

Abstract

1. Carcasses represent critical resources for many terrestrial organisms, including burying beetles, which rely on carcasses for survival and breeding. Carcass attributes can strongly influence the reproduction of burying beetles, yet most studies on their breeding ecology have used laboratory-reared carcasses of limited sizes, raising questions about whether these results are representative of natural patterns.

2. 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 such as clutch size, brood size, brood mass, and larval growth of the burying beetle *Nicrophorus nepalensis*.

3. 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 tissue nutritional composition, breeding outcomes and larval growth did not differ between the lab and wild 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.

4. 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, brood size, burying beetle, carcass, clutch size, nutritional composition, offspring quality-quantity trade-off, resource use efficiency

Introduction

Carcasses represent a rich resource for a wide variety of terrestrial organisms, including vertebrate scavengers, saprophagous invertebrates, and microbial decomposers (Barton et al., 2013; Rozen et al., 2008; Stiegler et al., 2020). These carcass-feeding organisms facilitate the recycling of carcass nutrients and make the resource available to other species (Tomberlin et al., 2017). For 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 (Scott, 1998). 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 (Creighton, 2005; Scott, 1998; Scott & Traniello, 1990; Smiseth et al., 2014; Trumbo, 1992). Moreover, parents can adjust their reproductive investment based on carcass size (Hopwood et al., 2016). For example, females lay more eggs on larger carcasses within a certain carcass size range (Müller et al., 1990), and parents regulate the brood size via filial cannibalism when carcass resource is limited (Bartlett, 1987). However, despite the resource benefits, large carcasses can be more difficult to utilize (Trumbo, 1992), and the energetic costs of processing carcass tissue also increase with carcass size. 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.

Besides carcass size, the source of carcass can also influence the 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 (Weldon et al., 2015), and these differences can alter larval survival and growth (Rozen et al., 2008; Shukla et al., 2018). 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 (Hocking et al., 2006; Scott, 1998). Different carcass taxa can vary in their tissue nutritional composition (May & El-Sabaawi, 2022), which may influence larval growth and development (Scriber & Slansky Jr, 1981). 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 (Bartlett, 1987; Creighton, 2005; Monteith et al., 2012). Such a trade-off between larval quality and quantity may vary with carcass size (Bartlett & Ashworth, 1988; Smiseth et al., 2014) because resource quantity can shape the life history traits of organisms (Boggs, 2009; Richardson & Smiseth, 2020; Tessier & Consolatti, 1991). 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 (Smiseth et al., 2014). 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 Woelber et al., 2018). 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 examined how breeding outcomes (clutch size, brood size, brood mass, etc.) and carcass use efficiency varied across a broad range of carcass size (weight was used as a proxy for size in this study) on lab (laboratory mice) and wild carcasses (wild mammals, birds, and reptiles). We further focused on the wild carcasses and compared the larval breeding outcomes and carcass use efficiency of *N. nepalensis* on the three wild carcass taxa. We expected that there would be an optimal carcass size for breeding, and the breeding outcomes may differ between lab and wild carcasses as well as among different wild carcass taxa. We next quantified the tissue nutritional composition of lab and wild carcasses and conducted a larval feeding experiment using carcass tissues from different sources and taxa. We expected that the larvae would perform better when feeding on diets with higher nutritional quality. Finally, we examined the larval quality-quantity trade-off on lab and wild carcasses. We expected a trade-off across a broad range of carcass sizes, and the trade-off pattern would differ between lab and wild carcasses.

Materials and Methods

Breeding experiments

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

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) (Sun et al., 2020). 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.

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 (2018). 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 nutritional composition. A total of seven lab mice, seven wild mammals, seven wild birds, and seven wild reptiles were dissected and analyzed.

