

1 **LOCAL ADAPTATION OF REPRODUCTIVE PERFORMANCE DURING**
2 **THERMAL STRESS**

3

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25 **Abstract**

26 Considerable evidence exists for local adaptation of critical thermal limits in
27 ectotherms following adult temperature stress, but fewer studies have tested for local
28 adaptation of sub-lethal heat stress effects across life history stages. In organisms
29 with complex life cycles, such as holometabolous insects, heat stress during juvenile
30 stages may severely impact gametogenesis, having downstream consequences on
31 reproductive performance that may be mediated by local adaptation, although this is
32 rarely studied. Here, we tested how exposure to either benign or heat stress
33 temperature during juvenile and adult stages, either independently or combined,
34 influences egg-to-adult viability, adult sperm motility and fertility in high and low
35 latitude populations of *Drosophila subobscura*. We found both population- and
36 temperature-specific effects on survival and sperm motility; juvenile heat stress
37 decreased survival and subsequent sperm motility and each trait was lower in the
38 northern population. We found an interaction between population and temperature
39 on fertility following application of juvenile heat stress; while fertility was negatively
40 impacted in both populations, the southern population was less affected. When the
41 adult stage was also subject to heat stress, the southern population exhibited
42 positive carry-over effects whereas the northern population's fertility remained low.
43 Thus, the northern population is more susceptible to sub-lethal reproductive
44 consequences following exposure to juvenile heat stress. This may be common in
45 other organisms with complex life cycles and current models predicting population
46 responses to climate change, which do not take into account the impact of juvenile
47 heat stress on reproductive performance, may be too conservative.

48 **Keywords:** spermatogenesis, climate change, range margin, phenotypic plasticity,
49 developmental acclimation, carry-over effects, evolution, inversions

50 **Introduction**

51 While a variety of abiotic factors are changing due to anthropogenic impacts on the
52 climate, perhaps the most critical are increases in the mean and variability of
53 temperature (IPCC, 2014). This is important because temperature has unique and
54 profound effects on organismal biochemistry and physiology. Selection acts on the
55 ability of organisms to function optimally within the range of temperatures that they
56 routinely encounter and to minimize fitness costs during exposure to suboptimal
57 temperatures (Angilleta, 2009). Ectotherms may be especially vulnerable to thermal
58 stress given that their basic physiology is limited by ambient temperatures
59 (Hochachka & Somero, 2002). Ectotherms also represent the vast majority of
60 terrestrial biodiversity (Wilson, 1992) and so understanding how they will respond to
61 increasing temperatures is of critical importance (Deutsch *et al.*, 2008).

62

63 For wide-ranging species that experience different thermal gradients in different
64 environments, populations may exhibit variation in thermal adaptations and
65 responses to suboptimal temperatures (e.g., Gardiner *et al.*, 2010; Yampolsky *et al.*,
66 2014). This may lead to local adaptation which predicts that populations inhabiting
67 warmer climes will function better at higher temperatures relative to populations from
68 cooler locations and vice versa (Angilleta *et al.*, 2003). Many studies have examined
69 responses to temperature stress in terrestrial ectotherms and quantified local
70 adaptation. In particular, critical thermal limits (CTL) - the temperatures at which the
71 ability of an organism to remain active under extreme conditions breaks down - have
72 received much attention. CTLs are thought to provide insight into species
73 distributions, ecology and evolution, with upper thermal limits positively related to
74 optimal performance temperatures (Terblanche *et al.*, 2007). However, recent

