Functional Ecology

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

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1 **Abstract**

2   1. Carcasses represent critical resources for many terrestrial organisms, including burying

3   beetles, which rely on carcasses for survival and breeding. Carcass attributes can strongly

4   influence the reproduction of burying beetles, yet most studies on their breeding ecology

5   have used laboratory-reared carcasses of limited sizes, raising questions about whether

6   these results are representative of natural patterns.

7   2. We conducted breeding and feeding experiments using a wide size range of lab (laboratory

8   mice) and wild carcasses (wild mammals, birds, and reptiles) to investigate how carcass

9   size, source, and taxon affect various breeding outcomes such as clutch size, brood size,

10   brood mass, and larval growth of the burying beetle *Nicrophorus nepalensis*.

11   3. Our results reveal a hump-shaped relationship between carcass size and breeding

12   performance, with optimal breeding outcomes occurring on medium-sized carcasses.

13   Furthermore, despite the variation in tissue nutritional composition, breeding outcomes and

14   larval growth did not differ between the lab and wild carcass sources or among the three

15   wild carcass taxa. Finally, we found a larval quality-quantity trade-off across the range of

16   carcasses examined, with carcass size shaping the larval life history traits.

17   4. Overall, these results elucidate how carcass resources may influence the breeding

18   performance of burying beetles. Importantly, our study provides solid evidence validating

19   decades of research using lab carcasses to study the reproductive ecology of burying beetles.

20

24 **Introduction**

25 Carcasses represent a rich resource for a wide variety of terrestrial organisms, including

26 vertebrate scavengers, saprophagous invertebrates, and microbial decomposers (Barton et al.,

27 2013; Rozen et al., 2008; Stiegler et al., 2020). These carcass-feeding organisms facilitate the

28 recycling of carcass nutrients and make the resource available to other species (Tomberlin et

29 al., 2017). For some species such as burying beetles (*Nicrophorus* spp.), carcasses are

30 particularly important because they serve as not only food resource but also breeding sites

31 where the offspring grow and develop under parental care (Scott, 1998). Carcass attributes,

32 therefore, can strongly influence the reproduction of burying beetles.

33 Carcass size is a key factor for the reproductive success of burying beetles because it

34 determines the amount of resource available for breeding. Brood size and brood mass are

35 generally greater on larger (heavier) carcasses (Creighton, 2005; Scott, 1998; Scott & Traniello,

36 1990; Smiseth et al., 2014; Trumbo, 1992). Moreover, parents can adjust their reproductive

37 investment based on carcass size (Hopwood et al., 2016). For example, females lay more eggs

38 on larger carcasses within a certain carcass size range (Müller et al., 1990), and parents regulate

39 the brood size via filial cannibalism when carcass resource is limited (Bartlett, 1987). However,

40 despite the resource benefits, large carcasses can be more difficult to utilize (Trumbo, 1992),

41 and the energetic costs of processing carcass tissue also increase with carcass size. Such cost-

42 benefit trade-offs suggest that reproductive performance might not necessarily be greater on

43 larger carcasses, yet no study has empirically examined whether there is an optimal carcass

44 size for breeding.

45 Besides carcass size, the source of carcass can also influence the reproduction of

46 burying beetles. Carcasses in the wild come from animals feeding on diverse diets in various

47 environments. However, most breeding experiments use laboratory mice and chicks, which are

48   usually fed fixed diets and reared in a controlled environment. Consequently, lab and wild

49   carcasses may have considerably different body compositions as well as skin and gut

50   microbiomes (Weldon et al., 2015), and these differences can alter larval survival and growth

51   (Rozen et al., 2008; Shukla et al., 2018). Therefore, experiments comparing the breeding

52   outcomes of burying beetles on lab versus wild carcasses are essential for evaluating whether

53   the results of past studies are representative of natural patterns. Furthermore, burying beetles

54   have been documented to breed on carcasses from a variety of taxonomic groups (Hocking et

55   al., 2006; Scott, 1998). Different carcass taxa can vary in their tissue nutritional composition

56   (May & El-Sabaawi, 2022), which may influence larval growth and development (Scriber &

57   Slansky Jr, 1981). However, it remains unknown how breeding outcomes and larval

58   performance may vary among different groups of wild carcasses.

59   Studies have shown that brood size and larval mass of burying beetles are often

60   negatively correlated with each other (Bartlett, 1987; Creighton, 2005; Monteith et al., 2012).

61   Such a trade-off between larval quality and quantity may vary with carcass size (Bartlett &

62   Ashworth, 1988; Smiseth et al., 2014) because resource quantity can shape the life history traits

63   of organisms (Boggs, 2009; Richardson & Smiseth, 2020; Tessier & Consolatti, 1991).

64   However, most studies on the offspring trade-off in burying beetles were conducted under a

65   limited range of carcass sizes, and the results have been mixed because of the differential

66   responses of brood size and larval mass to carcass size (Smiseth et al., 2014). Moreover, carcass

67   sources with different quality can influence larval performance and thereby alter the trade-off

68   patterns, yet few studies have examined this (but see Woelber et al., 2018). Therefore,

69   examining breeding outcomes across a wide range of carcass sizes from different carcass

70   sources (e.g., lab and wild carcasses) will help better understand how resource variation affects

71   the offspring life history trade-off in burying beetles.

