

## Heat stress effects on offspring compound across parental care

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## **Proceedings of the Royal Society B: Biological Sciences**

Accepted/In press: 03/02/2025

Peer reviewed version

Cyswllt i'r cyhoeddiad / Link to publication

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA): Malik, T., Tsai, M.-T., Jarrett, B., & Sun, S.-J. (in press). Heat stress effects on offspring compound across parental care. Proceedings of the Royal Society B: Biological Sciences.

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- 1 For submission to Proceedings of the Royal Society B
- Heat stress effects on offspring compound across parental care
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## 20 Abstract

- 21 Heatwaves associated with climate change threaten biodiversity by disrupting behaviours like
- 22 parental care. While parental care may buffer populations from adverse environments, studies
- 23 show mixed results, possibly due to heat stress affecting different care components. We
- 24 investigated how heat stress impacts parental care and offspring performance in the burying
- 25 beetle *Nicrophorus nepalensis* under control (17.8°C) and heat stress (21.8°C) conditions.
- 26 We focussed on two critical periods: pre-hatching care (carcass preparation) and post-
- 27 hatching care (offspring provisioning). To disentangle the vulnerability of these parental care
- 28 components to heat stress, we reciprocally transferred carcasses prepared under control or
- 29 heat stress to females breeding under both conditions. Heatwaves affecting only one care
- 30 period did not alter reproduction, but when both pre- and post-hatching periods experienced
- 31 heatwaves, reproductive success declined. Females exhibited higher energy expenditure
- during provisioning, evidenced by greater body mass loss. Notably, heat stress had long lasting effects on offspring via carcass preparation, resulting in smaller adult size and highe
- lasting effects on offspring via carcass preparation, resulting in smaller adult size and higher
   mortality. These results highlight the complexity of environmental stressors on parental care,
- 35 suggesting that different care components may respond differently to heat stress, and thus
- 36 need to be examined separately to better understand how parental care responds to, and
- 37 buffers against, temperature stress.
- 38 39

Keywords: climate change, plasticity, heat stress, reciprocal transplant, burying beetle,
 parental care

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## 46 Introduction

- 47 Global climate change poses significant threat to biodiversity, affecting ecosystems through
- 48 rising temperatures and more frequent extreme weather events (IPCC 2023; Scheffers *et al.*
- 49 2016). One of the most significant changes associated climate change is the increased
- 50 frequency and intensity of heat waves (Ma *et al.* 2021). Heat waves are defined as periods of
- 51 localised heat accumulation during consecutive days and nights of abnormally high
- 52 temperatures (Meehl & Tebaldi 2004). These heat waves can cause disruptions of life cycle
- 53 and physiological stress, especially for ectotherms like insects which are particularly
- 54 vulnerable to fluctuations in ambient temperature, directly impacting their physiology and
- 55 behaviour (Deutsch *et al.* 2008; Sánchez-Bayo & Wyckhuys 2019; Soroye *et al.* 2020).
- 56
- 57 The timing of heat waves can disproportionately impact species if it aligns with a particularly
- 58 sensitive period (Cinto Mejía & Wetzel 2023), like that of providing parental care (González-
- 59 Tokman et al. 2020; Weaving et al. 2022). For instance, exposure to extreme temperatures
- 60 during reproductive stages may impair key parental behaviours, leading to increased
- offspring mortality and reduced fitness (Cinto Mejía & Wetzel 2023; Zhang et al. 2015;
- 62 Zheng et al. 2017). Altricial offspring tend to be more vulnerable to environmental impacts,
- 63 needing aid in thermoregulation, especially considering nests cannot be moved to cooler
- 64 microhabitats as adults can (Pincebourde & Woods 2020). Parents can help buffer their
- 65 offspring from the negative effects of heat stress by plastically altering their suite of parental
- 66 care behaviours. Nest-building birds can alter the structure and composition of the nests they
- build to provide a more resilient thermal environment in which their offspring develop
- 68 (Mueller *et al.* 2019; Perez *et al.* 2020; Reid *et al.* 2000). After hatching, parents can also
- 69 increase the number of provisioning visits to mitigate heat stress (AlRashidi *et al.* 2011;
- 70 Bourne *et al.* 2021). In many cases, however, heat stress negatively impacts parental care
- behaviours, at a severe cost to the parents and to their offspring; parents may reduce their
- 12 levels of care to preserve and reallocate resources to their own survival (Wanless *et al.* 2023),
- and increased provisioning may not even prevent population decline (Wanless *et al.* 2023).
- 74

