying beetles can reproduce multiple times throughout their
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17 360 lifespans, and the patterns of single reproductive output may differ from the lifetime
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19 361 reproductive output [14]. Research quantifying the lifetime reproductive output will help better
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22 362 understand how carcass resources affect the over fitness of breeding individuals.

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25 363 Using a broad range of carcass sizes from both lab and wild sources, our study revealed
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27 364 a quadratic relationship between breeding performance and carcass size in burying beetle, with
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29 365 optimal breeding outcomes occurring on medium-sized carcasses. Breeding outcomes did not
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31 366 differ between lab and wild carcasses. Furthermore, despite the variation in tissue nutritional
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34 367 composition (particularly protein content) among wild mammal, bird, and reptile carcasses,
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36 368 larval traits, carcass use efficiency, and larval growth were generally similar among these wild
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39 369 carcass taxa. Finally, the larval quality-quantity trade-off existed across the range of lab and
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41 370 wild carcass sizes, with higher larval quantity (larval density) but lower quality (average larval
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43 371 mass) on smaller carcasses and lower larval quantity but higher larval quality on larger
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45 372 carcasses. Taken together, our study confirms that previous results from lab carcasses are fairly
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48 373 representative of natural patterns and provides a more complete picture of how carcass
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50 374 resources shape the breeding performance of burying beetles.

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56 376 **Acknowledgements**

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Conflict of interest

The authors declare no conflict of interest regarding this manuscript.

Author contributions

Gen-Chang Hsu and Syuan-Jyun Sun conceived the ideas; Gen-Chang Hsu, Wei-Jiun Lin, Yue-Jia Lee, and Syuan-Jyun Sun designed the experiments; Gen-Chang Hsu, Wei-Jiun Lin, Chi-Heng Hsieh, Yue-Jia Lee, and Syuan-Jyun Sun collected the data; Gen-Chang Hsu and Syuan-Jyun Sun analyzed the data; Gen-Chang Hsu and Syuan-Jyun Sun wrote the first draft of the manuscript; all authors revised the manuscript and approved the final version for publication.

Data accessibility

Data and code used in this manuscript are publicly available on Zenodo [46].

