

The fitness cost to females of exposure to males does not depend on water availability in seed beetles

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ABSTRACT:

Access to multiple males can benefit a female because it increases her fecundity and/or the performance of her offspring due to males providing material benefits and/or genetic gains from polyandry (i.e. cryptic female choice). However, the presence of more males can also impose costs on females that arise from an elevated mating rate and/or increased harassment. Understanding how different environments influence the relative magnitude of these costs and benefits is important to understanding how factors that affect the rate of male-female interactions, such as the sex ratio and density of each sex, will alter the evolution of traits due to shifts in the magnitude of sexual conflict and sexual selection. Here we explored whether the net fitness of female seed beetles (*Callosobruchus maculatus*) is affected by breeding in either a dry or wet environment when housed with differing numbers of males (either none, one or four). Consistent with costly male harassment, females housed with four males laid significantly fewer eggs than those housed alone or with a single male. However, there was no significant effect of the number of males on a female's egg laying rate, her lifespan, larval development rate or the egg-adult survival of offspring. Although females in the wet environment lived significantly longer, the decline in the rate of egg laying and egg-adult survival with maternal age was stronger in the wet than the dry environment. Crucially, there was no evidence that water availability affects the net fitness cost to females of being exposed to more males.

Keywords: *Callosobruchus maculatus*, costs of mating, fitness, sexual harassment, polyandry.

29 INTRODUCTION

30 Reproductive interactions between males and females always come with the benefit to
31 females of providing access to sperm that is needed for offspring production. However, the
32 presence of several males can also come at a cost for females. In most species the optimal
33 number of matings is lower for females than males (Arnqvist & Nilsson, 2000). This generates
34 sexual conflict over mating and can lead to male harassment (i.e. repeated unsuccessful
35 mating attempts by coercive males), which increases the costs of interacting with males (e.g.
36 reduced feeding opportunities, increased physical injury; Bateman, Ferguson, & Yetman, 2006;
37 Rönn, Katvala, & Arnqvist, 2006; Takahashi & Watanabe, 2010). In consequence, females might
38 sometimes opt to mate simply to decrease harassment (i.e. convenience polyandry) (Blyth &
39 Gilburn, 2006; Thornhill & Alcock, 1983). Although there are potential gains of mating multiply
40 for females, due to both direct benefits (e.g. greater access to male services and resources,
41 Hasson & Stone, 2009; Townsend, Clark, & McGowan, 2010) and indirect benefits (e.g. higher
42 genetic quality offspring, or the use of compatible sperm to elevate offspring viability,
43 Jennions & Petrie, 2000; Tregenza & Wedell, 2002), mating can impose direct costs that
44 accumulate with each successive mating (Crudgington & Siva-Jothy, 2000; Fedorka, Zuk, &
45 Mousseau, 2004; Wigby & Chapman, 2005). Consequently, the degree to which females
46 increase their mating rate when more males are present is likely to depend on balancing the
47 costs of resisting male mating attempts with the shift in the net fitness effects of each
48 successive mating. How the various costs and benefits balance out to influence female fitness
49 is important in determining what type of mating systems and reproductive behaviour evolve.

50 To date, few studies have tested whether the fitness consequences for females of
51 increased interactions with males varies because the local environment affects the relative
52 strength of sexual selection or sexual conflict arising from additional matings and/or resisting
53 more mating attempts (Sih, Montiglio, Wey, & Fogarty, 2017; Smith, 2007). Female mating

54 decisions are likely to be plastic if the fitness consequences of additional matings are context-
55 dependent (Rosenthal, 2017). For example, population density might influence the optimal
56 mating strategy of females to acquire direct and indirect benefits (Kokko & Rankin, 2006), such
57 that female mating preferences are density-dependent (Rosenthal, 2017; Welch, 2003). At low
58 densities females are predicted to be less selective due, in part, to reduced mate availability
59 and the increased time and energy costs of locating mates (Hutchinson & Halupka, 2004;
60 Kokko & Mappes, 2005). Higher densities will, however, tend to increase the number of male
61 mating attempts. This could lead to either a reduction in female willingness to remate (e.g.
62 Martin & Hosken, 2003) due to the increased costs of excess mating, or to an increase in
63 female mating rate to lower the short-term rate of harassment (e.g. Rowe *et al.*, 1994).