75 reviews have emphasised that studies of local thermal adaptation have not focused
76 on the most important life history traits, such as maintenance of fertility (Hoffmann,
77 2010). In previous studies, most of the traits measured to determine physiological
78 failure are related to lethal effects (e.g. survival) or only indirectly related to fitness
79 because they are general measures of mobility such as thermal knock-down.
80 However, the latter may not be correlated with subsequent reproductive success.
81 Moreover, reproductive processes, such as gamete production, are likely to be
82 negatively affected at less extreme temperatures than loss of mobility and
83 organismal death (Jørgensen *et al.*, 2006). The consequences of sub-lethal effects
84 on reproductive performance may be exceptionally important in short lived
85 organisms (i.e. many insects) because reversing thermal damage to reproductive
86 processes may require a large proportion of total lifespan (Sinclair & Roberts, 2005).
87 Thus, examining the consequences of thermal stress on reproductive performance is
88 important in understanding how organisms will respond to climate change.
89
90 Not only should reproductive fitness traits be a focus, but different life history stages
91 may experience different selection on these reproductive traits. Holometabolous
92 insects typically experience radically different juvenile and adult thermal niches and
93 therefore are expected to exhibit different susceptibilities to thermal stress (Angilleta,
94 2009; Hoffmann, 2010; Kingsolver *et al.*, 2011). Vagile stages will have the
95 opportunity to take advantage of environmental heterogeneity via behavioural
96 flexibility whereas developmental stages that are physically restricted have little or no
97 ability to move to less stressful environments.
98

99 The effects of thermal stress on reproductive performance may be particularly
100 relevant for males. For example, in *Drosophila*, spermatogenesis is predicted to be
101 more sensitive to thermal stress than oogenesis (David *et al.*, 2005). Given that
102 sperm production begins during the non-vagile juvenile stage in many insects
103 (Nijhout, 1998), such that mature sperm have already left the testes at the time of
104 emergence from the pupa (Wigglesworth, 1965), thermal stress during this time may
105 represent a profound episode of selection subject to local adaptation. Relatively few
106 studies in holometabolous insects have examined how thermal stress during earlier
107 life cycle stages could affect subsequent organism physiology (Kingsolver *et al.*,
108 2011). While recent work has begun to examine this, studies tend to measure egg-
109 to-adult viability (Kristensen *et al.*, 2015; Rohde *et al.*, 2016) but only rarely the
110 survivors' subsequent reproductive performance (Chirault *et al.*, 2015; Zhang *et al.*,
111 2015a; b) and even more rarely in the context of local adaptation (Fragata *et al.*,
112 2016). Some studies assess physiological responses following relatively short heat
113 shock (Chirault *et al.*, 2015; Zhang *et al.*, 2015a; b) but others ask what the fitness
114 consequences are after longer term exposure to mildly stressful temperatures during
115 development and whether this changes after laboratory adaptation (Fragata *et al.*,
116 2016).

117

118 Understanding the extent to which exposure to sub-lethal thermal extremes during
119 early life-history stages subsequently impacts survival and reproduction later in life is
120 critical to understanding the evolutionary potential and putative constraints on
121 thermal adaptation (Bowler & Terblanche, 2008). Positive carry-over effects (also
122 known as developmental acclimation) are one way that the non-vagile stages could
123 mitigate the cost of thermal stress in subsequent life-cycle stages, and may

124 represent an important component of thermal tolerance adaptation (Sgrò *et al.*,
125 2016). Such effects occur when exposure to thermal stress at an earlier life cycle
126 stage results in increased resistance/tolerance to extreme temperatures at later
127 stages (Angilleta, 2009) and may vary across geographic gradients, suggesting local
128 adaptation (de Jong *et al.*, 2010; Scharf *et al.*, 2010). In contrast, negative carry-over
129 effects occur when stress during development negatively impacts fitness
130 components at the adult stage (Bacigalupe *et al.*, 2007; Gaston & Spicer, 2013;
131 Schiffer *et al.*, 2013; O'Connor *et al.*, 2014). Two knowledge gaps exist in this
132 literature, however. Most studies examining carry-over effects have ignored
133 reproductive traits, which may be more sensitive to thermal stress. Moreover, when
134 performed, these studies have examined response to cold stress (Sgrò *et al.*, 2016).
135 However, capacity to deal with thermal changes is likely to be reduced as upper
136 critical limits are reached. This is because the range of variation in resistance to
137 thermal stress is considerably less at high temperatures than at cold, both between
138 species (Gaston & Chown, 1999; Addo-Bediako *et al.*, 2000) and within species
139 (Gaston & Chown, 1999; Terblanche *et al.*, 2007).