For each tissue sample, we dried approximately 100 mg (106 ± 18 mg) of wet tissue in a 40°C oven for 5 days until all water was removed. To determine the fat content, the dried tissue was thoroughly mixed with 100 µl of -20°C acetone and vortexed for one minute. The mixture was then placed in a -20°C fridge for a 30-minute reaction period (Saini et al., 2021). After the extraction, the mixture was centrifuged to separate the components, and the acetone was carefully removed. If the acetone appeared turbid after centrifugation, the solvent was discarded and replaced with fresh acetone for further extraction. The process was repeated until the solvent became clear. The residual solvent was then allowed to evaporate at room temperature for 12 hours. After the fat removal process, the final product was weighed to determine the protein content, and the fat content was determined by subtracting the protein weight from the dry weight.

Larval feeding experiments

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

Data analyses

1. Breeding outcomes and carcass use efficiency

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

To compare the brood size, brood mass, average larval mass, and carcass use efficiency on wild mammal, bird, and reptile carcasses, we fit generalized linear models (GLMs) with each of the aforementioned breeding outcomes as the response and wild carcass taxon as the fixed effect. Carcass weight, pronotum widths of the parents, and parent generation were included as the covariates in the models. The error distribution and link function for each of the responses were the same as the GLMMs. Because the carcass range was considerably 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 were excluded from the analysis.

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, and their interaction as the predictors. A significant negative slope indicates a larval quality-quantity trade-off.

We fit all aforementioned models using the `glmmTMB()` function in the R “glmmTMB” package (Brooks et al., 2017). Model assumptions were checked via the quantile residuals generated from the `simulateResiduals()` function in the R “DHARMa” package (Hartig, 2022). Predictor significance was assessed with the Wald chi-square test via the `Anova()` function (type II sums of squares) in the R “car” package (Fox & Weisberg, 2019). Post-hoc pairwise comparisons among carcass taxa with the Tukey multiplicity adjustment were conducted via the `emmeans()` function in the R “emmeans” package (Lenth, 2024). All analyses were performed in R version 4.3.3 (R Core Team, 2024). The scale of inference and sample sizes for the experiments are summarized in Table 1.

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 2) 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 2; 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 2; 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).

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).

242

243

Larval growth

244

245

246

247

248

249

250

251

252

253

Larval quality-quantity trade-off

254

255

256

257

258

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).

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 (Creighton, 2005; Eggert & Müller, 1992; Hopwood et al., 2016). 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 et al. (1990) 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 (Scott, 1998) or produce compounds harmful to eggs and larvae (Rozen et al., 2008).

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 (Eggert et al., 1998; Rozen et al., 2008), 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 growth in the feeding experiments did not vary significantly among the wild carcass taxa. In fact, we found 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 our breeding experiments. Interestingly, larvae did

tend to grow better on bird carcasses in the feeding experiments without parents. This suggests that parental care in burying beetles (e.g., carcass preparation and food provisioning) may help maintain 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 (Bartlett & Ashworth, 1988; Trumbo, 1990) and can arise from both larval competition and brood regulation by parents (Trumbo, 1990). 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 (Trumbo, 2006), 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 (Scott, 1998). However, multiple males and females may engage in cooperative breeding to better utilize large carcasses in the wild (Scott et al., 2007), although past results for the reproductive benefits of cooperation are mixed (Eggert & Sakaluk, 2000; Komdeur et al., 2013; Müller et al., 2007). Additionally, burying beetles in nature may face carcass

competition not only from microbes but also from various vertebrate scavengers and invertebrate carcass feeders (Chen et al., 2020; DeVault et al., 2003), and such interspecific competition can interact with carcass size to influence breeding success (Scott, 1994). 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.

Using a broad range of carcass sizes from both lab and wild sources, our study revealed for 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 smaller carcasses favoring larval quantity and larger carcasses favoring larval quality. Taken together, our study confirms that previous results from lab carcasses are fairly representative of natural patterns and provides a more complete picture of how carcass resources shape the breeding performance of burying beetles.