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Figures

Figure 1

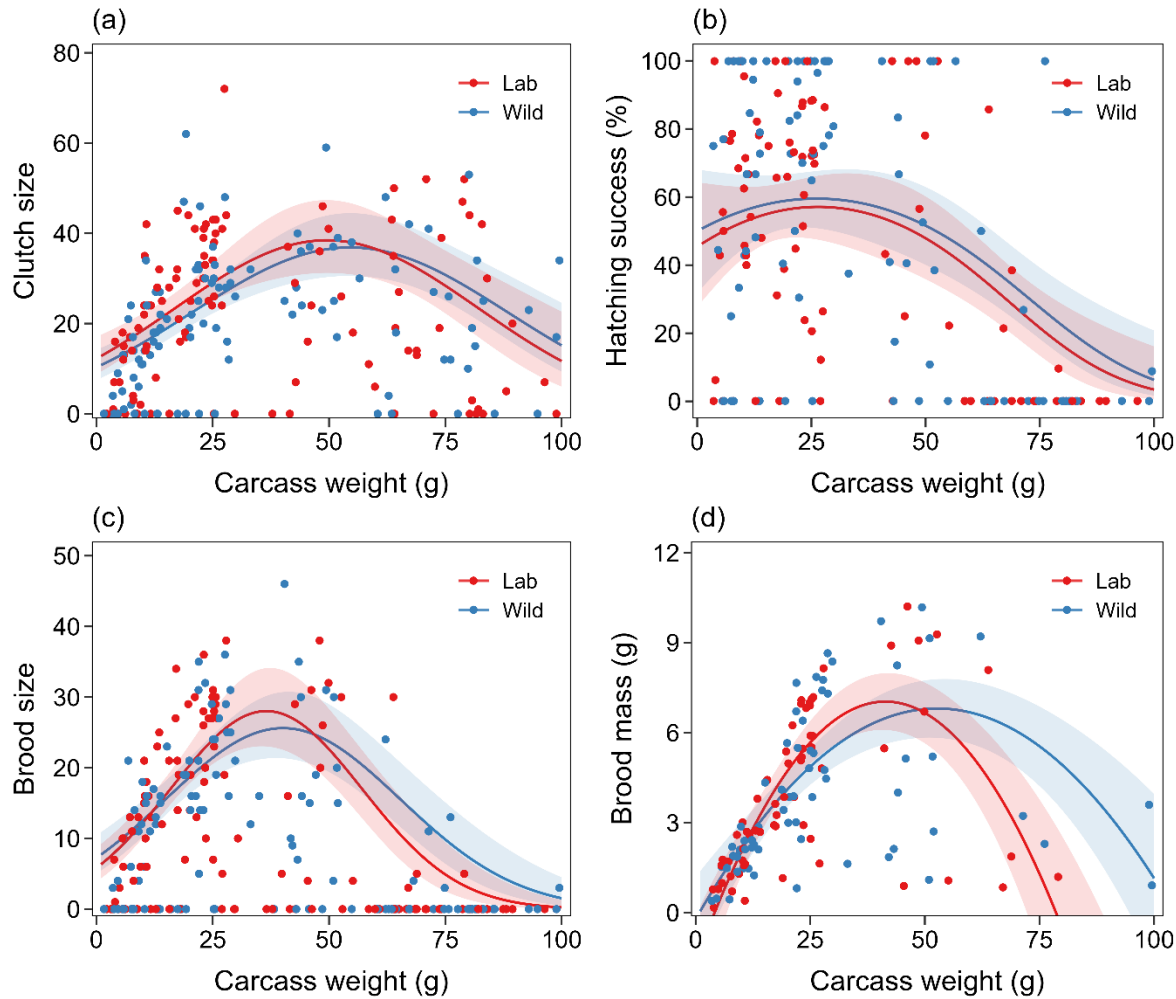
