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1 *For submission to Proceedings of the Royal Society B*

2 **Heat stress effects on offspring compound across parental care**

3

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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
32 during provisioning, evidenced by greater body mass loss. Notably, heat stress had long-
33 lasting effects on offspring via carcass preparation, resulting in smaller adult size and higher
34 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

40 **Keywords:** climate change, plasticity, heat stress, reciprocal transplant, burying beetle,
41 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
61 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
67 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
71 behaviours, at a severe cost to the parents and to their offspring; parents may reduce their
72 levels of care to preserve and reallocate resources to their own survival (Wanless *et al.* 2023),
73 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
76 in the same manner, or equally buffer offspring from the negative effects of heat stress to the
77 same degree (Furness & Capellini 2019; Remeš *et al.* 2015). For example, nesting birds build
78 a nest before any chick hatches, and can shape the nest according to local conditions, like
79 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
93 behaviours are crucial for the development and survival of the young (Trumbo 2017), but are
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
100 timing of parental care behaviours, further affecting offspring development and survival
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
103 reduced reproductive success (Moss & Moore 2021). However, Grew *et al.* (2019)
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 al.*
107 *et 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
114 on how temperature-induced changes in carcass preparation by parents, along with direct
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,
138 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
152 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**

156 *Nicrophorus nepalensis* beetles used in this experiment were descendants of a lab colony
157 established in 2022. The source beetles were collected from six different locations in northern
158 Taiwan (24.99°N, 121.62°E). Before breeding, all mites were removed from the beetles using
159 fine tweezers. The beetles were housed individually in plastic individual boxes (10.8 x 7.5 x
160 2.1 cm) filled with moist soil and were fed minced pork (~1.5 g) twice a week. Two weeks
161 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 × 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 x 2 x 2 cm) filled with moist soil and housed
166 individually until reaching adulthood. The beetles were raised in controlled laboratory
167 settings with a 10:14 light-to-dark cycle, 70% relative humidity, and a daily temperature
168 regime that mimicked the variations typical of their breeding season from November to April
169 in northern Taiwan (mean: 17.8°C, with daily variations between 16 and 20°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
182 individual box filled with moist soil and kept in an incubator set to 17.8°C as described
183 above.

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 ± 1.55 g; Mean \pm SD) of frozen mice (weighed to the nearest 0.01 g). Prior to
188 introduction, we also weighed each female to the nearest 0.0001 g. These breeding setups
189 were then incubated under two different temperature regimes: control and warming. The
190 warming treatment temperature was elevated by 4°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
197 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
201 bottom of the container (De Gasperin & Kilner 2016; Sun *et al.* 2020). This approach allows
202 for an accurate proxy for exact total egg production in *N. nepalensis* (Hsu *et al.* 2024). The
203 prepared carcass and the female were carefully removed and weighed again. The change in
204 beetle and carcass mass allowed us to estimate the extent of carrion consumption during the
205 carcass preparation stage. To determine the extent of carcass preparation, we took photos of
206 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
208 placed approximately 30 cm away from the carcass. We determined the roundness of carcass
209 by taking the average values derived from the two images, using ImageJ (see (De Gasperin *et*
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
214 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