64 The environment could also affect the magnitude of the direct benefits that females
65 receive from each mating (e.g. the quantity of beneficial substances transferred in ejaculates,
66 such as nutrients and hormonal triggers that elevate egg production; Arnqvist & Nilsson, 2000;
67 Yamane *et al.*, 2015). For example, low food availability might decrease male body condition
68 and reduce ejaculate size and composition (Perry & Rowe, 2010; Polak *et al.*, 2017, Iglesias-
69 Carrasco *et al.*, *in review*), and thereby lower the net direct benefits of mating. Similarly,
70 females in an environment with greater access to food have been shown to remate less often
71 due to the reduced value of obtaining mating gifts from males (Toft & Albo, 2015). Despite the
72 likely role of the environment in determining the net outcome of female mating decisions,
73 relatively few studies have asked how environmental changes affect male-female interactions
74 due to the potential for correlated changes in both male harassment and the benefits of
75 additional matings (but see Edvardsson 2007).

76 The seed beetle *Callosobruchus maculatus* is an ideal model species to investigate the
77 fitness consequences for females of interacting with different numbers of males. Although
78 females are often polyandrous the direct benefits of mating multiply are subtle (e.g. no nuptial

79 gifts). Previous studies in *C. maculatus* have yielded mixed results that make it difficult to
80 predict the net fitness effect on females of more males being present, due to the combination
81 of both direct and indirect benefits of additional matings, alongside the cumulative costs of
82 remating. For example, there is evidence that polyandrous females lay more eggs than those
83 mated to a single male (even after controlling for the number of matings) (Eady, Wilson, &
84 Jackson, 2000), possibly due to cryptic female choice. Intriguingly, there are also potential
85 direct benefits of mating. Several studies have suggested that fecundity benefits could be
86 attributed to nutrients and water in ejaculates (Eady, Hamilton, & Lyons, 2007; Savalli & Fox,
87 1999; Ursprung, Den Hollander, & Gwynne, 2009). *C. maculatus* are a pest of stored legumes,
88 and live in an environment where there is little or no access to water or food for adult beetles.
89 Water is therefore expected to be a limiting resource for females that constrains their
90 reproductive output and life span. Males of *C. maculatus* transfer ejaculates that are rich in
91 water (Edvardsson, 2007) so, if male ejaculates are the only water resource available to adult
92 females, we might expect the net benefits of remating to sometimes outweigh the costs,
93 thereby increasing the mating propensity of females. However, there are other potential costs
94 of mating that could reduce or eliminate the net benefits of polyandry. The costs of copulation
95 in the seed beetle include traumatic wounding of the female reproductive tract caused by the
96 male aedeagal spines and the transfer of toxic ejaculatory substances (Crudgington & Siva-
97 Jothy, 2000; Paul E. Eady et al., 2007; Gay, Eady, Vasudev, Hosken, & Tregenza, 2009). In
98 addition, the presence of more males leads to greater male harassment that can reduce
99 female lifespan (den Hollander & Gwynne, 2009). Surprisingly, despite extensive research in *C.*
100 *maculatus* we still do not know if the environment alters the balance between these putative
101 costs and benefits of mating and therefore the relative effect of variation in the number of
102 males that females encounter on their fitness (but see Edvardsson 2007).

103 Two previous studies in *C. maculatus* (Edvardsson, 2007; Ursprung et al., 2009) have
104 demonstrated that water availability increases a female's life span and fitness, while reducing