75 Parental care is comprised of a suite of behaviours that may or may not respond to heat stress

- in the same manner, or equally buffer offspring from the negative effects of heat stress to the
- same degree (Furness & Capellini 2019; Remeš et al. 2015). For example, nesting birds build
- a nest before any chick hatches, and can shape the nest according to local conditions, like
- temperature (Maziarz, 2019). But for species that exhibit less plasticity in the shape and form
- 80 of their nests, or use tree-holes (Maziarz *et al.* 2017), parents can only buffer offspring from
- 81 temperature with their interactions after hatching, which may mean the impact of heat stress
- 82 is greater in these species. Only by decomposing parental care into distinct components of
- 83 care can we obtain a greater mechanistic understanding of the impact of temperature on
- 84 parental care behaviours, to be able to predict which behaviours are most beneficial for
- 85 parental and offspring fitness, and to identify which behaviours could evolve to facilitate
- 86 persistence in a rapidly changing environment.
- 87
- 88 Here, we investigate the effects of elevated temperatures on two periods of parental care and
- 89 subsequent offspring performance in Nicrophorus nepalensis. The burying beetles,

90 *Nicrophorus* spp., exhibit elaborate parental care, including the preparation of carcasses as a 91 food source for their offspring (pre-hatching care), as well as direct feeding behaviour 92 provided to their young (post-hatching care; Potticary et al. 2024; Scott 1998). These behaviours are crucial for the development and survival of the young (Trumbo 2017), but are 93 94 potentially vulnerable to thermal stress. As ectotherms, burying beetles expend energy at a 95 rate determined by ambient temperature. Because parents and offspring feed from a shared 96 carcass resource, thermal stress may amplify conflict over care by increasing not only the 97 energetic demands (Angilletta 2009; Deutsch et al. 2008) of both parents and offspring, but 98 also the rate at which energy is expended on behaviours such as carcass maintenance, 99 provisioning, and begging. Additionally, thermal stress may impair parental coordination or timing of parental care behaviours, further affecting offspring development and survival 100 101 (Trumbo 2017). Previous studies have indicated that elevated temperatures can disrupt 102 parental care behaviours in burying beetles, leading to suboptimal carcass preparation and reduced reproductive success (Moss & Moore 2021). However, Grew et al. (2019) 103 104 demonstrated that parental effects were beneficial but were temperature dependent. It was 105 only under non-stressed conditions (15°C) that presence of female enhanced larval survival, 106 with no clear evidence of parental buffering at higher temperatures (20°C and 25°C) (Grew et 107 al. 2019). Collectively, these findings highlight consistent negative impacts of heat stress on 108 fitness, while also suggesting that the capacity of parents to mitigate these effects may vary 109 depending on the severity of thermal stress.

110

111 These results highlight that there is an urgent need to investigate how exactly climate change 112 influences parental care, and thus provide insight into the potential mechanisms by which 113 parents might buffer their offspring against rising and more extreme temperatures. We focus on how temperature-induced changes in carcass preparation by parents, along with direct 114 115 temperature effects, affect the growth and survival of their larvae. In the first experiment, we 116 evaluated the reproductive investment by exposing female beetles to control (17.8°C) and 117 heat stress (21.8°C) conditions during pre-hatching care. We investigated (i) whether heat 118 stress reduced the clutch size, (ii) whether heat stress makes carcass preparation more 119 energetically costly, and (iii) whether heat stress disrupts carcass preparation process. In the 120 follow-up experiment, we used a reciprocal transplant experiment that transferred carcass 121 prepared under either control or heat stress conditions to control-reared beetles during 122 breeding under both control and heat stress conditions (Fig 1). This design allows us to 123 investigate (i) the relative importance of carcass preparation and larval developmental 124 temperature on reproductive success, and (ii) whether parental care, partitioned as pre-125 hatching and/or post-hatching care, could mitigate the adverse impacts of heat stress. Our 126 study seeks to provide insights into the potential adaptive responses of burying beetles to 127 climate change and contribute to a broader understanding of how environmental stressors 128 impact parental care. 129

## 130 Materials and methods

## 131 Study system

- 132 *Nicrophorus nepalensis* is a subsocial beetle that exhibits biparental care, which is essential
- 133 for the development and survival of offspring (Malik *et al.* 2024). Parental care in this species