220 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
222 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
224 experiment. Each female was randomly assigned to one of the four experimental treatments.
225 Importantly, females and carcasses from the same source were never assigned together. The
226 treatments combined two factors: carcass preparation temperature (control or warming) and
227 developmental temperature (control or warming). Hence, for example, carcasses prepared
228 under warming conditions could only be assigned to treatments where developmental
229 temperature was either control or warming. Introducing females that had prepared carcasses
230 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 ± 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
241 the four experimental treatments, depending on the combination of carcass preparation
242 temperature (C) and developmental temperature (D), with each treatment having control
243 (control) and warming (warm) conditions: $C_{\text{control}} - D_{\text{control}}$ ($n = 21$), $C_{\text{warm}} - D_{\text{control}}$ ($n = 17$),
244 $C_{\text{control}} - D_{\text{warm}}$ ($n = 20$), and $C_{\text{warm}} - D_{\text{warm}}$ ($n = 20$) (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
260 transition of elytra colour from brown to black. Unsuccessful ones would die as larvae,
261 pupae, or even adults. For the successfully eclosed beetles, we determined their body size by
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
266 package ‘ggplot2’ (Wickham 2016). Generalised linear mixed models (GLMMs) were
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
269 required us to use negative binomial distribution for analysing clutch size, and to log
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
279 used GLMMs to analyse clutch size, carcass mass change, proportional body mass change,
280 and the roundness of prepared carcasses. In these analyses, we included temperature
281 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
283 been found to determine parental investment (Hopwood *et al.* 2016; Sun *et al.* 2019). Finally,
284 we included the family origin of the female that prepared the carcass as a random effect. For
285 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
300 distribution (logit link), and days to eclosion and body size with a gaussian distribution. For
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
311 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
314 sizes at warming compared to control conditions ($\chi^2 = 5.42$, d.f. = 1, $P = 0.020$; Fig. 2a), with
315 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
319 treatment, but did not lead to an increase in beetle body mass ($\chi^2 = 1.16$, d.f. = 1, $P = 0.282$;
320 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**

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

331

332 We found that carcass preparation temperature and developmental temperature interacted to
333 affect brood size (carcass preparation temperature x developmental temperature: $\chi^2 = 4.06$,
334 d.f. = 1, $P = 0.044$, Fig. 3a). While beetles assigned control and warmed carcasses produced
335 similar brood size at control temperatures (*post-hoc* comparison control v. warmed carcass,
336 estimate = -0.07, SE = 0.10, $z = -0.69$, $P = 0.494$), beetles assigned warmed carcasses
337 produced fewer larvae than beetles assigned control carcasses at warming temperatures (*post-*
338 *hoc* comparison control v. warmed carcass, estimate = 0.21, SE = 0.10, $z = 2.10$, $P = 0.036$);
339 the effect of warming at both carcass preparation and during offspring development
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
344 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
352 lower averaged larval mass ($\chi^2 = 36.69$, d.f. = 1, $P < 0.001$; Fig. 3c). Providing parental care
353 proved costly as beetles lost body mass after breeding, with proportionally greater losses
354 when rearing young at warming temperatures compared to control temperatures ($\chi^2 = 16.39$,
355 d.f. = 1, $P < 0.001$). Carcass preparation temperature did not significantly affect the
356 proportional body mass change ($\chi^2 = 1.66$, d.f. = 1, $P = 0.198$; Fig. 3d).

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

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

363
364 We found that carcass preparation temperature, but not developmental temperature,
365 determined the proportion of eclosion success ($\chi^2 = 3.85$, d.f. = 1, $P = 0.050$), with
366 proportionally fewer larvae successfully eclosed when feeding upon warmed carcasses (Fig.
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).

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
380 periods of parental care. We found that when heatwaves only affected one aspect of parental
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
383 was lower. It is important to note that these findings are applicable only to larvae that develop
384 from eggs to larvae under control temperatures. These results emphasize that the combined
385 contributions of pre- and post-hatching periods of care are equally important at buffering
386 offspring against heatwaves, highlighting the vulnerability of reproductive success to
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 & Xhiani 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
397 during post-hatching parental care. Nonetheless, our results demonstrate that this trend is
398 exacerbated by heat stress. Specifically, female mass loss was more pronounced when they
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
403 physiological and thermoregulatory processes, leaving fewer resources available for body
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
406 subsequent reproductive bouts. Although we did not directly measure parental care in this
407 study, previously study on *N. orbicollis* found no support that warming by 4°C induced
408 greater parental care from the caring females, indicating that females had already maximised
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
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
427 disentangle which parental care behaviours may be affected most by rising temperatures in
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
435 offspring development and survival (Fig. 4a,b). Indeed, the effects of warming in our study
436 were not limited to direct effects on larval development and survival within the experimental
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
443 burying carcasses deeper, the parents provide a cooler and more stable environment for the
444 larvae to develop, compared to those bury carcasses closer to the soil surface. This adaptive
445 behavioural plasticity has been found in dung beetles (Macagno *et al.* 2018), and given its
446 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
448 variations. Thus, we highlight the need to tease apart different components of parental care
449 and their separate roles in shaping short- and long-term fitness consequences to better
450 understand how species that care for their offspring will respond to a rapidly changing
451 climate.