72   In this study, we aimed to understand how various carcass attributes (size, source, and

73 taxon) influence the breeding outcomes, larval performance, and offspring quality-quantity

74 trade-off in burying beetles. We conducted breeding experiments on the species *Nicrophorus*

75 *nepalensis*, which has been shown to provide extensive parental care for offspring. First, we

76 examined how breeding outcomes (clutch size, brood size, brood mass, etc.) and carcass use

77 efficiency varied across a broad range of carcass size (weight was used as a proxy for size in

78 this study) on lab (laboratory mice) and wild carcasses (wild mammals, birds, and reptiles).

79 We further focused on the wild carcasses and compared the larval breeding outcomes and

80 carcass use efficiency of *N. nepalensis* on the three wild carcass taxa. We expected that there

81 would be an optimal carcass size for breeding, and the breeding outcomes may differ between

82 lab and wild carcasses as well as among different wild carcass taxa. We next quantified the

83 tissue nutritional composition of lab and wild carcasses and conducted a larval feeding

84 experiment using carcass tissues from different sources and taxa. We expected that the larvae

85 would perform better when feeding on diets with higher nutritional quality. Finally, we

86 examined the larval quality-quantity trade-off on lab and wild carcasses. We expected a trade-

87 off across a broad range of carcass sizes, and the trade-off pattern would differ between lab and

88 wild carcasses.

89

90   **Materials and Methods**

91   *Breeding experiments*

92   We conducted breeding experiments on *N. nepalensis* from the lab colony established in 2023.

93   Adult beetles were collected from Taipei and New Taipei City, Taiwan and reared in growth

94   chambers under a relative humidity of 70% and a 10:14 h light:dark cycle. The temperature

95   was set to mimic diurnal temperature fluctuation (mean: 17.8°C; range: 16–20°C). This

96   represents the natural temperature conditions during the breeding season (November–April) of

97   *N. nepalensis* in northern Taiwan. A male and a female were placed in a plastic breeding

98   container (14.2 cm in diameter and 6.3 cm in height) half-filled with moist commercial potting

99   mix (2 cm in depth, equivalent to 300 mL), and a defrosted carcass was then placed on the soil

100 surface. Frozen dead laboratory mice/rats were used as lab carcasses. Wild carcasses were

101 obtained from the Taiwan Roadkill Observation Network (https://roadkill.tw/eng/home) and

102 the Wild Bird Society of Taipei. These wild carcasses weighed from 1.6 to 99.5 grams and

103 consisted of small mammals, birds, and reptiles. The carcasses used for breeding experiments

104 were animals that had died within the past four months due to traffic collisions and other

105 accidental causes but not poisoning. Upon discovery, these carcasses were immediately

106 transferred to −20°C freezers for preservation. We paired each wild carcass with a lab carcass

107 of a similar weight (measured to the nearest 0.1 g using an electronic analytical balance

108 ATX224R, Shimadzu, Japan) and applied a sibship design where the two males and the two

109 females used in each lab-wild carcass were from the same family line, respectively, to control

110 for parental genotypes (the males and females came from genetically unrelated families). The

111 breeding containers were maintained under the same environmental conditions as those of the

112 lab colony. Five rounds of breeding experiments were conducted from May 2023 to March

113 2024 (each with a different beetle parent generation), consisting of a total of 121 lab-wild

114 carcass pairs (14, 76, and 31 wild mammal, bird, and reptile carcasses).

115   We recorded the clutch size of each breeding container at day 4 by counting the number

116   of eggs around the wall and at the bottom of the container from the outside. This minimized

117   the disturbance to the carcass and parents while providing an accurate estimate of the exact

118   clutch size ($r = 0.94$, $P < 0.001$, $n = 70$ broods) (Sun et al., 2020). Eleven days after beetle

119   pairing, we inspected the carcass to record the brood size (number of larvae) and brood mass

120   (total larval weight; measured to the nearest 0.0001 g). We calculated hatching success as brood

121   size divided by clutch size, average larval mass as brood mass divided by brood size, and larval

122   density as brood size divided by carcass weight. We also measured the total weight of breeding

123   containers at the beginning and at the end of the experiments to estimate the amount of carcass

124   tissue used by parents and larvae during the breeding process (larvae were removed from the

125   carcasses). Carcass use efficiency was calculated as the amount of carcass tissue used divided

126   by the initial carcass weight.

127

128   *Nutritional analysis of carcass tissue*

129   To quantify the nutritional composition of lab and wild carcasses, which is essential for

130   understanding how burying beetles use different types of carcasses, we estimated the protein

131   and fat content of carcass tissue by adopting a proximate analysis approach as described by Al

132   Shareefi and Cotter (2018). We dissected the carcasses by first skinning the animals and

133   retaining the trunks. Trunk tissue was then separated from the bones with a pair of fine tweezers

134   and a scalpel and divided into viscera (all organs inside the peritoneum) and muscles (all visible

135   muscle parts). We next used a meat tenderizer to pound the viscera and muscles evenly and

136   sampled three pieces of visceral and muscle tissue for each carcass for the analysis of

137   nutritional composition. A total of seven lab mice, seven wild mammals, seven wild birds, and

138   seven wild reptiles were dissected and analyzed.

139   For each tissue sample, we dried approximately 100 mg (106 ± 18 mg) of wet tissue in

140   a 40°C oven for 5 days until all water was removed. To determine the fat content, the dried

141   tissue was thoroughly mixed with 100 μl of −20°C acetone and vortexed for one minute. The

142   mixture was then placed in a −20°C fridge for a 30-minute reaction period (Saini et al., 2021).