- 134 consists of two primary stages: pre-hatching care and post-hatching care. During pre-hatching
- 135 care, parents prepare a small vertebrate carcass as a resource for their offspring by removing
- 136 fur or feathers and shaping the carcass into a compact ball. This process reduces decay and
- 137 microbial activity prior to larval hatching (Pukowski 1933). In the post-hatching stage,
- parents actively feed and protect the larvae until they disperse (Potticary *et al.* 2024; Scott
- 139 1998). Under natural temperature conditions during the beetle's breeding season in northern
- 140 Taiwan, carcass preparation typically lasts 3-4 days, while larval development lasts over 7-8
- 141 days (Malik *et al.* 2024). The entire period of parental care thus lasts approximately 10-12
- 142 days under typical environmental conditions (Malik *et al.* 2024; Müller & Eggert 1990;
- 143 Trumbo 1990).
- 144
- 145 Heatwaves, characterized by short-term but significant increases in temperature, may
- 146 selectively disrupt specific stages of parental care, such as carcass preparation and/or larval
- 147 development. Field observations at our study sites revealed that the average duration of heat
- 148 stress events was 4.21 days (median: 4 days), and in some cases, the heat stress persisted for
- 149 10-13 days (see Supplementary Fig. 1). These durations overlap with the timing of critical
- 150 parental care activities. Thus, our experimental manipulation of heat stress in only one of the
- 151 parental care stages (see below) aligns well with the field conditions typically experienced by
- beetles, where heat stress events are defined (see supplementary information) as the days
- 153 when the daily mean temperature exceeds 21.8°C.
- 154

# 155 Establishment of *Nicrophorus nepalensis* colony in the laboratory

- *Nicrophorus nepalensis* beetles used in this experiment were descendants of a lab colony
  established in 2022. The source beetles were collected from six different locations in northern
  Taiwan (24.99°N, 121.62°E). Before breeding, all mites were removed from the beetles using
  fine tweezers. The beetles were housed individually in plastic individual boxes (10.8 x 7.5 x
  2.1 cm) filled with moist soil and were fed minced pork (~1.5 g) twice a week. Two weeks
  after acclimation to lab conditions, we bred pairs of male and female *N. nepalensis* collected
- 162 from different sites, provisioning them with 22-30 g of frozen mice (n = 60 pairs). We bred
- 163 the beetles in cylindrical breeding boxes ( $14.2 \times 6.3$  cm) filled with 2cm of moist soil. At
- 164 larval dispersal, we determined brood size by counting all third-instar larvae, which were
- 165 then transferred to eclosion boxes  $(2 \times 2 \times 2 \text{ cm})$  filled with moist soil and housed
- individually until reaching adulthood. The beetles were raised in controlled laboratory
   settings with a 10:14 light-to-dark cycle, 70% relative humidity, and a daily temperature
- regime that mimicked the variations typical of their breeding season from November to April
- in northern Taiwan (mean:  $17.8^{\circ}$ C, with daily variations between 16 and  $20^{\circ}$ C (Tsai *et al.*
- 170 2020)). Each year, field beetles were collected and bred into the lab colony to maintain
- 171 genetic diversity.
- 172

# 173 Effects of temperatures on pre-hatching care

- 174 The experiments were conducted in May-June 2024 using the laboratory maintained beetle
- 175 colonies. In *Nicrophorus* spp., both parents typically participate in the preparation of the
- 176 carcass and care of the offspring, yet the males often prioritize seeking additional mating
- 177 opportunities and so are likely to leave the females alone to take care of the broods (Scott

178 1998). We focused our experiments on females (because they can breed independently once

179 mated) to avoid any confounding effects from male participation since males could influence

180 the reproductive fitness by competing with females over carcass resources. To generate

- 181 mated females, we paired up 118 males and females haphazardly. Each pair was placed in an
- individual box filled with moist soil and kept in an incubator set to 17.8°C as describedabove.
- 184

185 The next day, the males from each pair were removed, and only the mated females were

186 transferred to breeding boxes filled with 2cm of moist soil and provisioned with 23–29 g

187 (25.82  $\pm$  1.55 g; Mean  $\pm$  SD) of frozen mice (weighed to the nearest 0.01 g). Prior to

introduction, we also weighed each female to the nearest 0.0001 g. These breeding setups
were then incubated under two different temperature regimes: control and warming. The

warming treatment temperature was elevated by  $4^{\circ}$ C (21.8°C) compared to the control

191 (17.8°C), representing a projected extreme temperature increase by the end of this century.

- 192 During breeding attempts, the boxes were kept in complete darkness to simulate underground
- 193 conditions (Sun *et al.* 2020). In total, there were 78 control and 40 warming treatment
- 194 breeding attempts.