452

453

454 **Conflict of interest statement**

455 The authors declare no conflict of interest.

456

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462

463 **Data accessibility statement**

464 Data and relevant code for this work are stored in Zenodo: [10.5281/zenodo.13748681](https://zenodo.org/record/13748681)

465

466 **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

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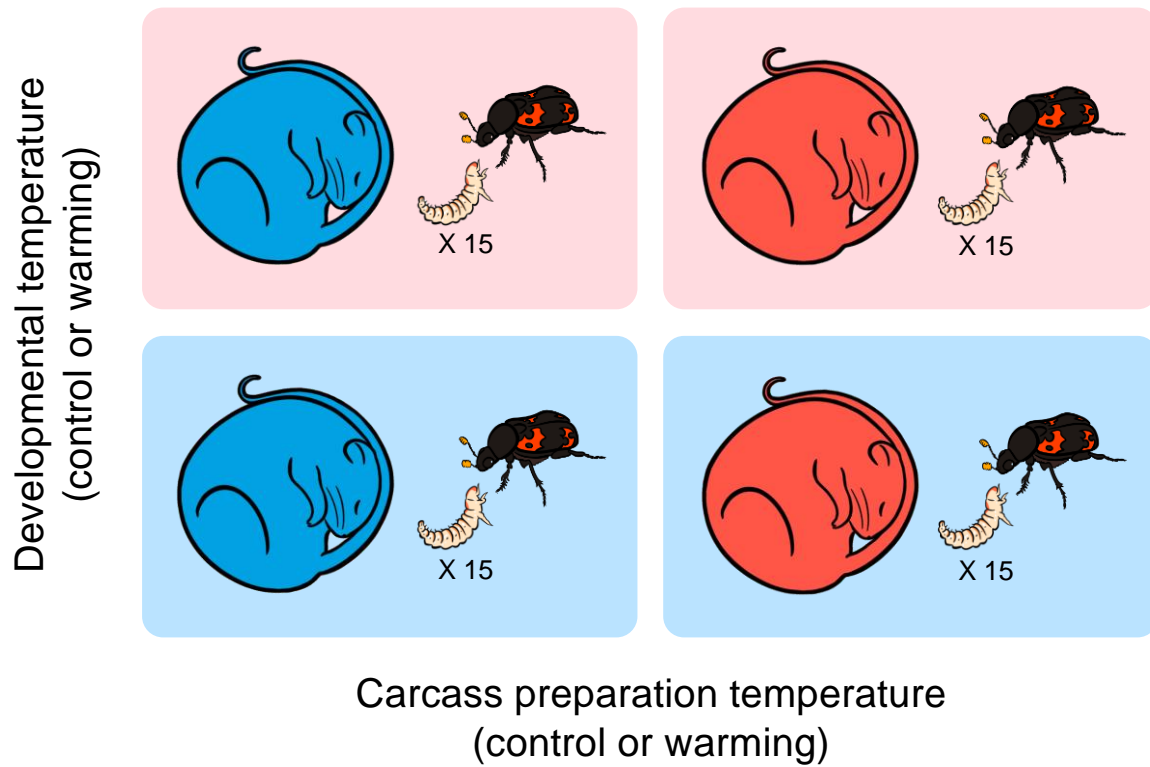
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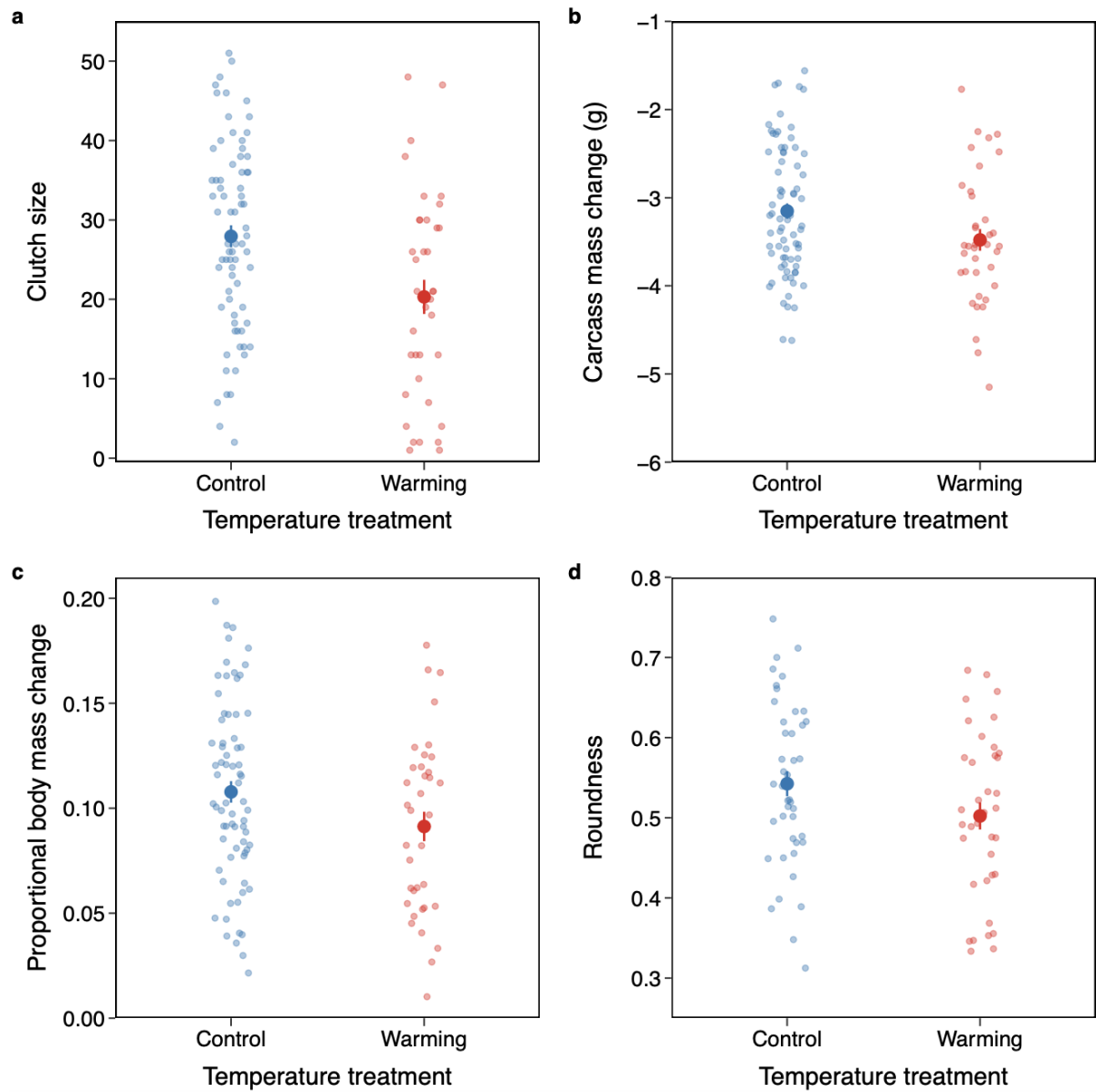
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627 Figure 1. Experimental set-up of the reciprocal transplant experiment testing the joint and
628 independent effects of temperatures (blue: control; red: warming) associated with carcass
629 preparation and larval development. Females that had prepared carcasses at control
630 temperatures were provided with either a carcass prepared by another beetle at control
631 temperatures or warming temperatures. Fifteen newly-hatched 1st larvae collected
632 haphazardly from different families were introduced as a group of genetic mix.
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635 Figure 2. Effects of temperature on investment in pre-hatching parental care. Temperature