140

141 In this study, we test for local adaptation of survival and reproductive traits in a
142 southern and a northern population of the holometabolous ectotherm, *Drosophila*
143 *subobscura*, manipulating heat stress during either juvenile or both juvenile and adult
144 stages. We focus on this species as it is a model for research on local adaptation to
145 increasing temperatures (Balanyá *et al.*, 2006). We predict that the southern
146 population will have greater reproductive fitness after continuous application of heat
147 stress during the juvenile stage whereas the northern population will have either
148 greater or similar fitness to the southern population at permissive temperatures.

149 Moreover, because traits related to reproduction should exhibit thermal impacts
150 before more frequently measured traits, such as survival, we predict the signal of
151 local adaptation will be stronger for reproductive traits compared to survival following
152 heat stress. Subsequent application of adult heat stress is predicted to affect
153 reproductive performance differently between the populations with the southern
154 population exhibiting positive, and the northern population exhibiting negative, carry-
155 over effects.

156

157 **Material and Methods**

158 *Fly stocks*

159 Stocks of *D. subobscura* originated from wild-caught individuals collected in August-
160 September 2011 from Uppsala, Sweden (59°85'N, 17°63'E) and from Valencia,
161 Spain (39°51'N, 0°42'W), in the same season, with outbred laboratory populations
162 established in mesh cages (50cm³) at 18°C under a 12h light/dark cycle.

163 Subsequently, isofemale lines were established in August 2012 from these two
164 populations (representing ca. 8 overlapping generations) from a single female per
165 line and kept at a population size of greater than 50 at 18°C. The 30 year historical
166 average maximum temperature of the hottest month at each site is 23°C in Uppsala
167 and 31°C in Valencia and previous work across the distribution of this species has
168 suggested that 18°C is a non-thermally stressful temperature for all populations
169 (Santos, 2007; Castañeda *et al.*, 2013). Previous work found that males become
170 sterile at 25°C or even lower (Krimbas, 1993) and our preliminary experiments
171 suggested that this temperature elicited substantial egg-to-adult mortality.

172

173 A maximum of twelve isofemale lines from the Valencia population and four
174 isofemale lines from the Uppsala population were used in the experiments, 3-7
175 months after they were established (representing 3-6 generations), as Valencia
176 exhibits a more diverse set of chromosomal inversion polymorphisms (Krimbas,
177 1993) and we wanted to capture as much genetic variation as possible. While
178 isofemale lines may produce inbreeding effects, levels of fertility and sperm motility
179 under benign conditions are relatively high (see Results) and our statistical models
180 specified overdispersion to account for isofemale line variation. Flies were kept in
181 vials containing a maize, sugar and agar food mix with additional live dry yeast for all
182 experiments, and kept under a 12h light/dark cycle. In all experiments, light CO₂
183 anaesthesia was used to collect and sex flies. Flies were collected as virgins, stored
184 for 6 days to become reproductively mature (Holman *et al.*, 2008) and then used in
185 experiments.

186

187 *Egg-to-adult viability*

188 Male and female pairs from each isofemale line were put in separate vials and kept
189 at 18 °C. Vials were inspected each day, and when more than 30 eggs had been laid
190 (ca. 2 days), the pair was removed, the final number of eggs counted, and vials
191 maintained at either 18°C or 23.5°C. We hereafter refer to the 18°C treatment as
192 benign (B) and the 23.5°C treatment as stressful (H). We aimed for a minimum of ten
193 vials per isofemale line per population per temperature treatment (total sample sizes:
194 B, Upp = 37 vials; H, Upp = 67; B, Val = 102; H, Val = 192). The vials were kept at
195 one of these two constant temperatures through development from egg to imago,
196 and the numbers of eggs that emerged as adults were counted.