143   After the extraction, the mixture was centrifuged to separate the components, and the acetone

144   was carefully removed. If the acetone appeared turbid after centrifugation, the solvent was

145   discarded and replaced with fresh acetone for further extraction. The process was repeated until

146   the solvent became clear. The residual solvent was then allowed to evaporate at room

147   temperature for 12 hours. After the fat removal process, the final product was weighed to

148   determine the protein content, and the fat content was determined by subtracting the protein

149   weight from the dry weight.

150

151   *Larval feeding experiments*

152   We conducted larval feeding experiments using the remaining dissected carcass tissue from the

153   nutritional composition analysis. We placed *ca.* 400 mg (401 ± 21 mg) of carcass tissue into

154   individual plastic containers filled with moist commercial potting mix (soil volume 3.2 × 3.2

155   × 2.7 cm). Newly hatched larvae (five days after female oviposition) were obtained from pairs

156   of breeding beetles (25 families) from the lab colony and one larva was introduced to each

157   container ($n = 188$). After five days of feeding, the larval mass at dispersal was recorded and

158   larval growth was measured as the larval weight gain during the experimental period.

159

160   *Data analyses*

161   1. Breeding outcomes and carcass use efficiency

162   To examine how clutch size, hatching success, brood size, brood mass, and carcass use

163   efficiency varied with carcass size on lab and wild carcasses, we fit generalized linear mixed

164   effects models (GLMMs) with each of the aforementioned breeding outcomes as the response,

165   carcass weight and carcass source as well as their interaction as the fixed effects, and lab-wild

166   carcass pair as the random effect. The pronotum widths of the parents and parent generation

167   were included as the covariates in the models. For clutch size and brood size, we used a

168   negative binomial error distribution with a log link function for model fitting to account for

169   data overdispersion; for hatching success, we used a binomial error distribution with a logit

170   link function; for brood mass, we used a Gaussian error distribution; for carcass use efficiency,

171   we used a beta error distribution with a logit link function. Because clutch size and brood size

172   contained many zero values, we additionally included a zero inflation structure in the models.

173   We determined whether a quadratic curve better described the relationship between each

174   response and carcass weight by comparing the GLMMs fitted with and without a quadratic

175   term for carcass weight via the likelihood ratio test. Results from the quadratic model were

176   reported if the test was significant ($\alpha = 0.05$).

177   To compare the brood size, brood mass, average larval mass, and carcass use efficiency

178   on wild mammal, bird, and reptile carcasses, we fit generalized linear models (GLMs) with

179   each of the aforementioned breeding outcomes as the response and wild carcass taxon as the

180   fixed effect. Carcass weight, pronotum widths of the parents, and parent generation were

181   included as the covariates in the models. The error distribution and link function for each of

182   the responses were the same as the GLMMs. Because the carcass range was considerably

183   smaller for reptiles (1.6–64.4 g) than for mammals (3.8–94.8 g) and birds (3.2–99.5 g), we

184   restricted the carcass weight range to that of reptiles (≤ 64.4 g) so that the results were more

185   comparable among the three wild taxa.

186

187   2.   Nutritional composition and larval growth

188   To compare the nutritional composition between the two carcass sources and the three wild

189   carcass taxa, we fit GLMMs with the proportion of protein/fat as the responses, carcass

190   source/taxon and tissue type (viscera vs. muscles) as the fix effects, and carcass ID as the

191   random effect (a total of four GLMMs). We used a beta error distribution with a logit link

192   function for model fitting in the GLMMs.

193   To compare the larval growth between the two carcass sources and the three wild

194   carcass taxa, we fit GLMMs with larval weight gain as the response, carcass source/taxon and

195   tissue type as the fix effects, and carcass ID and larval family as the random effects (a total of

196   two GLMMs). Larval mass at hatching was included in the models as a covariate. We used a

197   Gaussian error distribution for model fitting in the GLMMs. To further investigate the effect

198   of nutrient content on larval growth on both carcass sources and on wild carcasses only, we fit

199   GLMMs with larval weight gain as the response, proportion of protein, proportion of fat, and

200   tissue type as the fixed effects, and larval family as the random effects (a total of two GLMMs).

201   Larval mass at hatching was included as a covariate. Dead larvae were excluded from the

202   analysis.

203

204   3.   Larval quality-quantity trade-off

205   To evaluate the trade-off between offspring quality and quantity on lab and wild carcasses, we

206   fit a linear model with average larval mass as the response and larval density, carcass source,

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

208   quantity trade-off.

209

210   We fit all aforementioned models using the glmmtmb() function in the R "glmmTMB"

211   package (Brooks et al., 2017). Model assumptions were checked via the quantile residuals

212   generated from the simulateResiduals() function in the R "DHARMa" package (Hartig, 2022).

213   Predictor significance was assessed with the Wald chi-square test via the Anova() function

214   (type II sums of squares) in the R "car" package (Fox & Weisberg, 2019). Post-hoc pairwise

215   comparisons among carcass taxa with the Tukey multiplicity adjustment were conducted via

216   the emmeans() function in the R "emmeans" package (Lenth, 2024). All analyses were

217   performed in R version 4.3.3 (R Core Team, 2024). The scale of inference and sample sizes for

218   the experiments are summarized in Table 1.