636 effects on (a) clutch size, (b) carcass mass change, (c) proportional body mass change of

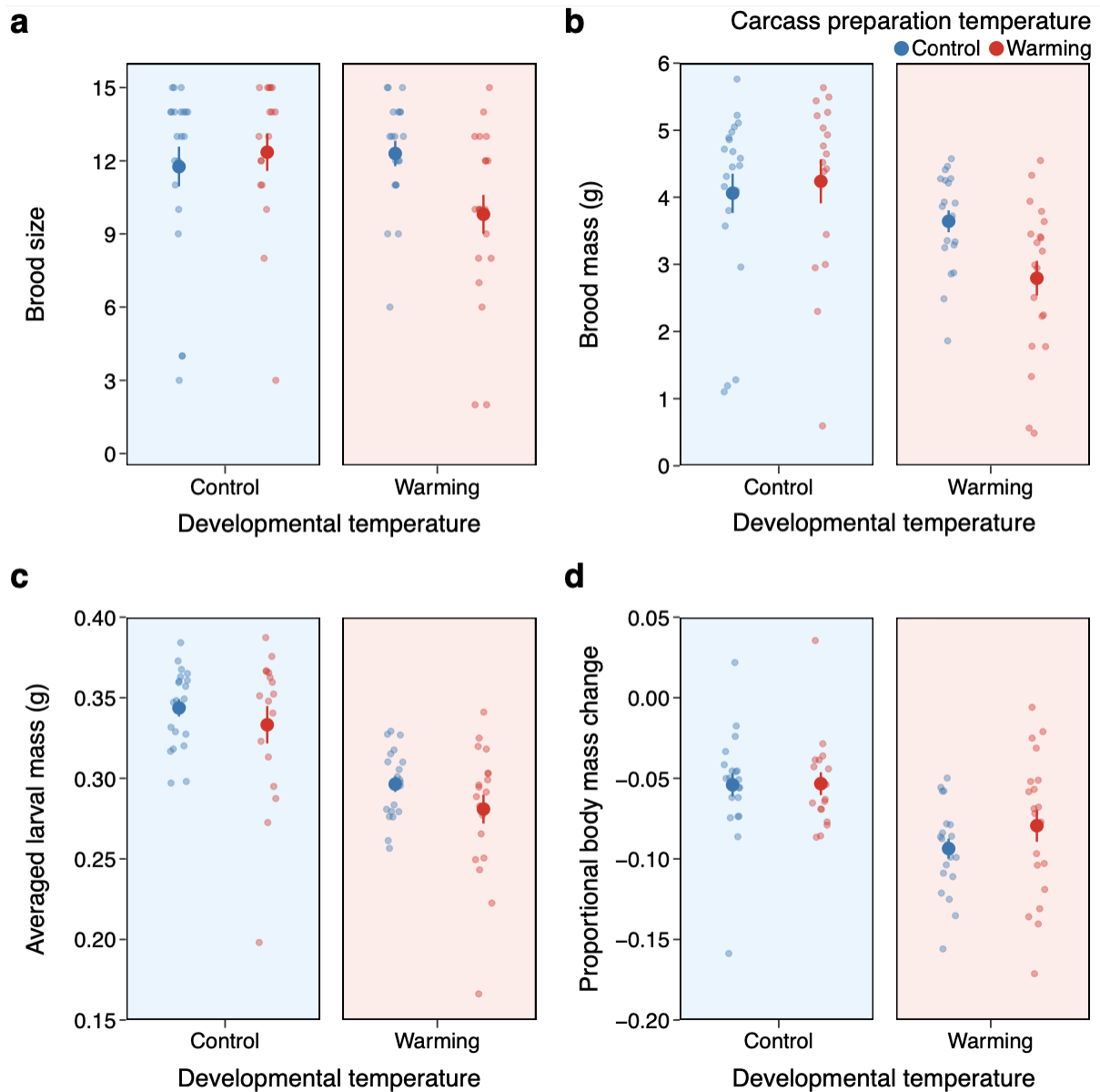
637 females, and (d) roundness of prepared carcass. Mean \pm SE are shown. Points are individual

638 broods ($n = 73$ and 37 for control and warming conditions, respectively).