197

198 All analyses were performed using R (v. 3.2.2, the R Foundation for Statistical
199 Computing, 2016). For each trait, the maximal model was simplified by step-wise
200 removal of non-significant factors, as judged by change in Akaike Information
201 Criterion. For ease of comparison among traits, data in figures are presented relative
202 to the mean performance of the Valencia population at the benign temperature, after
203 back transformation from the logit scale. For viability we used a generalized linear
204 models (GLM) of the proportion of survivors, with quasibinomial error structure to
205 account for over-dispersion and a logit link function. Temperature [B, H], population
206 [Val, Upp]) and their interaction, to detect local adaptation (Kawecki & Ebert, 2004),
207 were included in the analysis. The number of eggs was log transformed and treated
208 as a covariate in order to account for density effects.

209

210 *Sperm motility*

211 Spermatogenesis is predicted to be more strongly affected by thermal stress than
212 oogenesis. Male sterility is frequently quantified in *Drosophila* as lack of motile sperm
213 (Coyne & Orr, 1989) and here we examined the seminal vesicles for the presence of
214 motile sperm following either benign or stressful developmental temperatures. Flies
215 were generated as described above. Male flies were collected on the day of eclosion
216 and kept in vials of less than 20 flies for six days at their developmental temperature
217 (B or H) to reach sexual maturity. Seminal vesicles of a minimum of ten males per
218 line per population per temperature treatment were dissected (Snook, 1998), gently
219 squashed on a slide under a cover slip in phosphate buffered saline, and sperm
220 motility assessed (total sample sizes: B, Upp = 46; H, Upp = 73; B, Val = 126; H, Val
221 = 223). Males were considered sterile (coded as 0) if no sperm, or very few motile
222 sperm, were present; males were considered fertile (coded as 1) if motile sperm

223 were present. This response was analysed using a quasibinomial GLM as for
224 viability (but with no covariate).

225

226 *Fertility*

227 An ultimate measure of fertility is the ability to produce progeny. Here we were
228 interested to measure the consequences of the thermal environment at both
229 developmental and adult stages on fertility. We had four treatments for each
230 population: both juvenile and adult temperatures were benign (BB); both were
231 stressful (HH); juvenile temperature was stressful but adult temperature was benign
232 (HB); or juvenile temperature was benign and adult temperature stressful (BH). Flies
233 were generated as described above, being reared throughout development at either
234 the benign or stressful temperature and upon eclosion, virgin males and females
235 were collected and separated by sex into vials of less than 20 individuals for six days
236 to reach sexual maturity and were randomly assigned to either remain as adults at
237 their developmental temperature or to switch to the alternative temperature. At 6
238 days post-eclosion, a male and female were placed in a vial and monitored for larvae
239 production (we aimed for a minimum of 10 pairs per line per population per
240 temperature treatment; total sample size: for Uppsala, BB = 14, HH = 19, HB = 22,
241 BH = 21; for Valencia, BB = 50, HH = 48, HB = 89, BH = 73). Pairs were transferred
242 from the first vial after five days and placed in another vial for a further five days.
243 Pairs were considered fully sterile if no larvae were produced in either vial (coded as
244 0); pairs were considered fully fertile if larvae were produced in both vials (coded as
245 2); and pairs were considered partially fertile if larvae were produced in only one of
246 the two vials (coded as 1). This response was analysed using a binomial GLM (0, 1
247 or 2 successes out of 2 trials) because no over-dispersion was detected. The

248 temperature treatment in this case had four levels (BB, BH, HB, HH). We tested for
249 the predicted carry-over effects by making the appropriate contrast: the effect of HH
250 relative to HB was expected to depend on population (HH>HB in Val, HB>HH in
251 Upp).

252

253 **Results**

254 Temperature and population, but not their interaction, significantly influenced viability
255 from egg to adult (Fig. 1a) and sperm motility (Fig. 1b; Table 1; interaction term,
256 viability: df = 1, deviance = 0.065, p = 0.98; interaction term, sperm motility: df = 1,
257 deviance = 1.042, p = 0.31). Higher temperatures resulted in decreased survivorship
258 and sperm motility compared to lower temperature. Uppsala had lower viability and
259 sperm motility than Valencia at both temperatures.