219   **Results**

220   *Breeding outcomes and carcass use efficiency*

221   Clutch size, hatching success, brood size, and brood mass all showed a quadratic relationship

222   with carcass weight (clutch size: $\chi^2_2 = 44.6$, $P < 0.001$; hatching success: $\chi^2_2 = 32.1$, $P < 0.001$;

223   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

224   medium-sized carcasses (Fig. 1). Moreover, these breeding outcomes did not differ between

225   lab and wild carcasses (clutch size: $\chi^2_1 = 1.4$, $P = 0.39$; hatching success: $\chi^2_1 = 0.8$, $P = 0.37$;

226   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

227   use efficiency decreased with carcass weight ($\chi^2_2 = 64.5$, $P < 0.001$) but did not differ between

228   lab and wild carcasses ($\chi^2_1 = 0.003$, $P = 0.96$; Table 2; Fig. 2).

229   Brood size, brood mass, average larval mass, and carcass use efficiency did not differ

230   among wild mammal, bird, and reptile carcasses (brood size: $\chi^2_2 = 0.6$, $P = 0.75$; brood mass:

231     $\chi^2_2 = 3.6$, $P = 0.17$; average larval mass: $\chi^2_2 = 3.3$, $P = 0.19$; carcass use efficiency: $\chi^2_2 = 0.4$,

232     $P = 0.81$; Fig. 3).

233

234   *Nutritional composition of carcasses*

235   Protein content was similar between lab and wild carcasses (mean proportion: lab = 25.5%,

236   wild = 27.9%; $\chi^2_1 = 3.5$, $P = 0.06$; Fig. 4a) but differed among wild carcass taxa (mean

237   proportion: mammal = 28.7%, bird = 30.6%, reptile = 24.3%; $\chi^2_2 = 26.6$, $P < 0.001$; Fig. 4b).

238   Specifically, reptile carcasses had significantly lower protein content than mammal and bird

239   carcasses (Fig. 4b). Fat content was similar between lab and wild carcasses (mean proportion:

240   lab = 4.0%, wild = 3.7%; $\chi^2_1 = 1.1$, $P = 0.29$; Fig. 4c) and among wild carcass taxa (mean

241   proportion: mammal = 4.4%, bird = 4.4%, reptile = 2.1%; $\chi^2_2 = 3.5$, $P = 0.18$; Fig. 4d).

242

Larval growth

244   Growth was similar for larvae feeding on tissue from lab and wild carcasses ($\chi^2_1 = 0.1$, $P =$

245   0.74; Fig. 4e). Similarly, larval growth did not differ significantly among the three wild carcass

246   taxa ($\chi^2_2 = 5.2$, $P = 0.07$; Fig. 4f), although larvae feeding on wild bird carcasses tended to gain

247   more weight compared to those feeding on wild mammals and reptiles (Fig. 4f). When lab and

248   wild carcasses were combined, larval growth was not associated with either tissue protein

249   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

250   hand, larvae feeding on wild carcass tissue with higher fat content ($\chi^2_1 = 5.2$, $P = 0.02$), but not

251   protein content ($\chi^2_1 = 0.01$, $P = 0.92$), did grow better (Fig. S2c and d).

252

Larval quality-quantity trade-off

254   Average larval mass decreased with larval density on both lab and wild carcasses ($\beta = -0.096$,

255      $\chi^2_1 = 74.7$, $P < 0.001$; Fig. 5). The interaction between larval density and carcass source was

256   not significant ($\chi^2_1 = 1.2$, $P = 0.28$), indicating that the trade-off did not differ between lab and

257   wild carcasses (Fig. 5).

258

259   **Discussion**

260   We examined how breeding outcomes and carcass use efficiency of the burying beetle *N.*

261   *nepalensis* varied with carcass size on lab and wild carcasses. Clutch size, hatching success,

262   brood size, and brood mass all exhibited a quadratic relationship with carcass size, whereas

263   carcass use efficiency decreased with carcass size. Furthermore, these breeding outcomes and

264   carcass use efficiency did not differ between lab and wild carcasses. Despite the variation in

265   tissue nutritional composition (protein content) among wild mammal, bird, and reptile

266   carcasses, larval traits (brood size, brood mass, and average larval mass), carcass use efficiency,

267   and larval growth did not differ among the three wild carcass taxa. Finally, a negative

268   relationship existed between larval density and average larval mass on both lab and wild

269   carcasses, suggesting a trade-off between offspring quality and quantity. Taken together, our

270   results indicate that carcass size, but not carcass source or carcass taxon, is the main

271   determinant for the breeding performance and carcass resource use of burying beetles.

272   As expected, clutch size, hatching success, brood size, and brood mass all showed a

273   quadratic relationship with carcass size, with optimal breeding outcomes occurring on medium-

274   sized carcasses. The increase in breeding performance from small to medium carcasses is

275   consistent with previous studies on other burying beetle species (Creighton, 2005; Eggert &

276   Müller, 1992; Hopwood et al., 2016). Interestingly, when the parents bred on large carcasses,

277   their breeding performance decreased, along with a reduction in carcass use efficiency. This

278   may be because large carcasses are more energetically costly to process and females may lay

279   fewer eggs as a result of lower energy storage. In fact, Müller et al. (1990) found that clutch

280   size levels off beyond a certain carcass weight threshold, suggesting an energetic or

281   physiological constraint on beetles breeding on larger carcasses. Parents breeding on large

282   carcasses also face stronger competition with microbes, which can reduce the usable resource

283   for breeding (Scott, 1998) or produce compounds harmful to eggs and larvae (Rozen et al.,

284   2008).