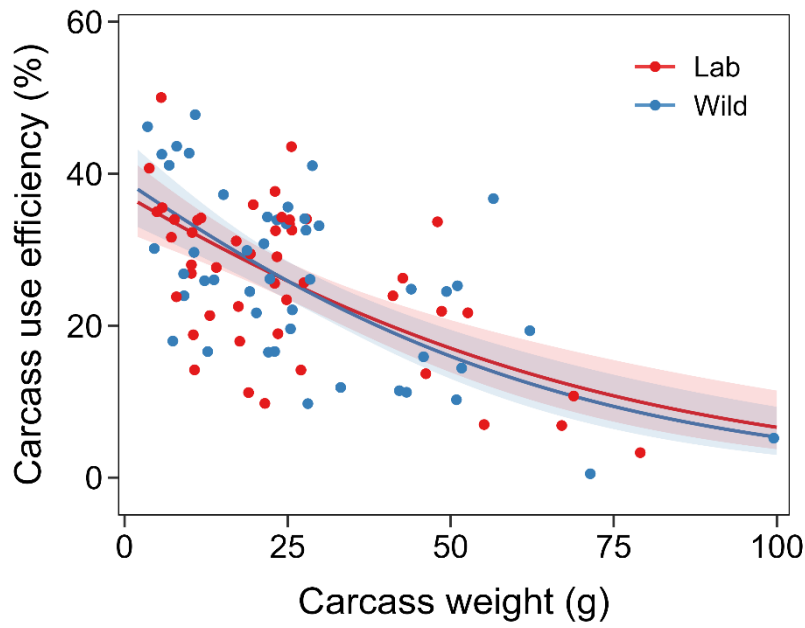
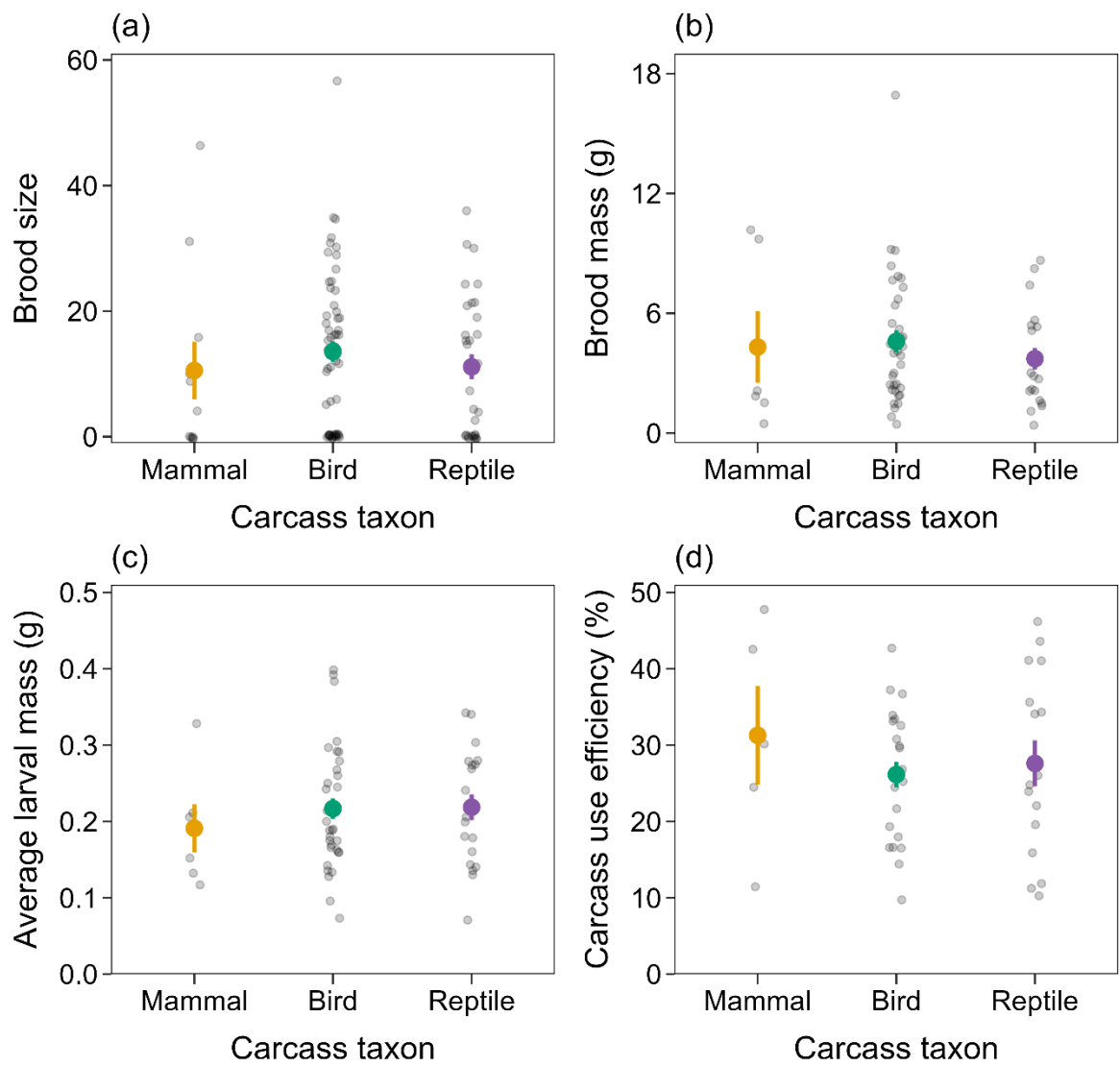