260

261 In contrast, temperature, population and their interaction significantly influenced
262 fertility (Fig. 2, Table 1). Stressful temperatures experienced only during
263 development (HB, Fig. 2) had a greater consequence on fertility than only
264 experiencing stressful temperatures as an adult (BH, Fig. 2) and this affect was
265 stronger on the Uppsala population than the Valencia population. We predicted that
266 the southern population would exhibit positive, whereas the northern population
267 would exhibit negative, carry-over effects. To test these predictions, we performed
268 two directional (and thus, one-tailed) t-tests, contrasting the effect of HH relative to
269 HB (HH>HB in Val, HB>HH in Upp). There was no difference in fertility for Uppsala
270 between stress in the juvenile stage only compared with stress in both stages ($t_{328} =$
271 0.99, P = 0.16). However, in Valencia, while heat stress negatively impacted fertility
272 during any stage, fertility was higher in individuals that experienced heat stress in

273 both juvenile and adult stages compared to heat stress only during the juvenile stage
274 ($t_{328} = 1.74$, $P = 0.041$).

275

276 **Discussion**

277 In this study, we aimed to reveal local adaptation of reproductive performance traits
278 under sub-lethal heat stress, with a particular emphasis on heat stress during the
279 juvenile stage. Furthermore, we aimed to evaluate whether acclimation to heat stress
280 during development impacted the late life response to these temperatures. We found
281 juvenile heat stress and population impacted egg-to-adult viability and sperm motility,
282 with the northern population having lower fitness than the southern population. We
283 found fertility exhibited local adaptation; although both populations were negatively
284 impacted during juvenile heat stress, the southern population was less effected. We
285 also found positive carry-over effects in fertility. Heat stress exposure during the
286 adult stage, after juvenile heat stress exposure, increased fertility in the southern, but
287 not northern, population. Overall, the northern population was significantly more
288 susceptible to juvenile and adult thermal stress across all fitness traits measured.
289 Our work fills a gap in research on temperature effects that has largely been
290 dominated by experiments focusing on CTLs of non-reproductive traits, primarily
291 following adult exposure to thermal, particularly cold, stress. In this work, we have
292 linked reproductive performance based responses to chronic sub-lethal heat stress,
293 particularly at the juvenile stage, to local adaptation. This work will provide novel
294 insights of how populations may respond to such thermal variation and may be
295 applicable across a variety of other animals. The generality of the results is likely
296 because many animals will lose fertility at temperatures much lower than fatal

297 thermal limits (Jørgensen *et al.*, 2006) and because, for many invertebrates, gamete
298 production begins during juvenile stages.

299

300 As predicted, we found no evidence of local adaptation in juvenile survival although
301 northern males had lower egg-to-adult viability. Likewise, sperm motility was
302 negatively impacted primarily by juvenile temperature and weakly by population, with
303 northern males having lower sperm motility overall than southern males but no
304 evidence of local adaptation. Sperm motility is a standard measure of male sterility
305 but is frequently measured as a binary trait, and thus may not reveal subtle changes
306 in sperm performance following thermal stress. The lower general fitness of the
307 Uppsala population may reflect range margin effects (Kawecki, 2008), consistent
308 with the decreased variation in chromosomal inversion polymorphisms as latitude
309 increases (Krimbas, 1993). Decreased genetic variation has been suggested to
310 occur at range margins as a consequence of some combination of Allee effects,
311 genetic drift and the lack of sufficient gene flow into populations at range limits to fuel
312 adaptive potential (Hoffmann & Blows, 1994; Bridle & Vines, 2007). Alternatively,
313 local adaptation may be decreased due to gene swamping, with dispersal from
314 (typically larger) more central populations continually introducing alleles into range
315 margins that are more appropriate for maximising fitness elsewhere (Hoffmann &
316 Blows 1994). The situation in *D. subobscura* is more complicated given the presence
317 of extensive variation in chromosomal inversion polymorphisms that prevent
318 recombination in inversion heterozygotes. While there is gene flow within the same
319 inversion arrangement (Simões *et al.*, 2012; Pegueroles *et al.*, 2013; Pratdesaba *et*
320 *al.*, 2015), there is substantial evidence that inversion polymorphisms themselves
321 are under selection (Balanyá *et al.*, 2006; Santos *et al.*, 2016).