105 her willingness to remate. These findings suggest that water is a limiting resource for females,
106 who might benefit from the water acquired from male ejaculates. However, if females make
107 use of environmental water this might reduce the advantage of additional matings through the
108 acquisition of water in ejaculates. Here we build on this previous knowledge to test whether
109 breeding in a dry versus wet environment affects the net fitness of females in a manner that
110 depends on how many males they are housed with (i.e. due to the combination of a change in
111 the level of male harassment and the number of mates and/or matings). Access to water in the
112 environment could lower the benefit to a female of gaining water from ejaculates, potentially
113 altering her behaviour and fitness in a manner that depends on the number of males
114 encountered. We predict that: 1) In both dry and wet conditions, females exposed to males
115 will have lower fitness than those housed alone, and that this negative effect increases with
116 the number of males; 2) If water is a critical resource, females housed alone in dry conditions
117 would have lower fitness than those in wet conditions; 3) Finally, we are especially interested
118 in how water availability influences the fitness consequences of being housed with multiple
119 males. Given the many potential ways in which mating with males (or resisting their advances)
120 influence female fitness it is, however, difficult to make concrete predictions: the outcome will
121 depend on the relative magnitude of the various costs and benefits.

122 METHODS

123 *Study species*

124 The seed beetle *Callosobruchus maculatus* is a pest species of stored legumes. In this
125 environment adults have no access to food or water and all resources required for adult
126 survival and reproduction are acquired from the host bean during the larval stage (Messina &
127 Slade, 1997). The seed beetle's life-cycle begins with females laying an egg on the surface of a
128 host bean. The hatching larva burrows into the bean and remains inside feeding on it for 3-4
129 weeks until it emerges as an adult. We used beetles originating from a stock kept at the

130 University of Western Australia since 2005 in cultures of >500 adults breeding on black-eyed
131 beans (*Vigna unguiculata*) (Dougherty et al., 2017). This stock was maintained in our lab in
132 cultures of >500 beetles at $27 \pm 1^\circ\text{C}$ with a 14:10 light:dark cycle for three generations prior to
133 our experiment. Stock larvae were raised on black-eyed beans and adults were provided with
134 neither food nor water.

135 *Ethical Note*

136 This work followed the ASAB/ABS guidelines for the treatment of animals in
137 behavioural research. Information about individuals' housing conditions are described below.
138 Housing conditions, handling and experimental monitoring were all conducted in a way so as
139 to maximize the animals' welfare. We complied with the Australian regulations for
140 experiments on invertebrates.

141 *Experimental design*

142 To investigate how the environment (i.e. water availability) influences the costs and
143 benefits for females of being exposed to different numbers of males we used a 2x3
144 experimental design where we manipulated water availability (wet or dry) and the number of
145 males (0, 1 or 4) housed with a female after an initial single mating to ensure she had sperm.
146 We had six experimental groups: 1) dry, no male (D0, $n = 49$); 2) wet, no male (W0, $n = 47$); 3)
147 dry, one male (D1, $n = 47$); 4) wet, one male (W1, $n = 44$); 5) dry, four males (D4, $n = 45$); 6)
148 wet, four males (W4, $n = 45$). Our dry treatment mirrors the natural or stock environment
149 where adult individuals lack access to water.

150 *Experimental procedure and measure of fitness traits*

151 To obtain virgins, 70 mated stock females were each placed individually in a petri dish
152 with 20 mung beans (*Vigna radiata*). Our stock performs equally well on mung or black-eyed
153 beans (McCorquodale, pers comm). We monitored the females constantly: each time they laid

154 an egg, the bean was removed and placed individually in an Eppendorf tube with a pin-hole in
155 the cap for airflow. We repeated this procedure until we had 400 beans each with a single egg.
156 The adults that started to emerge around 21 days later became the focal individuals for our
157 experiment. The eclosion date was recorded and individuals were given 24 h to attain sexual
158 maturity (Fox, Hickman, Raleigh, & Mousseau, 1995). We then placed a randomly chosen virgin
159 female with a virgin male in an Eppendorf tube and allowed them to mate. After a single
160 copulation, females were randomly assigned to one of our six treatments.