Figure 2



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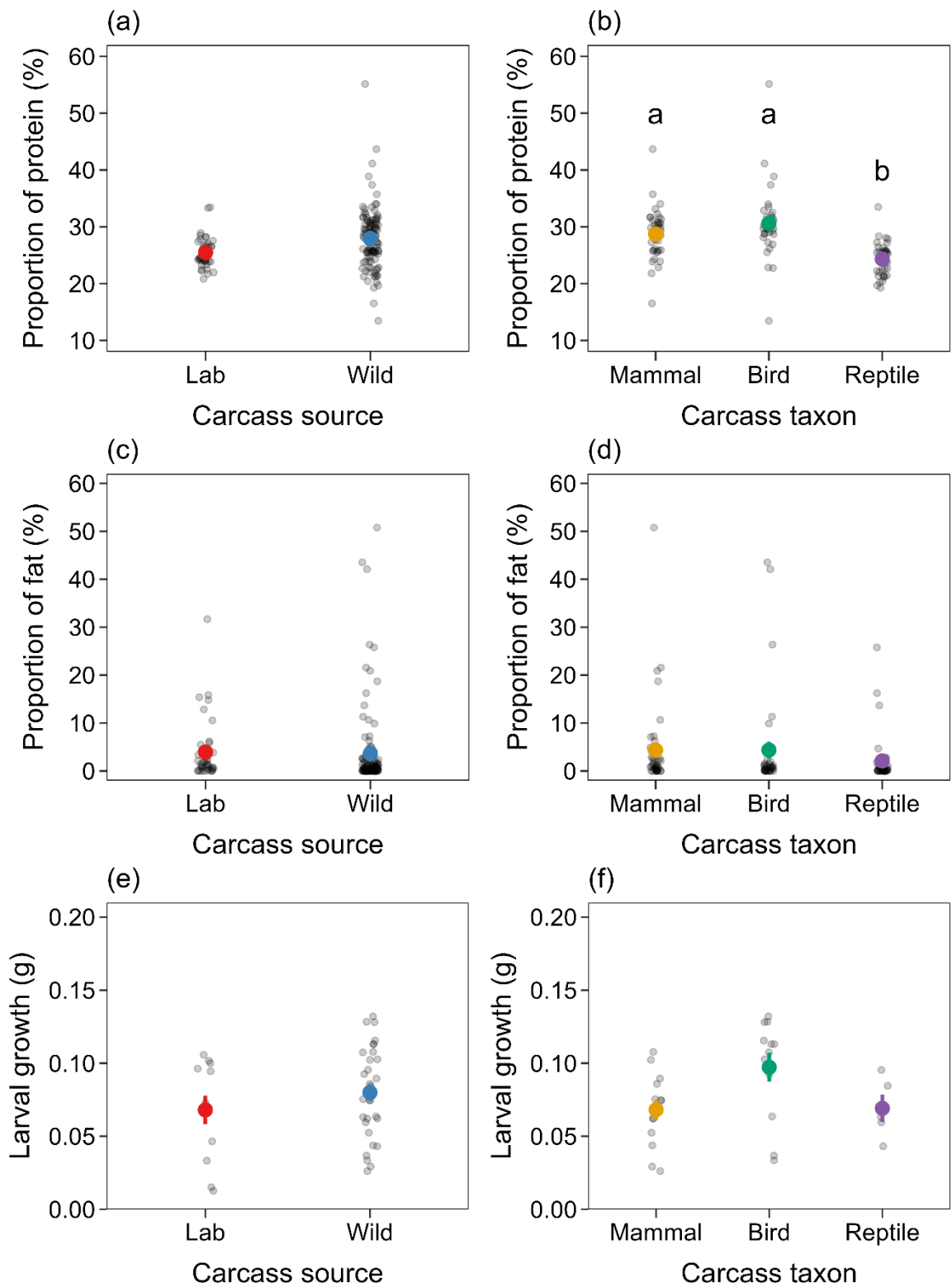
519 Figure 3