322

323 We did, however, demonstrate local adaptation to the thermal environment in our
324 other measure of fertility, as predicted. Fertility was negatively impacted by
325 application of juvenile heat stress, particularly for Uppsala compared to Valencia
326 pairs. Juvenile heat stress resulted in positive carry-over effects on fertility in
327 Valencia, but not Uppsala, when adults also experienced heat stress. These results
328 indicate that northern populations are more susceptible to sub-lethal reproductive
329 consequences following exposure to juvenile heat stress, and that such exposure
330 does not buffer reproductive performance against subsequent adult heat stress.

331

332 The effects of thermal stress on male and female reproductive function in the fertility
333 experiment cannot be separated. Now that we have demonstrated local adaptation in
334 fertility, future work should decompose the sex-specific effects of juvenile thermal
335 stress on gamete performance in more physiological detail, such as quantifying
336 apoptosis of oocytes and the effect of thermal stress on sperm number and length.
337 Female *D. subobscura* are monogamous (Smith, 1956; Holman *et al.*, 2008; Fisher
338 *et al.*, 2013) but, as with other *obscura* group species, males are sperm
339 heteromorphic, with males producing short, non-fertile sperm (parasperm) and
340 longer, fertile sperm (eusperm; Snook & Karr, 1998). In work on the closely related
341 polyandrous species, *D. pseudoobscura*, parasperm protect brother eusperm from a
342 hostile female reproductive tract (Holman & Snook, 2008). Sperm precedence
343 favours the last male, but after lengthy exposure to cold stress, sperm precedence
344 reverses to favour the first male (Giraldo-Perez *et al.*, 2016). Whether, and the extent
345 to which intra-ejaculate interactions and ejaculate-female reproductive tract

346 interactions change as a consequence of either juvenile or adult heat stress and
347 whether this is impacted by mating system is unknown.

348

349 We have previously found, across six *D. subobscura* populations from a 20°
350 latitudinal cline in Europe (of which the Valencia and Uppsala populations represent
351 the southern- and northern-most population respectively), a gradient in gene
352 expression in which adult males from southern populations have higher expression
353 of spermatogenesis genes than males from northern populations, under benign
354 (18°C) conditions ((Porcelli *et al.*, 2016). Given that females are monandrous, sperm
355 competition cannot explain increased investment in spermatogenesis in lower
356 latitude populations. As investment in spermatogenesis is less in Uppsala than
357 Valencia, it may be that northern males are not able to compensate as much as
358 southern males following juvenile heat stress. In support, we found evidence of
359 developmental acclimation in Valencia, but not Uppsala, populations.

360

361 If fertility reduction following juvenile heat stress is male-driven, then population
362 fitness can recover only if females remate with a fertile male. While females are
363 naturally monogamous, previous work has shown that *D. subobscura* females will
364 remate if they do not receive a functional ejaculate during copulation, a phenomenon
365 termed pseudo-polyandry (Fisher *et al.*, 2013). Thus, mitigating male sterility and
366 recovering population fitness may be possible if females can find a fertile mate,
367 although whether females will remate after receipt of a partially-fertile ejaculate is
368 unknown. Moreover, finding a fertile mate may be more difficult in northern, more
369 susceptible populations, because of the lower density of range margin populations
370 (including the *D. subobscura* Uppsala population; Krimbas, 1993). However, if

371 oogenesis is also damaged during juvenile heat stress, then even pseudo-polyandry
372 cannot rescue population fitness. The combination of higher latitude populations
373 being more susceptible to juvenile heat stress, decreasing both survival and
374 measures of reproductive performance (sperm motility and fertility), northern
375 populations investing less in spermatogenesis, and having low population density,
376 makes high latitude *D. subobscura* populations susceptible to extinction as both
377 average temperature and the frequency of extreme heat events increase (IPCC,
378 2014).