161 Females were individually placed in a 60 ml plastic container with approximately 40
162 mung beans. Water was supplied *ad libitum* to females in the wet treatment by placing soaked
163 cotton wool in a plastic vial lid, which was refilled every 24 hours. Females have previously
164 been shown to drink water when it is made available in this way (Edvardsson, 2007). Too few
165 males emerged at the same time as females, so for the first 24 hours after copulation (day 1),
166 all females were alone in their designated water treatment. For the next three days (day 2, 3,
167 4), in addition to their corresponding water treatment, females experienced one of the three
168 levels of exposures to males. Females were transferred daily to a new container with
169 approximately 40 mung beans that again, depending on her treatment group, housed either
170 zero, one or four males (drawn from a large stock of previously mated males). On day 5 all
171 males were removed and females remained in their day 4 container in their designated water
172 treatment until death. Female survival was monitored every 24 h and her lifespan was
173 recorded as the number of days a female survived after her first copulation. We counted the
174 number of eggs laid in each container to measure both egg laying rate (i.e. for days 2 and 3)
175 and 'lifetime' egg production (excluding day 1 eggs which were laid prior to exposure to
176 males). Once eggs on beans were counted, we returned the beans to the controlled
177 temperature room and 21 days later we began to check for emerging adults. We recorded the
178 eclosion date of the first emerging offspring per container to estimate development time.
179 Once offspring started to emerge they were counted and removed each day for 10 days. We

180 used these data to calculate the percentage of eggs that produced emergent offspring
181 (hereafter 'egg-adult survival').

182 *Statistical analyses*

183 Females spent the first 24 hours after their initial copulation in their designated water
184 treatment but without any males. Our test for an effect of male presence on female fitness is
185 therefore conservative. Our main aim was to test if and how access to water and exposure to
186 males interact, and we therefore excluded eggs collected on the first day from our analyses.
187 We tested how water availability and the exposure to males influenced egg laying in two ways:
188 'lifetime' egg production (i.e. from day 2 onward), and egg laying rate (i.e. eggs/day for day 2
189 and 3 in the second and third containers respectively). For 'lifetime' egg production we
190 specified a generalized linear model (GLM) with a Quasipoisson error distribution to account
191 for overdispersion. Water availability, level of exposure to males, and their interaction were
192 specified as fixed factors. For egg laying rate we specified a Generalized Linear Mixed Model
193 (GLMM) with a Poisson error distribution. Water availability, level of exposure to males, day
194 (second or third) and all two-way and three-way interactions were specified as fixed factors.
195 We included female identity as a random effect to control for individual variation, and an
196 observation level random effect to correct for overdispersion (Harrison, 2014). Following this
197 correction, our model was underdispersed (dispersion parameter = 0.517).

198 To determine whether water availability and level of exposure to males influenced
199 egg-adult survival we ran a GLMM with a binomial error distribution using the cbind function
200 (number of adults eclosing; number of unhatched eggs). We treated water availability, level of
201 exposure to males, the day the eggs were collected and all two and three-way interactions as
202 fixed factors. Female identity was treated as a random effect. We constructed a similar model
203 to look at the effects of water availability, level of exposure to males and time since mating on
204 offspring development time, but in this case the best fit model was a GLMM with a Poisson

205 error structure. We corrected for overdispersion using an observation level random effect
206 (Harrison, 2014).

207 We used Cox proportional hazard models (function `coxph`, R package *survival*,
208 Therneau & Grambsch, 2000) to test if water availability and male exposure affected female
209 survival.

210 Our general protocol to test whether water availability, level of exposure to males, and
211 day interacted to affect female fitness was to initially include two- and three-way interactions
212 in models. We then re-ran models without these interactions. If their removal did not reduce
213 the model fit (LLR test), we interpreted the main effects from the reduced model. If it did
214 reduce the fit, we interpreted the interactions by looking at the model parameters. All
215 analyses were conducted in R 3.4.

216 RESULTS

217 Model parameter estimates and test statistics are provided in Tables A1-A5 in the *Appendix*.
218 Tests for main and interaction effects are presented in the text below.

219 The number of males a female was exposed to had a significant effect on her lifetime egg
220 production ($\chi^2 = 6.730$, $df = 2$, $P = 0.035$, Figure 1a, Table A1). Females exposed to four males
221 laid significantly fewer eggs than those that were alone ($P = 0.006$) or with only one male ($P =$
222 0.048). There was, however, no significant difference in the total number of eggs laid by
223 females that were alone or with only one male ($P = 0.444$). Water availability did not affect the
224 total number of eggs laid ($\chi^2 = 0.141$, $df = 1$, $P = 0.707$), nor did it have a modifying effect on
225 the consequence of a greater level of exposure to males (water*number of males: $\chi^2 = 4.755$,
226 $df = 2$, $P = 0.093$).