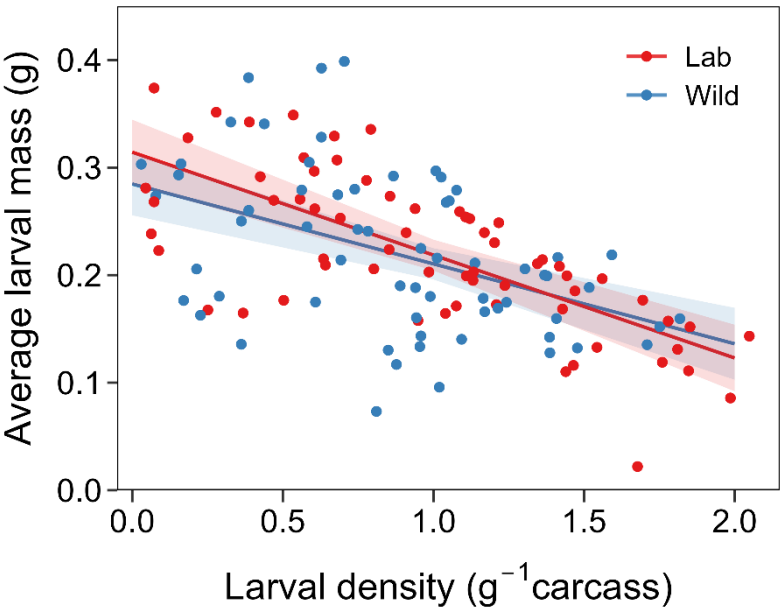
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Figure 4



526 Figure 5



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Figure legends

Figure 1. The relationship between carcass weight and clutch size (a), hatching success (b), brood size (c), and brood mass (d) on lab and wild carcasses. Note that the observations without any larva were excluded from the brood mass analysis. Lines represent the statistically significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded areas represent the 95% confidence intervals.

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. Lines represent the statistically significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded areas represent the 95% confidence intervals.

Figure 3. Brood size (a), brood mass (b), average larval mass (c), and carcass use efficiency (d) on wild mammal, bird, and reptile carcasses. Points represent the means and error bars represent the standard errors. Note that the observations without any larva were excluded from the brood mass analysis.

Figure 4. Tissue protein and fat content (a–d) and larval growth (e and f) on lab and wild carcasses as well as on wild mammal, bird, and reptile carcasses. Points represent the means and error bars represent the standard errors. Letters denote significant difference with Tukey multiplicity adjustment ($\alpha = 0.05$).

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550 Figure 5. The relationship between larval density and average larval mass on lab and wild
551 carcasses. Lines represent the statistically significant relationships predicted from GLMMs (α
552 = 0.05); shaded areas represent the 95% confidence intervals.

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554 Tables

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

Model response	<i>n</i>	Predictor		
		Carcass weight	Carcass source	Weight × Source
Clutch size	210*	$\chi^2_2 = 44.6,$ $P < 0.001$	$\chi^2_1 = 1.4,$ $P = 0.39$	$\chi^2_2 = 1.9,$ $P = 0.24$
Hatching success	176 [†]	$\chi^2_2 = 32.1,$ $P < 0.001$	$\chi^2_1 = 0.8,$ $P = 0.37$	$\chi^2_2 = 0.3,$ $P = 0.88$
Brood size	238	$\chi^2_2 = 63.3,$ $P < 0.001$	$\chi^2_1 = 0.009,$ $P = 0.93$	$\chi^2_2 = 3.5,$ $P = 0.17$
Brood mass	129 [‡]	$\chi^2_2 = 91.9,$ $P < 0.001$	$\chi^2_1 = 0.001,$ $P = 0.99$	$\chi^2_2 = 11.0,$ $P = 0.004$
Carcass use efficiency	95 [§]	$\chi^2_1 = 64.5,$ $P < 0.001$	$\chi^2_1 = 0.003,$ $P = 0.96$	$\chi^2_1 = 0.3,$ $P = 0.57$

558 *Clutch size was not recorded in the first round of breeding experiments.