285   Contrary to our prediction, we found no major difference in the breeding outcomes and

286   carcass use efficiency of *N. nepalensis* on lab versus wild carcasses. A potential explanation is

287   that the parents manipulated the carcasses (e.g., by secreting antimicrobial compounds) such

288   that the eggs and larvae experienced similar growing environments regardless of carcass source.

289   Studies have shown that parental care is crucial for larval performance in burying beetles

290   (Eggert et al., 1998; Rozen et al., 2008), and we speculate that parental food preparation and

291   regurgitation may offset the difference between the two carcass sources. Further experiments

292   comparing breeding outcomes on lab and wild carcasses with versus without parents will help

293   verify our speculation. The analyses did reveal an interaction between carcass size and carcass

294   source for brood mass. In fact, the patterns were mostly similar between lab and wild carcasses

295   on small and medium carcasses, whereas the difference on large carcasses was mainly driven

296   by two observations on large wild carcasses (the interaction became non-significant when these

297   two observations were removed; $P = 0.38$, Fig. S3). Overall, our results support the validity of

298   research using lab-reared organisms as breeding carcasses to study the reproductive biology of

299   burying beetles.

300   Our tissue nutritional analysis showed that protein content was higher in wild mammal

301   and bird carcasses than in wild reptile carcasses, whereas fat content was similar among these

302   taxa. Yet, despite the variation in tissue protein content, larval growth in the feeding

303   experiments did not vary significantly among the wild carcass taxa. In fact, we found that it

304   was fat content, not protein content, that affected larval growth on wild carcasses. Since fat

305   content did not vary among the three wild carcass taxa, we did not observe major difference in

306   larval growth. This may also partially explain why larval traits and carcass use efficiency were

307   similar among the three wild carcass taxa in our breeding experiments. Interestingly, larvae did

308   tend to grow better on bird carcasses in the feeding experiments without parents. This suggests

309   that parental care in burying beetles (e.g., carcass preparation and food provisioning) may help

310   maintain breeding performance on a variety of carcasses in the wild. But without parental care,

311   carcass taxon may potentially influence individual larval performance.

312   The negative relationship between average larval mass and larval density on both lab

313   and wild carcasses indicates a trade-off between offspring quality and quantity regardless of

314   carcass source. Similar trade-off patterns have been shown in previous studies (Bartlett &

315   Ashworth, 1988; Trumbo, 1990) and can arise from both larval competition and brood

316   regulation by parents (Trumbo, 1990). Stronger interspecific competition under a higher larval

317   density may reduce individual larval growth, leading to lower average larval mass. On the other

318   hand, parents may regulate brood size by culling excess larvae to reduce larval competition

319   (Trumbo, 2006), thereby leading to greater larval growth and higher average biomass.

320   Furthermore, the slope of the negative relationship between average larval mass and larval

321   density did not depend on carcass source, agreeing with our findings that brood size and brood

322   mass did not differ between lab and wild carcasses. Interestingly, we found that the average

323   larval mass increased with carcass size for small and medium carcasses, whereas larval density

324   decreased (Fig. S1). This suggests that the larval life history traits of burying beetles can shift

325   depending on breeding resource availability, with smaller carcasses favoring larval quantity

326   (per capita carcass resource) and larger carcasses favoring larval quality.

327   Our results illustrate the role of carcass size in the breeding outcomes of a single parent

328   pair. This is the most common breeding system in burying beetles on small- and medium-sized

329   carcasses (Scott, 1998). However, multiple males and females may engage in cooperative

330   breeding to better utilize large carcasses in the wild (Scott et al., 2007), although past results

331   for the reproductive benefits of cooperation are mixed (Eggert & Sakaluk, 2000; Komdeur et

332   al., 2013; Müller et al., 2007). Additionally, burying beetles in nature may face carcass

333   competition not only from microbes but also from various vertebrate scavengers and

334   invertebrate carcass feeders (Chen et al., 2020; DeVault et al., 2003), and such interspecific

335   competition can interact with carcass size to influence breeding success (Scott, 1994). Field

336   experiments using a wide range of carcass sizes will help elucidate how intraspecific and

337   interspecific interactions as well as the interplay between biotic interactions and carcass size

338   jointly shape the breeding performance of burying beetles.

339   Using a broad range of carcass sizes from both lab and wild sources, our study revealed

340   for the first time the quadratic relationship between breeding performance and carcass size in

341   burying beetle, with optimal breeding outcomes occurring on medium-sized carcasses.

342   Breeding outcomes did not differ between lab and wild carcasses. Furthermore, despite the

343   variation in tissue nutritional composition (particularly protein content) among wild mammal,

344   bird, and reptile carcasses, larval traits, carcass use efficiency, and larval growth were generally

345   similar among these wild carcass taxa. Finally, the larval quality-quantity trade-off existed

346   across the range of lab and wild carcass sizes, and larval life history traits may shift depending

347   on carcass size, with smaller carcasses favoring larval quantity and larger carcasses favoring

348   larval quality. Taken together, our study confirms that previous results from lab carcasses are

349   fairly representative of natural patterns and provides a more complete picture of how carcass

350   resources shape the breeding performance of burying beetles.