379

380 While this work is specific for *D. subobscura*, such results may be quite common.
381 Many organisms spend a portion of their life cycle as relatively immobile juveniles, so
382 thermal stress during this life cycle stage may have profound negative fitness
383 consequences beyond survival because gamete production typically starts during
384 juvenile stages. Such subsequent negative fertility effects on adults should
385 compound reductions in population size, which can then have substantial
386 downstream effects via, for example, genetic drift (Polechova & Barton, 2015). As
387 range margin populations tend to have less genetic diversity to begin with, and the
388 range of variation in resistance to thermal stress is considerably less at high
389 temperatures than at cold (Gaston & Chown, 1999; Addo-Bediako *et al.*, 2000;
390 Terblanche *et al.*, 2007), sub-lethal effects on reproductive traits may make these
391 populations even more susceptible as the climate continues to get warmer and the
392 frequency of extreme heat events increases. Local adaptation may provide some
393 resilience to climate change and we found such local adaptation for juvenile heat
394 stress and reproductive performance in a southern population. Variation in
395 organismal tolerance throughout the life cycle should be incorporated in models that

396 predict impacts of climate change. To date, heavy reliance has necessarily been
397 placed on predictions based on latitudinal trends in upper and lower critical or lethal
398 thermal limits (Lancaster, 2016) derived from data compilations (e.g. Addo-Bediako
399 *et al.*, 2000; Sunday *et al.*, 2011). A recent study on lizards, with a sessile embryonic
400 stage, developed a life cycle model for demographic consequences based on
401 microclimates at high spatio-thermal resolution and experimentally derived
402 embryonic thermal tolerances (Levy *et al.*, 2015). Levy and colleagues found that
403 models that ignored embryonic consequences (egg-to-adult viability) severely
404 underestimated the demographic effects of (previously unrecognised) thermal
405 events. Such detailed analysis that also incorporates any subsequent fertility effects
406 remains to be done. Given that we show substantial effects beyond egg-to-adult
407 survival, models without this consideration may prove even more unduly
408 conservative.

409

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561

562 Table 1. Generalized linear model of the effects of temperature, population and their
 563 interaction on (a) egg-to-adult viability, (b) sperm motility and (c) fertility. p values are
 564 reported either exactly or < 0.001 . For egg-to-adult viability, the starting number of
 565 eggs was also included in the model. Non-significant terms have been dropped.
 566 Residual deviance is included where the quasibinomial model was used.

567

source	d.f.	Deviance	Deviance ratio	p
(a) egg-to-adult viability				
log(eggs)	1	67.232	6.1864	0.01
Temperature	1	302.528	27.8375	< 0.001
Population	1	59.993	5.5203	0.01
Residual	394	4937.1		
(b) sperm motility				
Temperature	1	76.95		<0.001
Population	1	4.49		0.03
(c) fertility				
Temperature combination	3	148.731	28.77	<0.001
Population	1	11.224	6.5128	0.01
Temperature*Population	3	14.657	2.8348	0.038
Residual	328	587.43		

568

569 Figure legends.

570 Figure 1. The effect of juvenile heat stress and population influence a) egg-to-adult
571 viability and b) sperm motility. Mean \pm SE relative to Valencia (southern population)
572 at the benign temperature, following back transformation from the logit scale.

573

574 Figure 2. Heat stress during the juvenile life cycle stages negatively impacts fertility
575 in Valencia (southern population) pairs significantly less than Uppsala (northern
576 population) pairs and subsequent adult heat stress improves fertility in Valencia, but
577 not Uppsala, pairs. B= benign and H = heat stress; BB = benign temperature for both
578 juvenile and adult stages, BH = benign temperature during juvenile stages but heat
579 stress as adults, HB = heat stress during juvenile stages but not during the adult
580 stage; HH = heat stress during juvenile and adult stages. Mean \pm SE relative to
581 Valencia at the benign temperature for BB, following back transformation from the
582 logit scale.