227

228 The daily egg laying rate decreased over time (Figure 1b, Table A2), and this decline was
229 significantly greater for females with access to water than those without (day*water: $X^2 =$
230 8.461, df = 1, $P = 0.004$). No other interactions had a significant effect on the egg laying rate
231 (water*mating: $X^2 = 2.899$, df = 2, $P = 0.235$; day*number of males: $X^2 = 0.027$, df = 4, $P = 0.987$;
232 water*number of males*day: $X^2 = 2.256$, df = 4, $P = 0.324$).

233 Egg-adult survival was affected by a three-way interaction between the day of laying, the
234 water treatment and the level of exposure to males ($X^2 = 14.535$, df = 4, $P = 0.006$, Figure 2,
235 Table A3). To investigate this interaction further we analysed each water treatment separately.
236 In the dry environment, the egg-adult survival was lower for the eggs laid on day 4 than for
237 those laid on day 2 or 3 ($X^2 = 32.525$, df = 2, $P < 0.001$, Table 3.1). There was no effect of the
238 exposure to males or any interaction between the day and male exposure (both P -values $>$ -
239 0.358). For the wet treatment, how the level of exposure to males affected egg-adult survival
240 depended on the day which eggs were laid (i.e. a two way interaction between male exposure
241 treatment and day: $X^2 = 31.398$, df = 4, $P < 0.001$). On day 2 egg-adult survival decreased when
242 females were exposed with more males, on day 3 egg-adult survival was similar across all male
243 exposure treatments, and on day 4 egg-adult survival was greater when females were exposed
244 to more males.

245 Larval development time was not affected by the day of egg laying, water availability, the
246 exposure to males or any of their interactions (all $P > 0.931$; Figure 3, Table A4).

247 Finally, females with access to water lived for significantly longer ($X^2 = 46.71$, df = 1, $P < 0.001$),
248 but there was no effect of the level of exposure to males on survival ($X^2 = 4.59$, df = 2, $P =$
249 0.100) nor did it mediate the effect of access to water (interaction: $X^2 = 0.51$, df = 2, $P = 0.775$)
250 (Table A5, Figure 4).

251 **DISCUSSION**

252 As predicted, we found that a greater level of exposure to males imposed a fitness cost
253 on females because it had a negative effect on lifetime egg production. There was, however,
254 no detectable effect of the number of males on female lifespan, egg-laying rate, or offspring
255 egg-adult survival or development time. Contrary to our initial prediction that the environment
256 (water availability) would modify the fitness consequences of sexual conflict and sexual
257 selection, while access to water had a significant positive effect on female lifespan, there were
258 no significant interactions between the level of exposure to males and access to water for any
259 of the other four components of fitness that we measured.

260 *Exposure to males and female fitness*

261 We predicted that encountering a greater number of males would negatively affect a
262 female's fitness. Females housed with four males did indeed have lower lifetime egg
263 production than those housed alone or with a single male, but this effect was independent of
264 the water availability treatment. There was no other effect of the number of males present on
265 the other fitness components that we measured, including offspring survival and development
266 rate. The lower fecundity of the females exposed to several males suggests that there are
267 direct costs of mating multiply for females and/or that females suffered from increased male
268 harassment. At present, we are unable to tease apart these two potential costs of an increase
269 in exposure to males, but we discuss the likely role of each. Further, there was no evidence for
270 any paternal effects (i.e. indirect genetic benefits, although non-genetic effects are also
271 possible; see Gasparini, Devigili, & Pilastro, 2012) of being housed with multiple males on
272 offspring fitness. Introducing males after 24hrs, rather than immediately after an initial mating,
273 could reduce the difference in offspring development time and survival due to different levels
274 of male exposure. However, there was a significant effect of the level of exposure to males on
275 female egg production, so our design was still powerful enough to detect biological meaningful
276 effects of variation in access to males on female fitness.