195 Ninety hours later, we determined clutch size and carcass preparation for each brood. At this 196 stage, egg-laying was largely completed, and the carcass was fully prepared (i.e., fur removal

and rolling into a ball (Malik *et al.* 2024). This timeline was chosen based on our prior

- 198 observations, ensuring consistency in carcass preparation across different temperature
- 199 treatments despite potential accelerated degradation or egg development at higher
- 200 temperatures (Malik *et al.* 2024). We counted the number of eggs that were visible at the
- bottom of the container (De Gasperin & Kilner 2016; Sun *et al.* 2020). This approach allows for an accurate proxy for exact total egg production in *N. nepalensis* (Hsu *et al.* 2024). The
- for an accurate proxy for exact total egg production in *N. nepalensis* (Hsu *et al.* 2024). The prepared carcass and the female were carefully removed and weighed again. The change in
- beetle and carcass mass allowed us to estimate the extent of carrion consumption during the
- carcass preparation stage. To determine the extent of carcass preparation, we took photos of each prepared carcass using two identical cameras (TG7 – OM System). The carcasses were
- 207 photographed against a white background from both the side and the top, with each camera
- placed approximately 30 cm away from the carcass. We determined the roundness of carcass
   by taking the average values derived from the two images, using ImageJ (see (De Gasperin *et*
- by taking the average values derived from the two images, using imageJ (see (De Gasperin ei
- 210 *al.* 2016; Sun & Kilner 2020) for details).
- 211

# 212 Experimental manipulation of carcass preparation and developmental temperatures

213 To investigate the temperature effects of carcass preparation and larval development, we used

a reciprocal transplant experiment that transferred carcasses prepared at different

215 temperatures (hereafter carcass preparation temperature) for control-reared beetles to breed

- 216 upon at different temperatures (hereafter developmental temperature, i.e., the temperature
- 217 experienced by larvae post-hatching). The carcasses, prepared at either control or warming
- 218 temperatures from previous experiment, were placed inside new breeding boxes filled with
- 219 2cm of moist soil. These were set aside for later use in rearing experimental broods (see

- below). Since this study focuses on the effect of temperatures on carcass preparation and
- 221 larval development, females that prepared carcasses at warming temperatures were not used
- to avoid the carry-over effect of warming. Thus, we initially allowed extra females to prepare
- 223 carcasses in the control environment to ensure a sufficient number of females for the
- experiment. Each female was randomly assigned to one of the four experimental treatments.Importantly, females and carcasses from the same source were never assigned together. The
- treatments combined two factors: carcass preparation temperature (control or warming) and
- developmental temperature (control or warming). Hence, for example, carcasses prepared
- 228 under warming conditions could only be assigned to treatments where developmental
- temperature was either control or warming. Introducing females that had prepared carcasses
- and laid eggs under control conditions also ensured that the beetles were of similar
- 231 physiological conditions in the middle of reproduction prior to the transfer.
- 232 When egg started hatching at 96 hours, different broods of newly hatched larvae from 233 carcasses prepared at control conditions were pooled in a Petri dish, fifteen larvae were 234 haphazardly selected and transferred to their designated experimental treatments using fine 235 tweezers. We chose 15 individuals because this number falls within the brood range exhibited 236 in our lab and field individuals when bred in the lab (ranging between 1 and 34,  $14.7 \pm 9.6$ , n 237 = 60). This pool of unrelated larvae reduces potential confounding maternal effects such as 238 sib-sib genetic influences and ongoing maternal care provided to the same genetic offspring, 239 although it does not remove all maternal effects (e.g., maternal mRNAs that are potentially 240 already present in eggs) (Jarrett et al. 2018). The larvae were haphazardly assigned to one of the four experimental treatments, depending on the combination of carcass preparation 241 242 temperature (C) and developmental temperature (D), with each treatment having control 243 (control) and warming (warm) conditions:  $C_{control} - D_{control}$  (n = 21),  $C_{warm} - D_{control}$  (n = 17), 244  $C_{control} - D_{warm}$  (n = 20), and  $C_{warm} - D_{warm}$  (n = 20) (Fig. 1). There is no evidence that burying 245 beetle parents can recognise kin but rather, acceptance is contingent on larvae arriving within 246 the expected time window from oviposition (Müller & Eggert 1990). The larvae used in the 247 experiment were time-matched with the expected hatch date of the focal female's eggs to 248 ensure acceptance by the females and to standardize developmental timing across all 249 treatments. The larvae were placed gently on each carcass simultaneously. The boxes were 250 then returned to their designated developmental temperatures. We checked the development 251 of larvae daily from day 8 and recorded the day of larval dispersal into the nearby soil. At this 252 stage, the entire brood was counted and weighed to the nearest 0.001 g using an analytical 253 balance (Shimadzu, Model: ATX224R). Subsequently, all females were removed from the 254 boxes, their body mass measured again, and their size was estimated by measuring the 255 pronotum width (to the nearest 0.01 mm) with a vernier caliper. The change in body mass has 256 been used widely as an indicator of the cost associated with parental investment (Ma et al. 257 2022). Following these measurements, the dispersing larvae were individually placed and 258 incubated in eclosion boxes under control conditions. Upon eclosion, we determined if, and 259 when, the offspring successfully eclosed as new adult, as evidenced by the complete transition of elytra colour from brown to black. Unsuccessful ones would die as larvae, 260 pupae, or even adults. For the successfully eclosed beetles, we determined their body size by 261 262 measuring the pronotum width to the nearest 0.01 mm.