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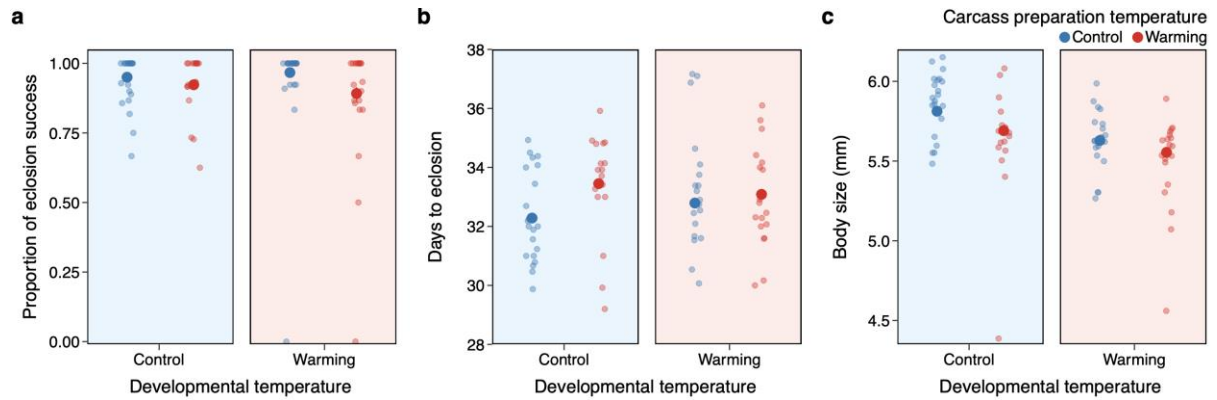
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Figure 3. Effects of carcass preparation temperature and developmental temperature on reproductive performance. The reproductive success is measured as (a) brood size (b) brood mass, which determines the larval quality as (c) averaged larval mass. (d) Proportional body mass change is recorded as the proportional change in body mass of females from the completion of carcass preparation to larval dispersal. Mean \pm SE are shown. Points are individual broods ($n = 21, 17, 20,$ and 20 for $C_{\text{control}} - D_{\text{control}}, C_{\text{warm}} - D_{\text{control}}, C_{\text{control}} - D_{\text{warm}},$ and $C_{\text{warm}} - D_{\text{warm}},$ respectively).

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

660 **Supplementary information**

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663 **Additional Methods and Results**

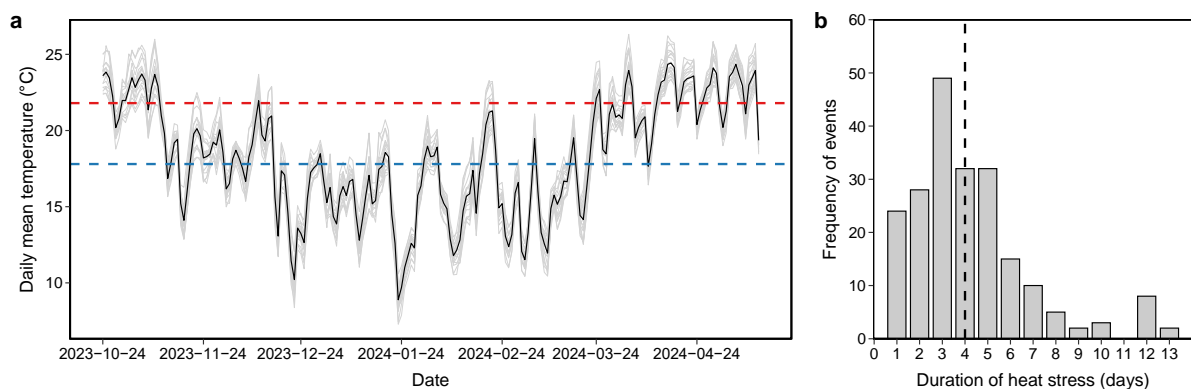
664 *Ambient temperature variations in natural habitats*

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

676 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 $18.33 \pm 3.81^\circ\text{C}$ (in line
678 with our use of 17.8°C control temperature). During the breeding season, the averaged
679 duration of heat stress lasted 4.21 days, with a median of 4 days.

680



681

682 **Supplementary Figure 1. Heat stress frequency and distribution in natural habitats of burying**
683 **beetle *N. nepalensis*.** (a) Fluctuations of daily mean temperature from October 2023 to April
684 2024, a typical breeding season for beetles in Taiwan. The red and blue dashed lines indicate
685 21.8°C and 17.8°C , representing the control and heat stress conditions, respectively, used in
686 this study. (b) Frequency of heat stress events with their length in days (heat stress event is
687 defined as the days when the daily mean temperature exceeds 21.8°C). The dashed line
688 indicates the median.

689