277 Mating imposes direct costs on females in several species due to physical damage
278 during copulation and/or the transfer of toxic ejaculate substances (Chapman, Liddle, Kalb,
279 Wolfner, & Partridge, 1995; Crudgington & Siva-Jothy, 2000; Johnstone & Keller, 2000;
280 Yamane, Miyatake, & Kimura, 2008). In *C. maculatus*, for example, male genital spines can
281 wound females during copulation which facilitates the circulation of seminal fluids into the
282 body cavity (Dougherty & Simmons, 2017; Hotzy, Polak, Rönn, & Arnqvist, 2012). However, the
283 acquisition of direct material benefits from males upon mating can sometimes compensate for
284 these costs of copulation (Arnqvist & Nilsson, 2000). For example, mating multiply can increase
285 female life span and fecundity when females only have brief access to males (den Hollander,
286 2007), suggesting that the male-derived material benefits from mating can sometimes offset
287 any costs of genital injury and toxic effects of ejaculates. In *C. maculatus* it has been suggested
288 that hydration is the main direct material benefit of mating (Ursprung et al., 2009), which
289 could partly offset costs associated with damage to the female reproductive tract during
290 copulation (Crudgington & Siva-Jothy, 2000). Our results for female survival partly support
291 these previous findings, in that access to water elevated female lifespan suggesting that water
292 is an important limiting resource for female self-maintenance.

293 In our study, as always occurs in nature, the opportunity to mate with more males
294 involved greater exposure to males. Male harassment has been suggested to lower female
295 fitness in both vertebrates and invertebrates (e.g. fish: Ojanguren & Magurran, 2007;
296 damselflies: Takahashi & Watanabe, 2010; seed beetles: Laurene *et al.*, 2009), related to a
297 range of costs for females including loss of feeding time (Dadda, Pilastro, & Bisazza, 2005) and
298 higher energy expenditure when resisting male mating attempts (Watson, Arnqvist, &
299 Stallmann, 1998) that often lead to a reduction in fecundity (Crudgington & Siva-Jothy, 2000;
300 Eady *et al.*, 2007). In our study a plausible explanation for the lower offspring production of
301 females housed with four males is that it is partly due to greater male harassment and the cost
302 of evading males. Females might also engage in superfluous matings to avoid the costs of

303 rejection (i.e. convenience polyandry). This could elevate a female's mating rate such that any
304 direct benefit of re-mating (e.g. hydration) is outweighed by associated costs (e.g. physical
305 damage) that lower her net fecundity. If so, we might expect other components of fitness,
306 such as female survival, to also be affected. This was not the case, however, as exposure to
307 more males did not affect early egg laying rate or female survival.

308 *Effect of water availability and exposure to males on female fitness*

309 Water has been suggested to be a critical environmental factor driving the mating
310 behaviour of *C. maculatus*, since females gain hydration benefits by mating (Ursprung et al.,
311 2009). In our experiment, *both* sexes either did or did not have access to water as adults,
312 which mirrors what occurs naturally as there is no habitat segregation by sex. Access to water
313 could influence the relative cost of being exposed to more males in several ways. First, female
314 *C. maculatus* have a lower propensity to remate after they receive a large ejaculate (Fox,
315 Stillwell, Wallin, & Hitchcock, 2006; Miyatake & Matsumura, 2004), which increases their
316 fecundity (Moya-Laraño & Fox, 2006). Males with access to water might incorporate more into
317 their ejaculates and thereby transfer larger ejaculates. This would make remating less
318 beneficial for females and increase the net cost of being exposed to more males. Second,
319 females with direct access to water have less to gain from the hydration benefit of ejaculates,
320 again reducing the benefits of remating. If females are phenotypically plastic in their mating
321 behaviour, both mechanisms should reduce their propensity to mate multiply, decreasing the
322 number of costly copulations (albeit while simultaneously increasing the absolute material
323 benefits of obtaining more water if they do remate). Fewer copulations and the increased
324 acquisition of useful material in ejaculates should be beneficial for females. In support of the
325 benefit of greater access to water, although we cannot tease apart the importance of the two
326 mentioned mechanisms, we found that adult females with access to water lived significantly

327 longer than those without access. Previous studies have also found a fecundity benefit to
328 females provided with water (Edvardsson, 2007; Ursprung et al., 2009).