263

## 264 Statistical analysis

265 All statistical analyses were performed in R version 4.1.2, with figures generated using the package 'ggplot2' (Wickham 2016). Generalised linear mixed models (GLMMs) were 266 267 performed with the *glmer* function in the 'lme4' package (Bates et al. 2015). We checked for 268 normal distribution of model residuals using the 'DHARMa' package (Hartig 2022). This required us to use negative binomial distribution for analysing clutch size, and to log 269 270 transform brood mass prior to analysis since our initial diagnostics indicated that the data was 271 overdispersed, with variance exceeding the mean, which violated the model assumptions. For 272 all models, we reported likelihood ratios for the main effect and interaction between carcass 273 preparation temperature and developmental temperature using the Anova function in the 'car' 274 package (Fox *et al.* 2021). Once an interaction was found statistically significant (P < 0.05), 275 we conducted post-hoc pairwise comparisons using the *emmeans* function in the 'emmeans' 276 package (Lenth 2021).

277

278 To examine the effect of temperatures on parental investment during pre-hatching care, we

used GLMMs to analyse clutch size, carcass mass change, proportional body mass change,and the roundness of prepared carcasses. In these analyses, we included temperature

treatment (control/warming) as an explanatory variable. We also included the initial carcass

282 mass (i.e., prior to breeding) and female body size as covariates, since these variables have

been found to determine parental investment (Hopwood et al. 2016; Sun et al. 2019). Finally,

we included the family origin of the female that prepared the carcass as a random effect. For each temperature treatment, we used between one and nine individuals per family,

286 haphazardly selected from 25 different families.

287

288 To examine the reproductive performance of burying beetles, we used GLMMs to analyse 289 brood size, brood mass, averaged larval mass, and the proportion of body mass change. The 290 brood size was analysed with a poisson distribution, whereas the rest of dependent variables 291 were analysed with a gaussian distribution. We included carcass preparation temperature and 292 developmental temperature and their interaction as explanatory variables. We also included 293 carcass mass and roundness as additional fixed effects to account for potential effects due to 294 variation in the allocated carcass, and the family origin of the female that provided care as a 295 random effect.

296

297 To examine the fitness consequences on beetle offspring, we used GLMMs to analyse the 298 proportion of eclosion success, days to eclosion, and body size of individual offspring when 299 they became adults. We analysed the proportion of eclosion success with a binomial distribution (logit link), and days to eclosion and body size with a gaussian distribution. For 300 301 these analyses, we began by including carcass preparation temperature and developmental 302 temperature and their interaction as explanatory variables. For the analysis of days to 303 eclosion, we also considered the sex and body size as covariates; for the analysis of body 304 size, we also considered the sex. Since multiple individuals could come from the same brood, 305 we included brood ID as a random effect. 306

## 307 Results

- 308 Effects of elevated temperatures on pre-hatching parental care
- 309 To disentangle the effects of heat stress on reproduction when exposure occurs at distinct
- 310 stages of parental care, we exposed breeding females of *N. nepalensis* to contrasting control
- and warming conditions during the pre- and post-hatching stages and evaluated the resulting
- 312 reproductive outcomes. As carcass preparation and egg-laying were completed, we found
- 313 significant disruptions at the higher temperature (Fig. 2). Beetles produced smaller clutch
- sizes at warming compared to control conditions ( $\chi^2 = 5.42$ , d.f. = 1, P = 0.020; Fig. 2a), with
- none of the eggs hatched under warming conditions, consistent with our previous finding
- 316 (Malik *et al.* 2024). Additionally, during carcass preparation, beetles consumed more of the
- 317 carcass under warming compared to control conditions ( $\chi^2 = 4.22$ , d.f. = 1, P = 0.040; Fig. 318 2b). This increased consumption resulted in a reduction in carcass weight in the warming
- 2b). This increased consumption resulted in a reduction in carcass weight in the warming treatment, but did not lead to an increase in beetle body mass ( $\chi^2 = 1.16$ , d.f. = 1, P = 0.282;
- Fig. 2c). The carcasses prepared under warming were also of lower quality, being less well-
- 321 prepared than those under control conditions ( $\chi^2 = 3.89$ , d.f. = 1, P = 0.049; Fig. 2d).
- 322

# 323 Effects of carcass preparation temperature and developmental temperature on

## 324 reproduction

To investigate whether maternal behavioural adjustments during high-temperature carcass preparation buffer subsequent thermal effects on larval development, we experimentally transferred carcasses prepared by beetles under either warming or control conditions and allowed other beetles to breed upon them at these temperatures (see Methods). Each female received an experimental brood of 15 newly-hatched larvae from the control condition, which were reared until larval dispersal.