351

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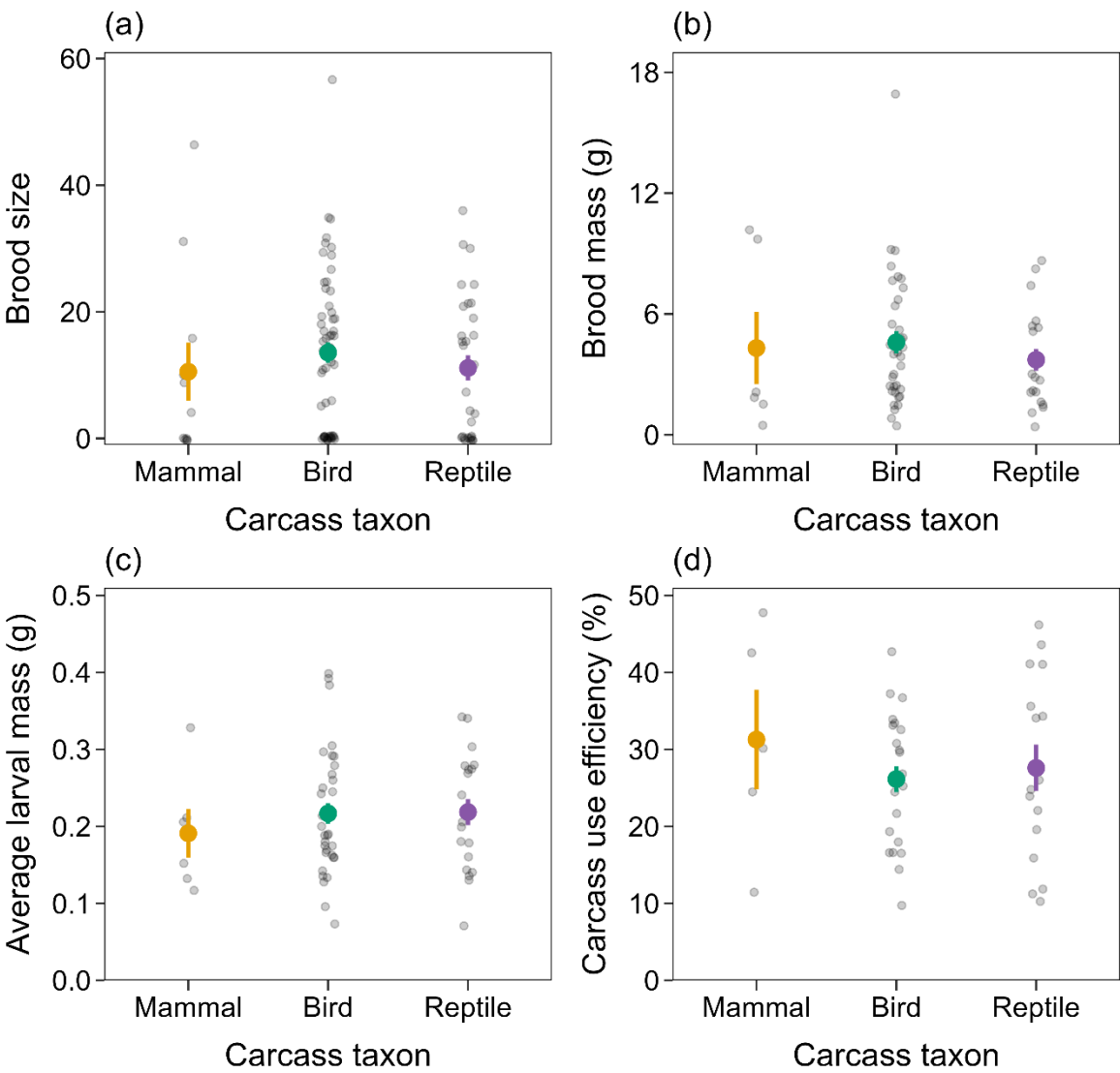
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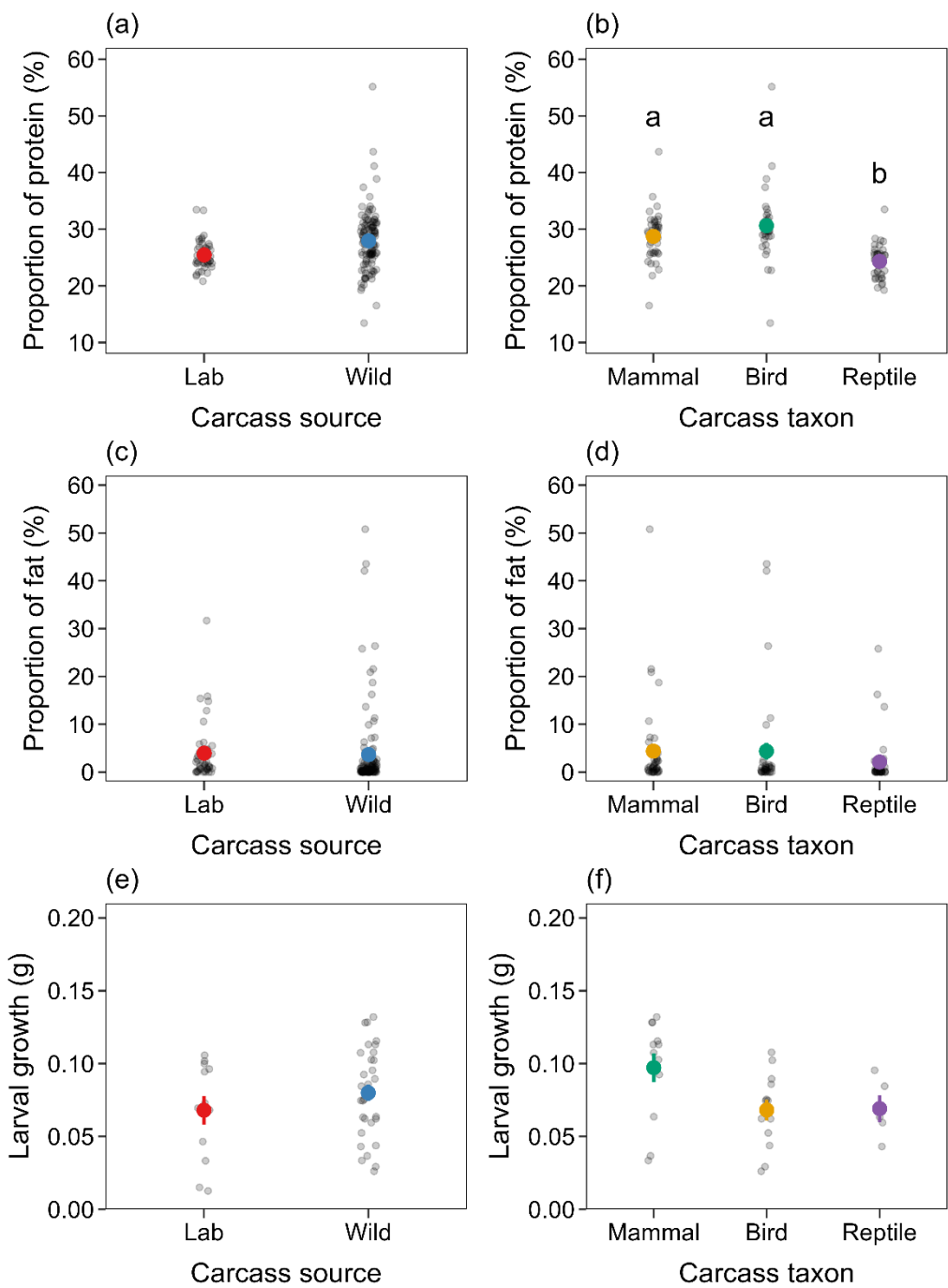
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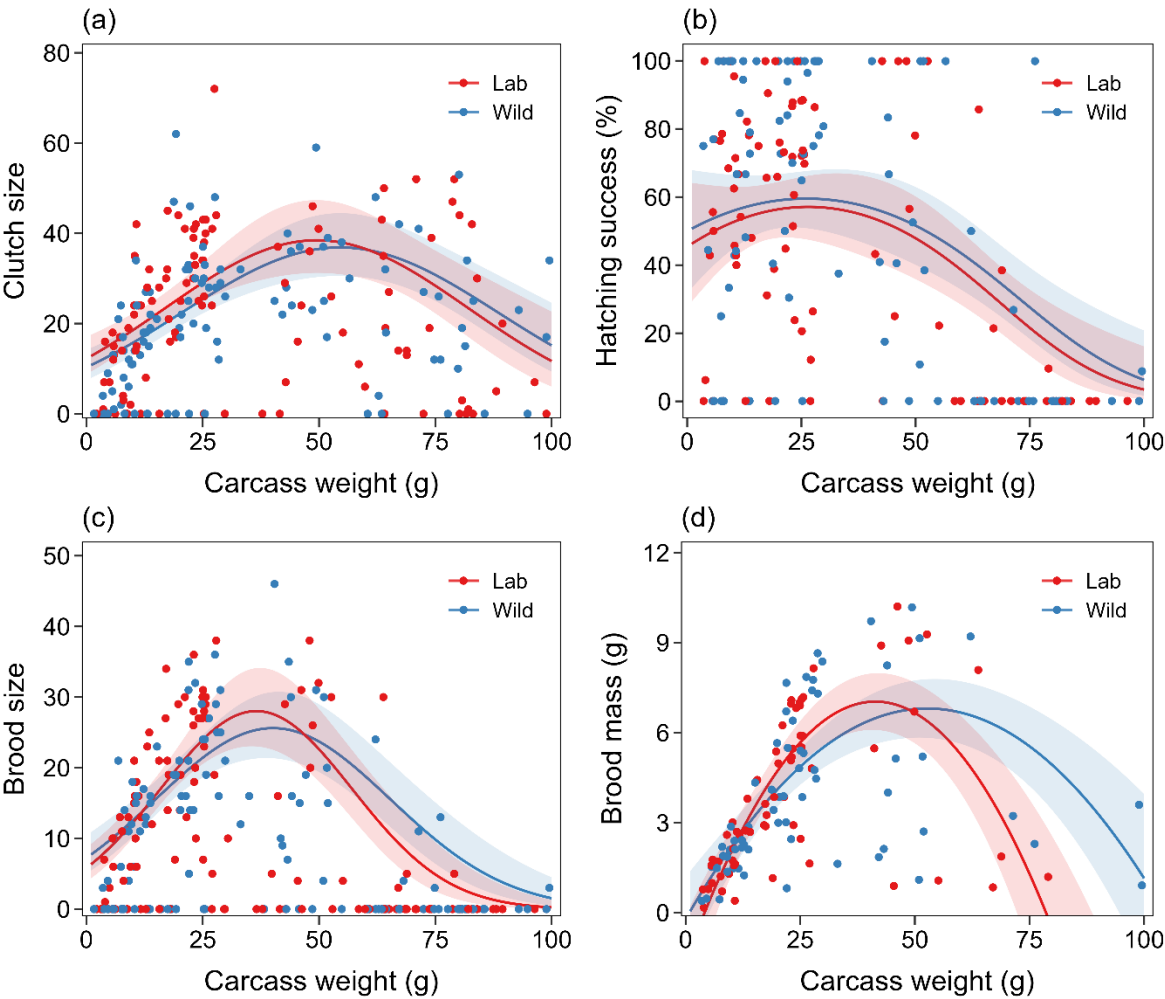