329 Unexpectedly, females housed with four males showed a similar reduction in offspring
330 production compared to those maintained alone or with one male regardless of whether or
331 not they had access to water. There was no detectable moderating effect of the environment
332 on the combined effects of male presence on female fitness due to changes in harassment,
333 mating rates and the material benefits of mating. However, a *post hoc* exploratory analysis
334 (suggested by a reviewer) suggests that there might be an effect of water availability on the
335 fitness cost of exposure to males when only considering females exposed to either no male or a
336 single male ($P = 0.036$ for the interaction) . Even so, we treat this result with caution because it
337 is based on an unplanned test promoted by the observed trend. Nonetheless, it suggests that
338 the effects of male exposure might be non-linear, which is worth exploring in future studies. In
339 general, we expect changes in the environment to alter the balance between the costs of
340 copulation and harassment, and any benefits of mating (e.g. Plath *et al.*, 2003; Edenbrow *et*
341 *al.*, 2011; Fox *et al.*, 2006) , and thereby affect female behavioural responses to male
342 harassment (e.g. Rowe *et al.*, 1994). Specifically, in seed beetles females with access to water
343 are less inclined to remate than those without access after short-term exposure to males
344 (Edvardsson, 2007; Ursprung et al., 2009), reducing the costs associated with excess
345 copulations. But, to our knowledge, what happens when females are continuously housed and
346 harassed by males has not yet been explored. One possibility is that in a wet environment
347 mating attempts by male *C. maculatus* are more frequent and longer chases ensue due to a
348 greater reluctance of females to mate. This might increase disturbance of females during
349 oviposition and induce females to engage in superfluous matings thereby ameliorating the
350 aforementioned benefits to females of greater access to water by adults. Future studies need
351 to experimentally manipulate water availability for each sex separately to determine how this
352 affects the rate of male harassment and the fitness of females. We did not pursue this

353 approach in the current study because we were primarily interested in the net effect of a
354 natural environmental change in water availability for adults. In the wild access to water
355 always changes for both sexes simultaneously.

356 *Female age effects*

357 Independently of any effect of greater exposure to males there was a reduction in both egg
358 laying rate and egg-adult survival over time. These findings are likely to be related to maternal
359 age (Fox, 1993). Previous studies have found that older females lay fewer, smaller eggs with
360 reduced eclosion success (e.g. Fox 1993, but see Moore and Harris 2003), presumably due to
361 the depletion of female breeding resources (Richards & Myers, 1980). Surprisingly, this age-
362 related reduction in the number of eggs laid and their eclosion success was significantly
363 greater for females with access to water. In general, females deprived of suitable oviposition
364 substrate lay fewer eggs (Messina & Fry, 2003), and egg-adult survival is reduced when host
365 beans are of lower quality (Fox, Waddell, & Mousseau, 1994). One explanation for our findings
366 is therefore that, while water is beneficial for adults, it increased the humidity in the test
367 containers reducing the quality of the beans for developing larvae, reducing egg-adult survival
368 (e.g. growth of fungus due to high humidity) and lowering the rate of egg laying. This
369 explanation can be tested by measuring offspring traits when reared on beans in a humid and
370 a dry environment.

371 *Conclusions*

372 In *C. maculatus* the environment can affect the materials transferred in ejaculates,
373 which should affect the fitness gained by females who remate, as well as the costs of male
374 harassment for females. In our study, despite previously demonstrated effects of water
375 availability on female fitness and male ejaculate properties, we found little evidence for a
376 difference in the relative fitness of females housed with or without water, as there was no
377 effect on our measure of lifetime egg production. More importantly, while being exposed to

378 more males significantly reduced female lifetime fecundity this negative effect was not
379 moderated by adult access to water. Future studies should investigate the, albeit artificial,
380 effect of experimentally induced sex-specific changes in water availability to disentangle how
381 this changes the costs and benefits for females of greater exposure to males. This might reveal
382 that simultaneous changes in costs and benefits cancel out. Such a study should also be
383 designed to tease apart the different effects of mating and resisting harassment on female
384 fitness. In addition, it is important to explore whether the balance between the costs and
385 benefits of male-female interactions change in more realistic and natural environments, where
386 sexual encounters are less frequent and hence male harm is potentially reduced (e.g. Yun *et*
387 *al.*, 2017).