331

332 We found that carcass preparation temperature and developmental temperature interacted to affect brood size (carcass preparation temperature x developmental temperature:  $\chi^2 = 4.06$ , 333 d.f. = 1, P = 0.044, Fig. 3a). While beetles assigned control and warmed carcasses produced 334 335 similar brood size at control temperatures (post-hoc comparison control v. warmed carcass, estimate = -0.07, SE = 0.10, z = -0.69, P = 0.494), beetles assigned warmed carcasses 336 337 produced fewer larvae than beetles assigned control carcasses at warming temperatures (post*hoc* comparison control v. warmed carcass, estimate = 0.21, SE = 0.10, z = 2.10, P = 0.036); 338 the effect of warming at both carcass preparation and during offspring development 339

- 340 compounded to reduce brood size.
- 341

342 Similarly, brood mass was affected by the interaction between carcass preparation

- 343 temperature and development temperature (carcass preparation temperature x developmental
- temperature:  $\chi^2 = 4.16$ , d.f. = 1, P = 0.041, Fig. 3b). At control temperatures, beetles had
- 345 similar brood mass regardless of carcass preparation temperature (*post-hoc* comparison
- 346 control v. warmed carcass, estimate = -0.05, SE = 0.097, t = -0.53, P = 0.601). However, at
- 347 warming temperatures, beetles had lower brood mass when assigned warmed carcasses (post-
- 348 *hoc* comparison control v. warmed carcass, estimate = 0.22, SE = 0.099, t = 2.27, P = 0.027),
- 349 which follows given the reduction in brood size (Fig. 3a)
- 350

- 351 Higher developmental temperature alone, not carcass preparation temperature, resulted in
- lower averaged larval mass ( $\chi^2 = 36.69$ , d.f. = 1, P < 0.001; Fig. 3c). Providing parental care 352
- proved costly as beetles lost body mass after breeding, with proportionally greater losses 353 when rearing young at warming temperatures compared to control temperatures ( $\chi^2 = 16.39$ , 354
- d.f. = 1, P < 0.001). Carcass preparation temperature did not significantly affect the 355 proportional body mass change ( $\chi^2 = 1.66$ , d.f. = 1, P = 0.198; Fig. 3d).
- 356
- 357

#### 358 Effects of carcass preparation temperature and developmental temperature on 359 offspring at adulthood

To investigate the cascading effect of carcass preparation temperature and developmental 360 temperature on offspring, we determined the proportion of larvae that successfully eclosed as 361 362 adults, time to eclosion, and their body size.

- 363
- 364 We found that carcass preparation temperature, but not developmental temperature,
- determined the proportion of eclosion success ( $\gamma^2 = 3.85$ , d.f. = 1, P = 0.050), with 365
- proportionally fewer larvae successfully eclosed when feeding upon warmed carcasses (Fig. 366
- 367 4a). Of all larvae that survived to adulthood, the duration from larval dispersal to eclosion
- 368 was unaffected by carcass preparation and developmental temperature (Fig. 4b). However,
- 369 larvae feeding on warmed carcasses eclosed as smaller adults compared to those feeding on
- 370 control carcasses (Fig. 4c).
- 371

#### 372 Discussion

373 Elevated temperatures associated with climate change are posing significant challenges to 374 organisms worldwide. While rapid behavioural responses to climate change through 375 individual plasticity are now widely accepted (Charmantier et al. 2008), the impact of higher 376 temperatures on the effectiveness of parental care is increasingly studied and remains 377 equivocal. This is potentially due to a lack of understanding of which aspects of parental care 378 are most fragile to high temperatures. To address this issue, we used the burying beetle 379 Nicrophorus nepalensis to mechanistically investigate the impact of heatwaves on two time periods of parental care. We found that when heatwaves only affected one aspect of parental 380 381 care (pre- or post-hatching periods of care), reproductive success was equal to that of the 382 control treatment. But when a heatwave affected both periods of care, reproductive success was lower. It is important to note that these findings are applicable only to larvae that develop 383 384 from eggs to larvae under control temperatures. These results emphasize that the combined contributions of pre- and post-hatching periods of care are equally important at buffering 385 offspring against heatwaves, highlighting the vulnerability of reproductive success to 386

- 387 cumulative thermal stress.
- 388

389 Our results showed – consistent with many other studies – that temperature increase led to 390 reduction of reproductive investment, likely because of a greater energetic demand and/or the 391 need for more intensive care efforts to maintain suitable conditions for offspring. Consistent

- 392 with data from the congeneric N. orbicollis, we show that female body mass increased when
- 393 preparing a carcass, and decreased after caring for offspring (Trumbo & Xhihani 2015). We
- 394 note that females that provided post-hatching care all prepared carcasses in the control