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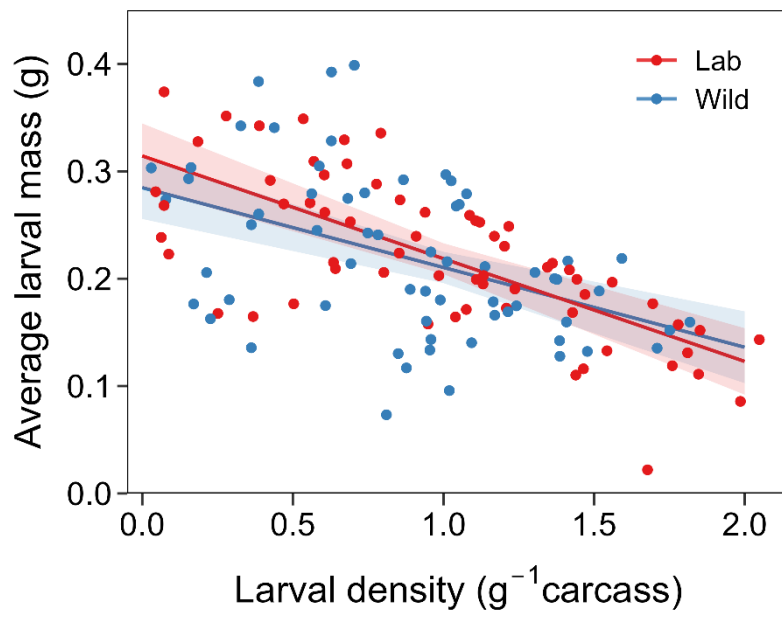
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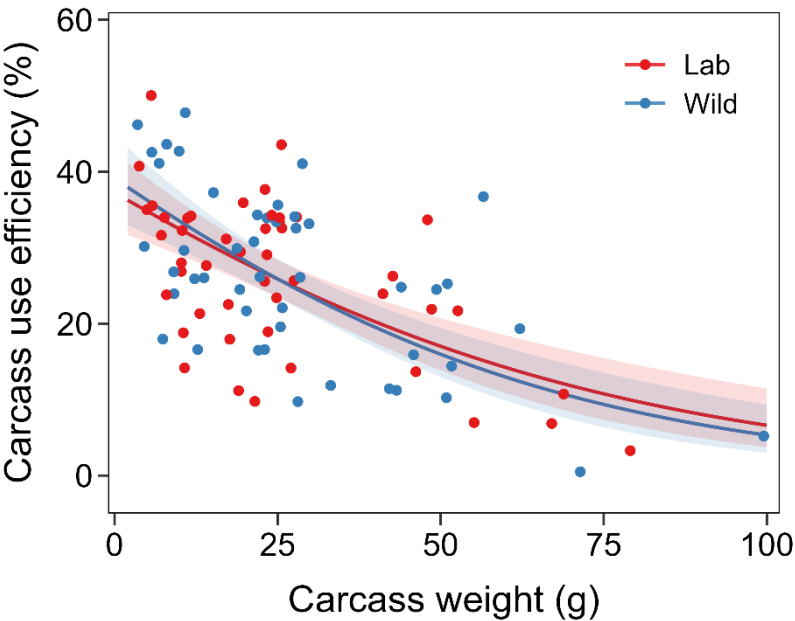
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471　**Figure legends**