References

- Al Shareefi, E. & Cotter, S.C. (2018). The nutritional ecology of maturation in a carnivorous insect. *Behavioral Ecology*, 30, 256-266.
- Bartlett, J. (1987). Filial cannibalism in burying beetles. *Behavioral Ecology and Sociobiology*, 21, 179-183.
- Bartlett, J. & Ashworth, C. (1988). Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology*, 22, 429-434.
- Barton, P.S., Cunningham, S.A., Lindenmayer, D.B. & Manning, A.D. (2013). The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. *Oecologia*, 171, 761-772.
- Boggs, C.L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, 23, 27-37.
- Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A. et al. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal*, 9, 378-400.
- Chen, B.F., Liu, M., Rubenstein, D.R., Sun, S.J., Liu, J.N., Lin, Y.H. et al. (2020). A chemically triggered transition from conflict to cooperation in burying beetles. *Ecology Letters*, 23, 467-475.
- Creighton, J.C. (2005). Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology*, 16, 1031-1036.
- DeVault, T.L., Rhodes, J., Olin E & Shivik, J.A. (2003). Scavenging by vertebrates: behavioral, ecological, and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems. *Oikos*, 102, 225-234.
- Eggert, A.-K. & Müller, J.K. (1992). Joint breeding in female burying beetles. *Behavioral Ecology and Sociobiology*, 31, 237-242.

377 Eggert, A.-K., Reinking, M. & Müller, J.K. (1998). Parental care improves offspring survival
378 and growth in burying beetles. *Animal Behaviour*, 55, 97-107.

379 Eggert, A.K. & Sakaluk, S.K. (2000). Benefits of communal breeding in burying beetles: a
380 field experiment. *Ecological Entomology*, 25, 262-266.

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

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

385 Hocking, M., Ring, R. & Reimchen, T. (2006). Burying beetle *Nicrophorus investigator*
386 reproduction on Pacific salmon carcasses. *Ecological Entomology*, 31, 5-12.

387 Hopwood, P.E., Moore, A.J., Tregenza, T. & Royle, N.J. (2016). Niche variation and the
388 maintenance of variation in body size in a burying beetle. *Ecological Entomology*, 41,
389 96-104.

390 Komdeur, J., Schrama, M.J., Meijer, K., Moore, A.J. & Beukeboom, L.W. (2013). Cobreeding
391 in the burying beetle, *Nicrophorus vespilloides*: tolerance rather than cooperation.
392 *Ethology*, 119, 1138-1148.

393 Lenth, R.V. (2024). emmeans: Estimated Marginal Means, aka Least-Squares Means.

394 May, E.M. & El-Sabaawi, R.W. (2022). Life stage and taxonomy the most important factors
395 determining vertebrate stoichiometry: A meta-analysis. *Ecology and Evolution*, 12,
396 e9354.

397 Monteith, K.M., Andrews, C. & Smiseth, P.T. (2012). Post-hatching parental care masks the
398 effects of egg size on offspring fitness: a removal experiment on burying beetles.
399 *Journal of Evolutionary Biology*, 25, 1815-1822.

400 Müller, J.K., Braunisch, V., Hwang, W. & Eggert, A.-K. (2007). Alternative tactics and
 401 individual reproductive success in natural associations of the burying beetle,
 402 *Nicrophorus vespilloides*. *Behavioral Ecology*, 18, 196-203.

403 Müller, J.K., Eggert, A.-K. & Furlkröger, E. (1990). Clutch size regulation in the burying beetle
 404 *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae). *Journal of Insect Behavior*,
 405 3, 265–270.

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

408 Richardson, J. & Smiseth, P.T. (2020). Effects of variation in resource acquisition during
 409 different stages of the life cycle on life-history traits and trade-offs in a burying beetle.
 410 *Journal of Evolutionary Biology*, 32, 19-30.

411 Rozen, D., Engelmoer, D. & Smiseth, P.T. (2008). Antimicrobial strategies in burying beetles
 412 breeding on carrion. *Proceedings of the National Academy of Sciences*, 105, 17890-
 413 17895.