395 environment, eliminating potential carryover effects of heat stress during carcass preparation. 396 This absence of pre-hatching heat stress likely reduced the costs females may have incurred during post-hatching parental care. Nonetheless, our results demonstrate that this trend is 397 exacerbated by heat stress. Specifically, female mass loss was more pronounced when they 398 399 reared the offspring in the warming conditions than the control, despite consuming more of 400 the carcass under warming (Fig. 3d). This indicates that the heightened metabolic demands 401 induced by heat stress may have offset the nutritional benefits of increased carcass 402 consumption. Elevated temperatures likely increase energy expenditures through enhanced physiological and thermoregulatory processes, leaving fewer resources available for body 403 404 mass accumulation. These increased energy demands may deplete the resources available for 405 current parental care and future reproductive investment, ultimately risking the success of subsequent reproductive bouts. Although we did not directly measure parental care in this 406 407 study, previously study on *N. orbicollis* found no support that warming by 4°C induced greater parental care from the caring females, indicating that females had already maximised 408 409 their time in care (Moss & Moore 2021).

410

411 Given the challenges posed by heat stress, should we conclude that parents have limited

412 ability to help buffer the adverse thermal environments of the offspring? Our carcass

413 reciprocal transplant experiment suggests that parents can partially rescue offspring from the

414 detrimental impact of heat during the two periods of parental care we manipulated. In fact,

415 we showed that females provided with warm carcasses to breed under control conditions and 416 females provided with control carcasses to breed under warming conditions were both able to

- 416 females provided with control carcasses to breed under warming conditions were both able 417 produce similar brood size and mass, compared to the control carcass at control conditions.
- 418 However, females provided with warm carcasses were unable to effectively rear offspring
- 419 under heat stress, and as such had significantly smaller broods (Fig. 3a). Together, these
- 420 findings highlight both pre-hatching care (e.g., carcass nest preparation) and post-hatching
- 421 care (e.g., offspring provisioning and carcass maintenance (Scott 1998)) can partially mitigate
- 422 against heat stress, but also the limitation of parental care in buffering offspring against

423 longer-term heat stress spanning throughout the breeding event.

424

425 Different aspects of parental care may be able to evolve independently in response to a 426 change in temperature (Jarrett et al. 2024, Walling et al. 2008). Our study has started to disentangle which parental care behaviours may be affected most by rising temperatures in 427 428 the hope we can predict which behaviours may play a part in adaptation to climate change. 429 Carcass preparation in burying beetles forms an essential part of pre-hatching care, which is 430 critical for the development of their young, since it provides both a food source and a nest. 431 Our findings showed that preparing carcasses under heat stress can be more costly than under 432 control conditions, and that the quality of carcass preparation suffers as a result. The 433 suboptimal carcass conditions under heat stress can lead to reduced nutritional quality and 434 increased exposure to competing microbes (Stiegler et al. 2020), negatively impacting offspring development and survival (Fig. 4a,b). Indeed, the effects of warming in our study 435 were not limited to direct effects on larval development and survival within the experimental 436 437 period, but extended even further until the offspring eclosed to adulthood. Specifically, larvae 438 that developed on warmed carcasses, but not warming environments, struggled to

439 successfully eclose (Fig. 4a); and even if they survived, they eclosed as smaller adults (Fig.

- 440 4c), which can subsequently reduce their fitness advantages during future breeding attempt.
- 441 Other possible mechanisms of adaptive behavioural plasticity, such as carcass burial, may
- 442 also help buffer offspring against thermal stress particularly under climate warming. By
- burying carcasses deeper, the parents provide a cooler and more stable environment for the
- larvae to develop, compared to those bury carcasses closer to the soil surface. This adaptive
  behavioural plasticity has been found in dung beetles (Macagno *et al.* 2018), and given its
- similarity with burying beetles in burial behaviour, future studies may determine whether
- 447 parental care in burying beetles also allow for such adaptive response to temperature
- variations. Thus, we highlight the need to tease apart different components of parental careand their separate roles in shaping short- and long-term fitness consequences to better
- understand how species that care for their offspring will respond to a rapidly changingclimate.
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- 453

## 454 **Conflict of interest statement**

- 455 The authors declare no conflict of interest.
- 456

# 457 Acknowledgement

- 458 We would like to thank members of the Sun Lab, particularly Wei-Jiun Lin, Yi-Ta Wu, Yu-459 Shieng Huang, Brendan Lan, Bright Hubert Mbuya, and Chen-Ju Wang for helping field
- 459 Shieng Huang, Brendan Lan, Bright Hubert Mouya, and Chen-Ju wang for helping field 460 survey; we thank Shu-Ping Tseng for the loan of a camera (OM System); we also thank
- 461 Megan M.Y. Chang for help making Figure 1.
- 462