559 [†]Observations with a zero clutch size were excluded from the analysis.

560 [‡]Observations with a zero brood size were excluded from the analysis.

561 [§]Carcass use was not measured in the first and second round of the breeding experiments; observations with a
 562 zero brood size were excluded from the analysis.

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Supplementary Materials

Title: Carcass size, not source or taxon, dictates breeding performance and carcass use
in burying beetle

Authors: Gen-Chang Hsu, Wei-Jiun Lin, Chi-Heng Hsieh, Yue-Jia Lee, Syuan-Jyun
Sun

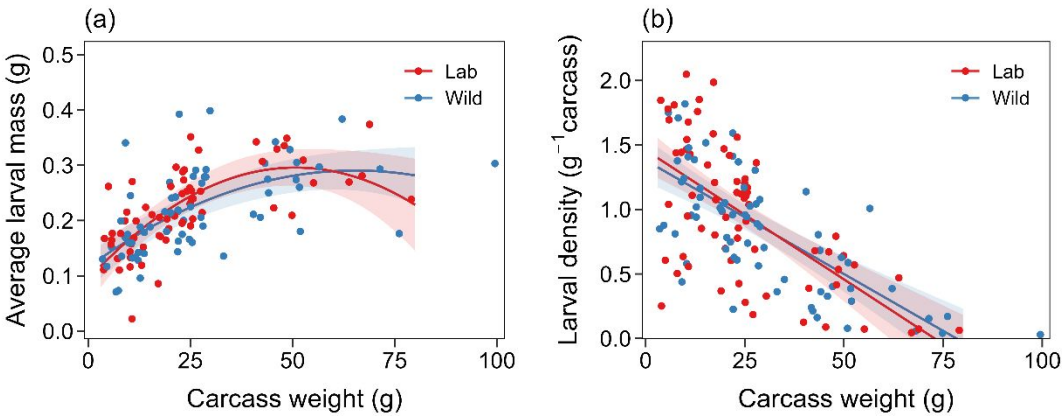


Figure S1. The relationship between carcass weight and average larval mass (a) and larval density (b) on lab and wild carcasses. Lines represent the statistically significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded areas represent the 95% confidence intervals.