472　Figure 1. The relationship between carcass weight and clutch size (a), hatching success (b),

473　brood size (c), and brood mass (d) on lab and wild carcasses. Note that the observations without

474　any larva were excluded from the brood mass analysis. Lines represent the statistically

475　significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded areas represent the 95%

476　confidence intervals.

477

478　Figure 2. The relationship between carcass weight and carcass use efficiency on lab and wild

479　carcasses. Note that the observations without any larva were excluded from the analysis. Lines

480　represent the statistically significant relationships predicted from GLMMs ($\alpha = 0.05$); shaded

481　areas represent the 95% confidence intervals.

482

483　Figure 3. Brood size (a), brood mass (b), average larval mass (c), and carcass use efficiency (d)

484　on wild mammal, bird, and reptile carcasses. Points represent the means and error bars

485　represent the standard errors. Note that the observations without any larva were excluded from

486　the brood mass analysis.

487

488　Figure 4. Tissue protein and fat content (a–d) and larval growth (e and f) on lab and wild

489　carcasses as well as on wild mammal, bird, and reptile carcasses. Points represent the means

490　and error bars represent the standard errors. Letters denote significant difference with Tukey

491　multiplicity adjustment ($\alpha = 0.05$).

492

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

501   Table 2. A summary of the GLMM results for the breeding outcomes and carcass use efficiency

502   of *Nicrophorus nepalensis*. The pronotum widths of the parents and parent generation were

503   included as the covariates in all models.

Model response	n	Predictor		
		Carcass weight	Carcass source	Weight $\times$ 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§	$\chi^2_1 = 64.5$, $P < 0.001$	$\chi^2_1 = 0.003$, $P = 0.96$	$\chi^2_1 = 0.3$, $P = 0.57$

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

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

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

507   §Carcass use was not measured in the first and second round of the breeding experiments; observations with a

508   zero brood size were excluded from the analysis.

509