# 463 **Data accessibility statement**

- 464 Data and relevant code for this work are stored in Zenodo: 10.5281/zenodo.13748681
- 465466 Author contributions
- 467 Conceptualisation: BJMJ, SJS; Data Collection: TGM, MTT, SJS; Analysis & Visualisation:
- 468 all authors; Writing-Original Draft: all authors; Writing-Review & Editing: all authors;
- 469 Resources & Funding: SJS
- 470

# 471 **Funding**

- 472 S.-J.S. was supported by NTU New Faculty Founding Research Grant, National Science and
- 473 Technology Council 2030 Cross-Generation Young Scholars Program (112-2628-B-002-
- 474 013-; 113-2628-B-002-028-), and Yushan Fellow Program provided by the Ministry of
- 475 Education (MOE-108- YSFMS-0004-012-P1). B.J.M.J was supported by a Human Frontiers
- 476 Science Program fellowship (LT000879/2020).
- 477
- 478

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- 611 612

## 625 Figures



# Carcass preparation temperature (control or warming)

## 626

627 Figure 1. Experimental set-up of the reciprocal transplant experiment testing the joint and

628 independent effects of temperatures (blue: control; red: warming) associated with carcass

629 preparation and larval development. Females that had prepared carcasses at control

temperatures were provided with either a carcass prepared by another beetle at control

631 temperatures or warming temperatures. Fifteen newly-hatched 1<sup>st</sup> larvae collected

haphazardly from different families were introduced as a group of genetic mix.



Temperature treatmentTemperature treatment635Figure 2. Effects of temperature on investment in pre-hatching parental care. Temperature636effects on (a) clutch size, (b) carcass mass change, (c) proportional body mass change of637females, and (d) roundness of prepared carcass. Mean  $\pm$  SE are shown. Points are individual638broods (n = 73 and 37 for control and warming conditions, respectively).



643

Figure 3. Effects of carcass preparation temperature and developmental temperature on 644 645 reproductive performance. The reproductive success is measured as (a) brood size (b) brood 646 mass, which determines the larval quality as (c) averaged larval mass. (d) Proportional body 647 mass change is recorded as the proportional change in body mass of females from the

648 completion of carcass preparation to larval dispersal. Mean  $\pm$  SE are shown. Points are

649 individual broods (n = 21, 17, 20, and 20 for C<sub>control</sub> – D<sub>control</sub>, C<sub>warm</sub> – D<sub>control</sub>, C<sub>control</sub> – D<sub>warm</sub>, 650 and C<sub>warm</sub> – D<sub>warm</sub>, respectively).



Figure 4. Effects of carcass preparation temperature and developmental temperature on the

655 fitness consequences of offspring. The fitness consequences are measured as (a) the

- proportion of eclosion success, (b) days to eclosion, and (c) body size as adults. Mean  $\pm$  SE
- are shown. For visualisation purposes, data points are grouped by individual beetles from the
- same brood.
- 659

- 660 Supplementary information
- 661

## 662

## 663 Additional Methods and Results

## 664 Ambient temperature variations in natural habitats

665 We evaluated the variations of daily ambient temperature in our field trapping sites in Taipei and New Taipei City, northern Taiwan. We used hanging traps, each baited with a fresh 666 667 mouse carcass, to sample field Nicrophorus nepalensis activity alongside the ambient temperature at which the beetles were active during breeding season. The trapping was 668 conducted once per month, starting from October 2023 to May 2024. A total of 20 sites were 669 selected, with each site having three sets of traps spaced 150 m apart to avoid interference. In 670 the geographic centre of each site we also set up a temperature data logger (TOMST), which 671 672 continuously recorded ambient temperature at soil surface every 15 min. Upon capture, we 673 noted the presence of *N. nepalensis* per trap, collected all *N. nepalensis* to breed into the lab

- 674 colony, and replenished the trap the next month.
- 675

By recording the microclimate of natural habitats of burying beetles, we found that the daily

677 mean temperature ranged from 7.28 to 26.32°C, with an average of  $18.33 \pm 3.81$ °C (in line

678 with our use of 17.8°C control temperature). During the breeding season, the averaged

duration of heat stress lasted 4.21 days, with a median of 4 days.

680



681

Supplementary Figure 1. Heat stress frequency and distribution in natural habitats of burying beetle *N. nepalensis*. (a) Fluctuations of daily mean temperature from October 2023 to April 2024, a typical breeding season for beetles in Taiwan. The red and blue dashed lines indicate 21.8°C and 17.8°C, representing the control and heat stress conditions, respectively, used in this study. (b) Frequency of heat stress events with their length in days (heat stress event is defined as the days when the daily mean temperature exceeds 21.8°C). The dashed line indicates the median.