414 Saini, R.K., Prasad, P., Shang, X. & Keum, Y.-S. (2021). Advances in lipid extraction
 415 methods—a review. *International Journal of Molecular Sciences*, 22, 13643.

416 Scott, M.P. (1994). Competition with flies promotes communal breeding in the burying beetle,
 417 *Nicrophorus tomentosus*. *Behavioral Ecology and Sociobiology*, 34, 367-373.

418 Scott, M.P. (1998). The ecology and behavior of burying beetles. *Annual Review of*
 419 *Entomology*, 43, 595-618.

420 Scott, M.P., LEE, W.J. & Van Der Reijden, E. (2007). The frequency and fitness consequences
 421 of communal breeding in a natural population of burying beetles: a test of reproductive
 422 skew. *Ecological Entomology*, 32, 651-661.

423 Scott, M.P. & Traniello, J.F. (1990). Behavioural and ecological correlates of male and female
 424 parental care and reproductive success in burying beetles (*Nicrophorus* spp.). *Animal*
 425 *Behaviour*, 39, 274-283.

426 Scriber, J. & Slansky Jr, F. (1981). The nutritional ecology of immature insects. *Annual Review*
 427 *of Entomology*, 26, 183-211.

428 Shukla, S.P., Plata, C., Reichelt, M., Steiger, S., Heckel, D.G., Kaltenpoth, M. et al. (2018).
 429 Microbiome-assisted carrion preservation aids larval development in a burying beetle.
 430 *Proceedings of the National Academy of Sciences*, 115, 11274-11279.

431 Smiseth, P.T., Andrews, C.P., Matthey, S.N. & Mooney, R. (2014). Phenotypic variation in
 432 resource acquisition influences trade-off between number and mass of offspring in a
 433 burying beetle. *Journal of Zoology*, 293, 80-83.

434 Stiegler, J., Von Hoermann, C., Müller, J., Benbow, M.E. & Heurich, M. (2020). Carcass
 435 provisioning for scavenger conservation in a temperate forest ecosystem. *Ecosphere*,
 436 11, e03063.

437 Sun, S.-J., Catherall, A.M., Pascoal, S., Jarrett, B.J., Miller, S.E., Sheehan, M.J. et al. (2020).
 438 Rapid local adaptation linked with phenotypic plasticity. *Evolution Letters*, 4, 345-359.

439 Tessier, A.J. & Consolatti, N.L. (1991). Resource quantity and offspring quality in *Daphnia*.
 440 *Ecology*, 72, 468-478.

441 Tomberlin, J.K., Barton, B.T., Lashley, M.A. & Jordan, H.R. (2017). Mass mortality events
 442 and the role of necrophagous invertebrates. *Current Opinion in Insect Science*, 23, 7-
 443 12.

444 Trumbo, S.T. (1990). Regulation of brood size in a burying beetle, *Nicrophorus tomentosus*
 445 (Silphidae). *Journal of Insect Behavior*, 3, 491-500.

446 Trumbo, S.T. (1992). Monogamy to communal breeding: exploitation of a broad resource base
 447 by burying beetles (*Nicrophorus*). *Ecological Entomology*, 17, 289-298.

448 Trumbo, S.T. (2006). Infanticide, sexual selection and task specialization in a biparental
449 burying beetle. *Animal Behaviour*, 72, 1159-1167.