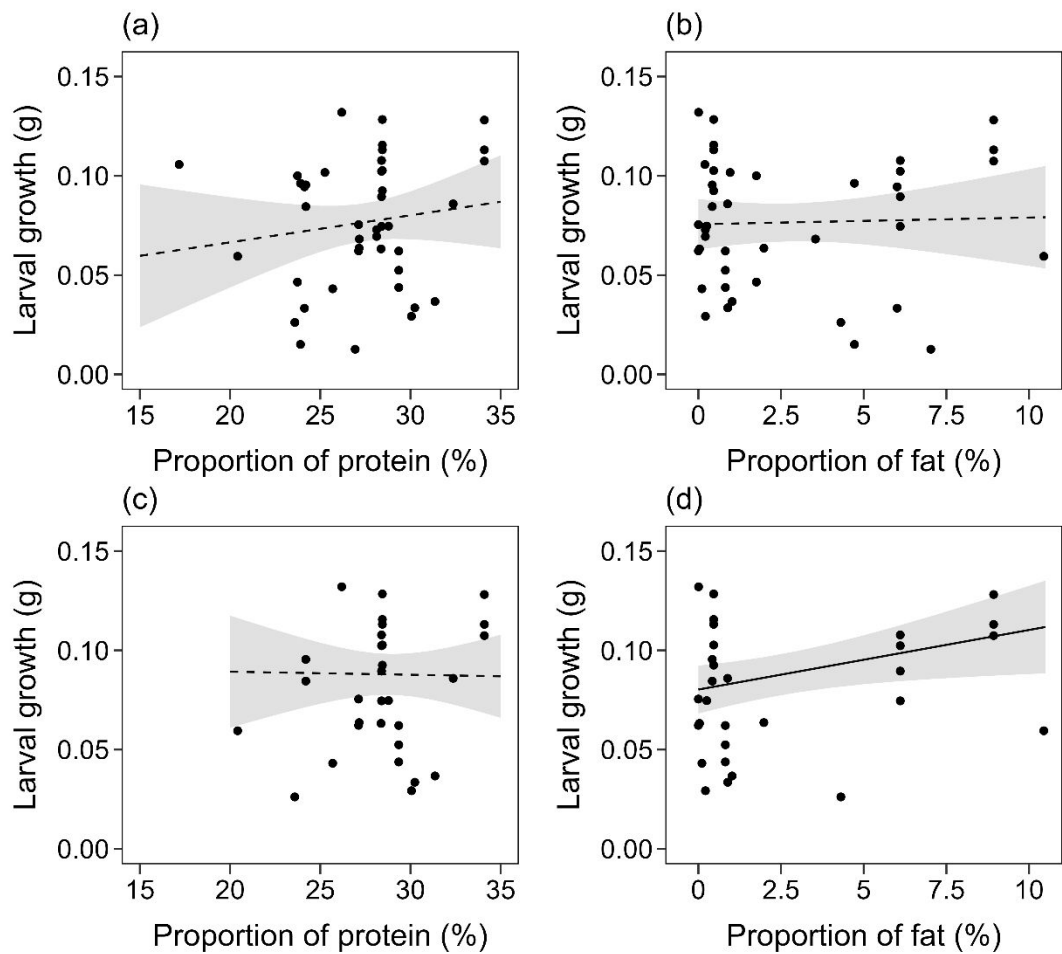


Figure S2. The relationship between tissue nutrient content and larval growth. Panel (a) and (b) include observations from both lab and wild carcasses; panel (c) and (d) include only observations from wild carcasses. Solid and dashed lines represent the significant and non-significant relationships predicted from GLMMs, respectively ($\alpha = 0.05$); shaded areas represent the 95% confidence intervals.

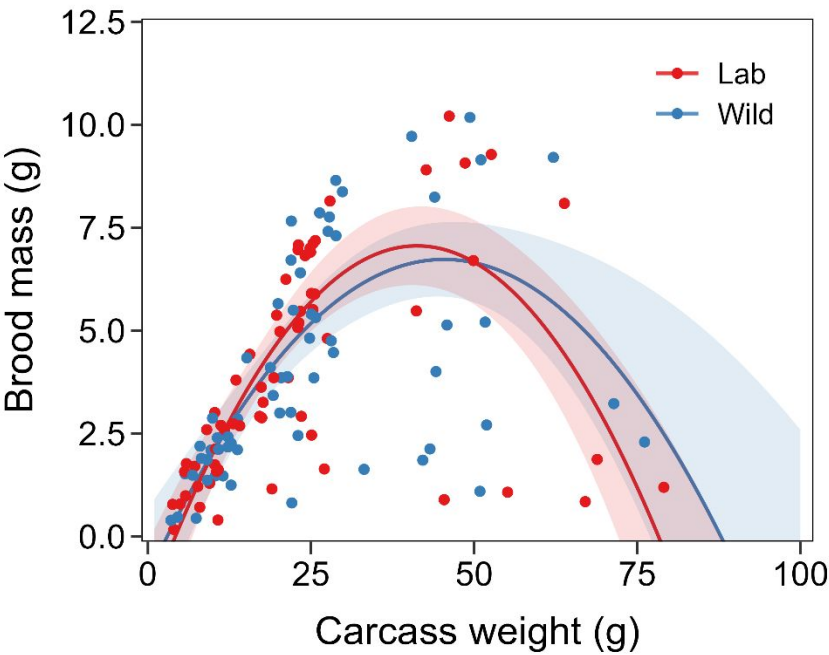


Figure S3. The relationship between carcass weight and brood mass on lab and wild carcasses with two observations on large wild carcasses removed. Lines represent the statistically significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded areas represent the 95% confidence intervals.