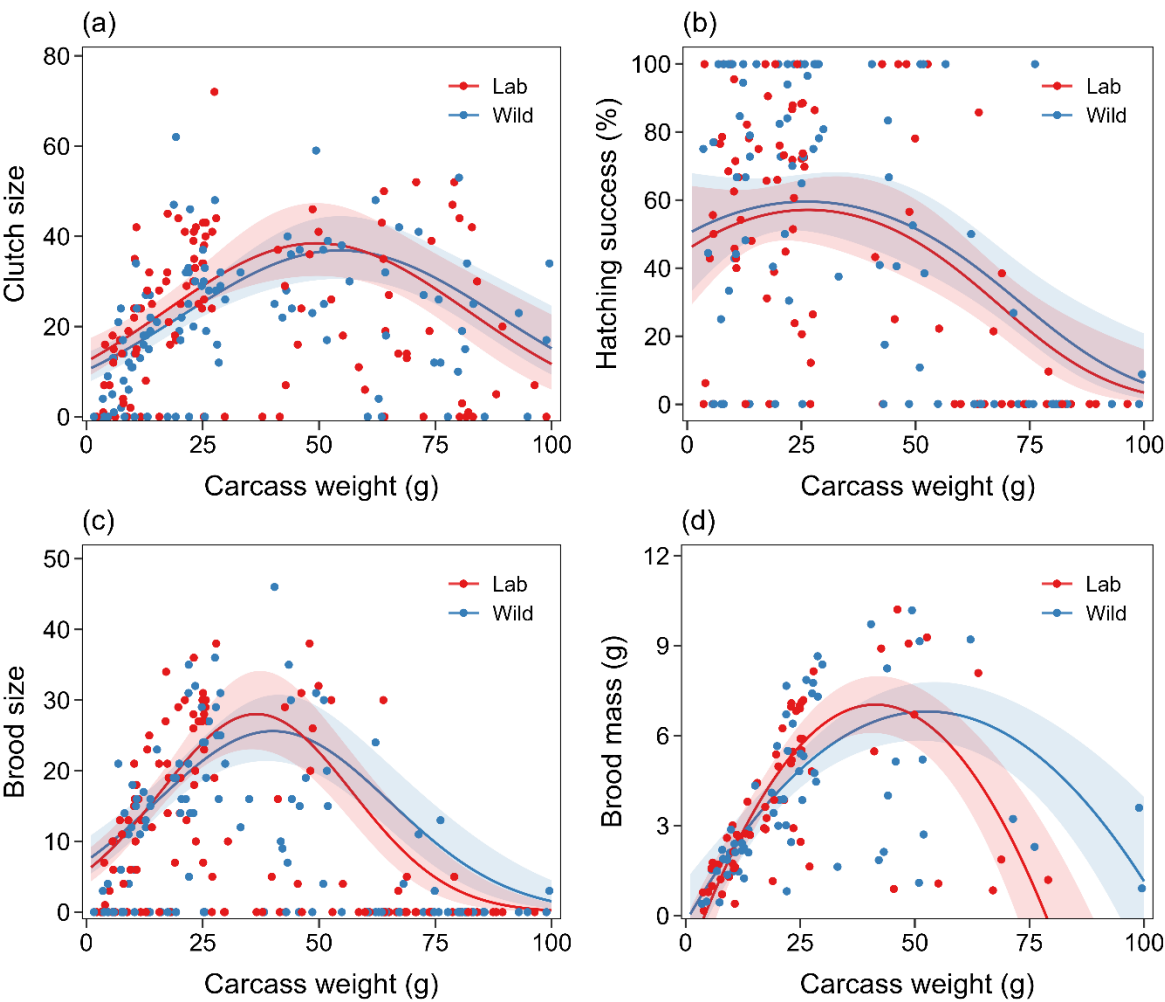
450 Weldon, L., Abolins, S., Lenzi, L., Bourne, C., Riley, E.M. & Viney, M. (2015). The gut
451 microbiota of wild mice. *PLoS One*, 10, e0134643.

452 Woelber, B.K., Hall, C.L. & Howard, D.R. (2018). Environmental cues influence parental
453 brood structure decisions in the burying beetle *Nicrophorus marginatus*. *Journal of*
454 *Ethology*, 36, 55-64.

455

456 **Figures**

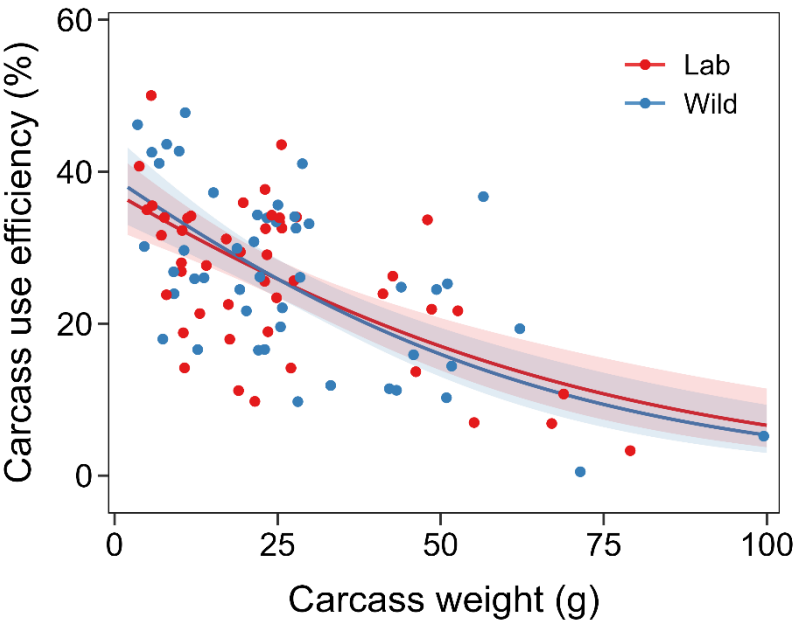
457 **Figure 1**



458

459

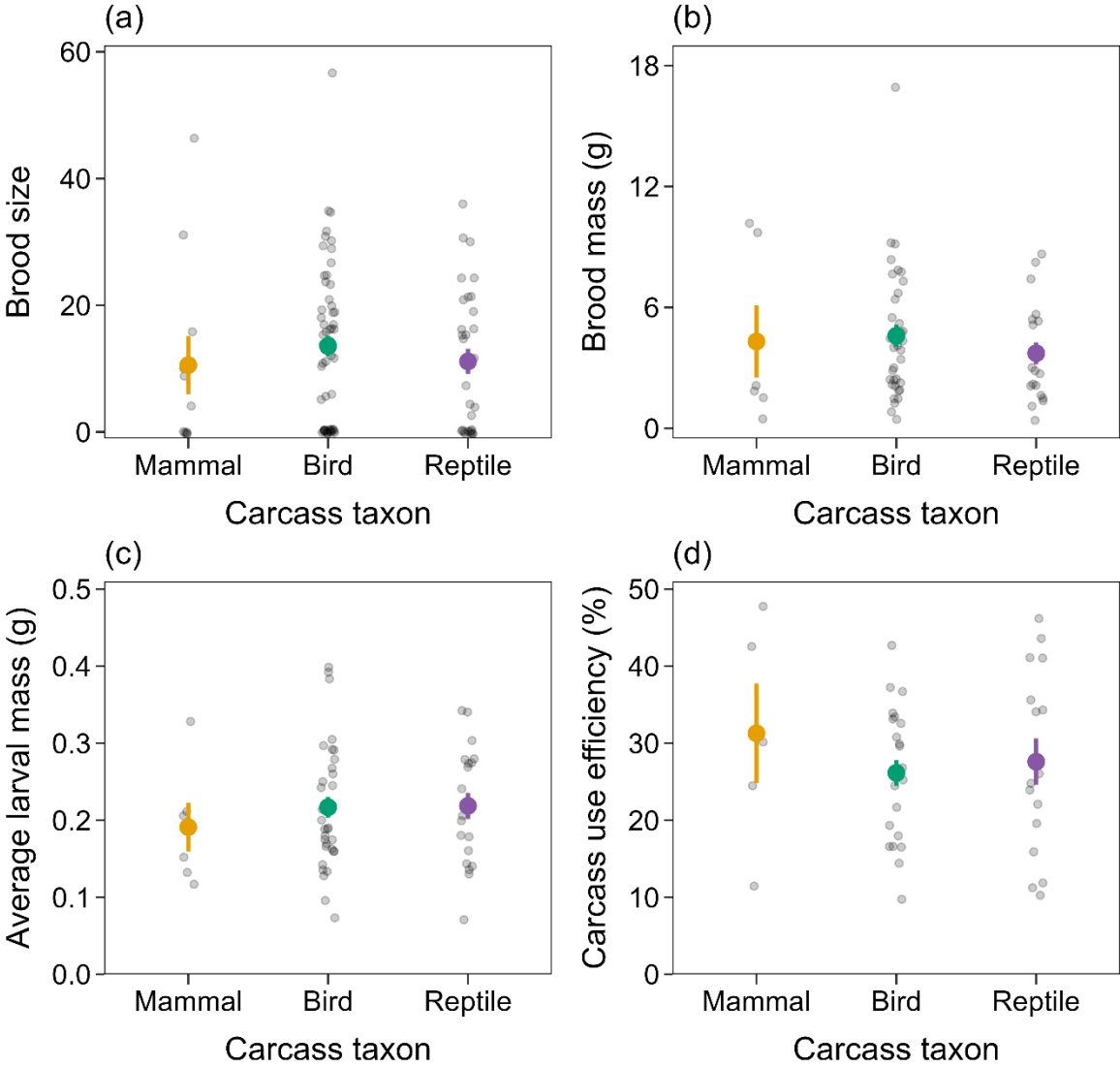
460 Figure 2



461

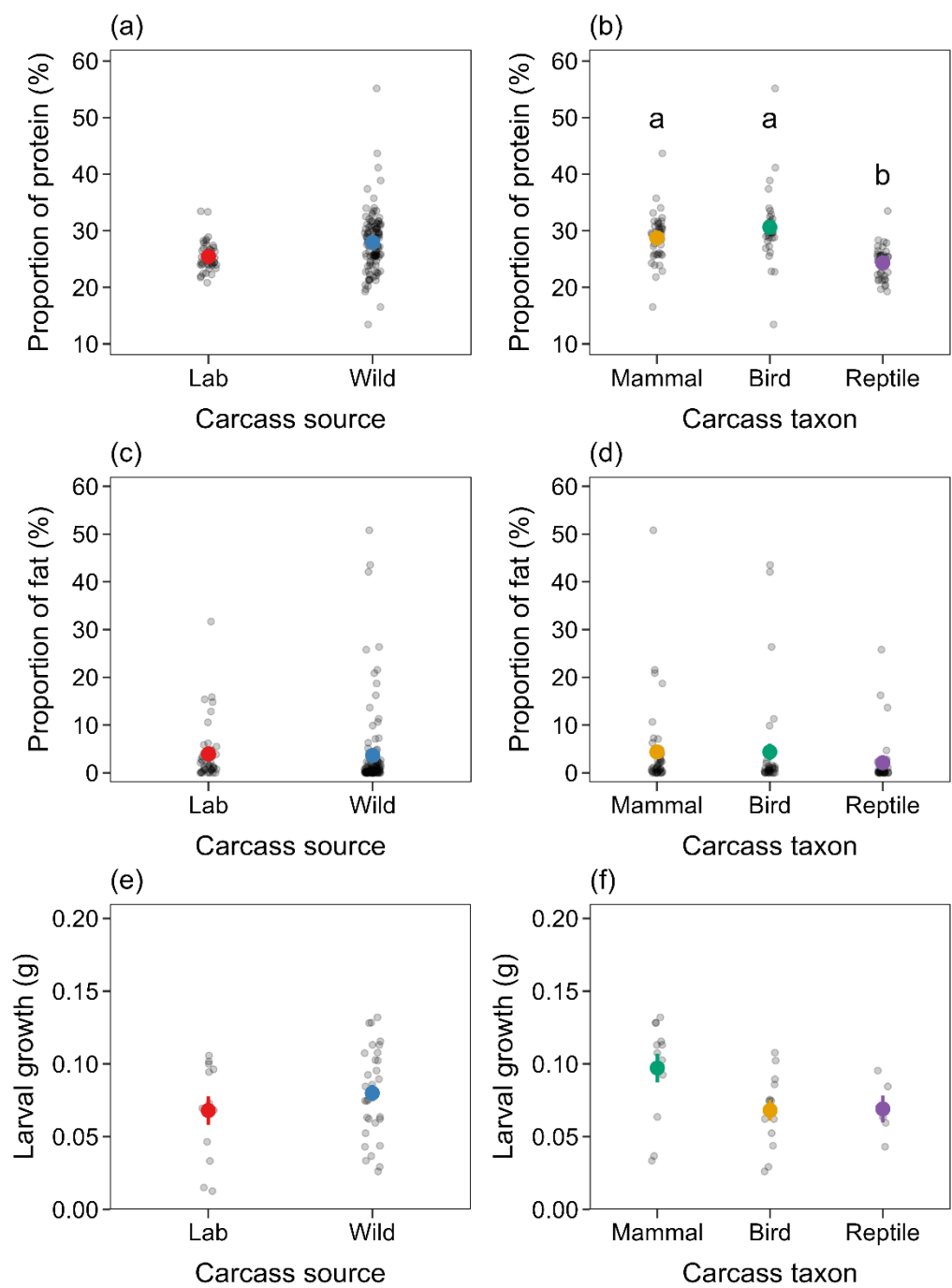
462

463 Figure 3



464

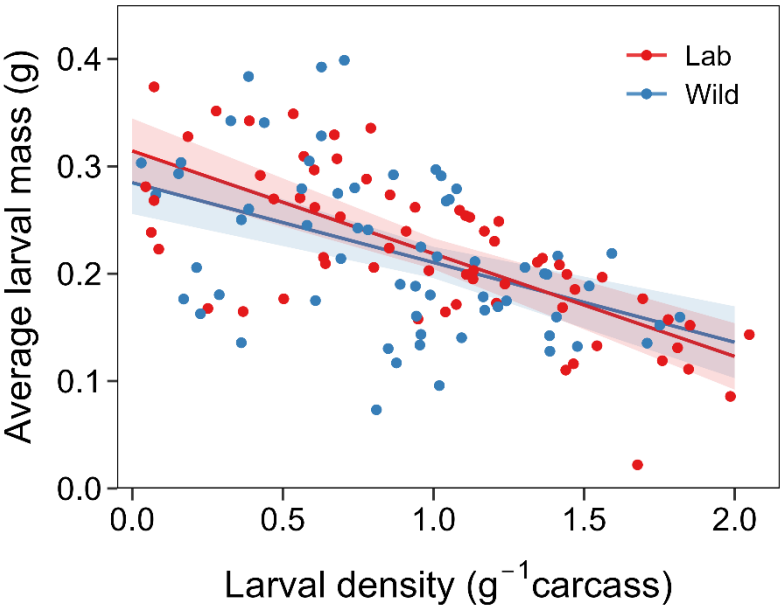
465



467

468

469 Figure 5



470

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 ($\alpha = 0.05$).

493 Figure 5. The relationship between larval density and average larval mass on lab and wild
494 carcasses. Lines represent the statistically significant relationships predicted from GLMMs (α
495 = 0.05); shaded areas represent the 95% confidence intervals.

496

497 **Tables**

498 Table 1. Replication statement for the experimental design in this study.

| Scale of inference | Scale at which the factor of interest is applied | Number of replicates at the appropriate scale |
|--|---|---|
| Breeding performance of burying beetles | Male-female breeding pair | 242 breeding containers |
| Larval growth of burying beetles | Individual larva | 188 larval feeding containers |
| Nutritional composition of vertebrate carcasses | Individual carcass | 26 lab and wild carcasses (3 visceral and 3 muscle tissue samples for each carcass) |

499

500

Table 2. 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.