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570 **APPENDIX 1.**

571 Model parameter estimates and test statistics:

572 Table A1. Effect of water availability and exposure to males on the lifetime number of eggs.

573 GLM model output from full (including interaction terms) and reduced (main effects only)

574 models (see text), and statistical comparisons of model fits. Significant values are in bold.

Lifetime number of eggs

	Estimate	Std. Error	t-value	p	
Full model					
(Intercept)	3.939	0.048	81.16	< 0.001	
Water (Yes)	0.129	0.067	1.93		
Male number (1 male)	0.062	0.068	0.91		
Male number (4 males)	-0.06	0.071	-0.848		
Water*Male number (1 male)	-0.201	0.097	-2.07	0.039	
Water*Male number (4 males)	-0.156	0.100	-1.56	0.119	
Reduced model					
(Intercept)	3.997	0.039	101.73	< 0.001	
Water (Yes)	0.015	0.041	0.38	0.707	
Male number (1 male)	-0.004	0.049	-0.76	0.444	
Male number (4 male)	-0.139	0.050	-2.77	0.006	
Comparison of fit					
Model	Residual (df)	Residual deviance	Deviance	F	p
Full	271	1830.9			
Reduced	273	1859.1	-28.194	2.378	0.095

575

576 Table A2. Effect of water availability and exposure to males, as well as days since mating on

577 rate of egg laying. GLMM model outputs from full (including interaction terms) and reduced

578 (main effects only) models and statistical comparison of model fits (see text). Significant values

579 are in bold.

Rate of egg laying

	Estimate	Std. Error	z-value	p
Full model				
(Intercept)	2.741	0.06	45.62	< 0.001
Water (yes)	0.103	0.085	1.21	
Male number (1 male)	-0.005	0.086	-0.05	
Male number(4 males)	0.017	0.087	0.19	
Day 3	-0.219	0.075	-2.92	
Water * Male number (1 male)	-0.152	0.123	-1.24	0.216
Water * Male number (4 males)	-0.179	0.123	-1.46	0.144
Water * Day 3	-0.232	0.108	-2.15	0.032
Male number (1 male) * Day 3	0.019	0.107	0.18	0.854

Male number (4 males) * Day 3	-0.008	0.108	-0.07	0.941
Water * Male number (1 male) * Day 3	0.139	0.155	0.90	0.368
Water * Male number (4 males) * Day 3	0.048	0.156	0.31	0.759
Reduced model				
(Intercept)	2.816	0.043	65.62	<0.001
Water (yes)	-0.084	0.041	-2.04	0.042
Male number (1 male)	-0.039	0.049	-0.78	0.433
Male number (4 males)	-0.064	0.052	-1.28	0.199
Day 3	-0.298	0.032	-9.17	<0.001

Comparison of fit

Model	df	Log-likelihood	Deviance	χ^2	p
Full	7	3649.2	-1802.5		
Reduced	14	3681.7	-1796.6	11.73	0.109

580

581 Table A3. Effect of water availability, exposure to males and days since mating on the
582 percentage of eggs emerging as adults. GLMM model outputs from full models (including
583 interaction terms). Significant values are in bold.

Egg to adult survival (i.e. eggs emerging as adults, %)

	Estimate	Std. Error	z-value	p
Full model				
(Intercept)	2.752	0.173	15.87	<0.001
Water (yes)	-0.892	0.225	-3.97	
Male number (1 male)	-0.331	0.239	-1.39	
Male number (4 males)	-0.407	0.238	-1.71	
Day 3	-0.054	0.206	-0.26	
Day 4	-1.270	0.159	-8.04	
Water*Male number (1 male)	-0.157	0.314	-0.50	0.617
Water*Male number (4 males)	-0.361	0.311	-1.16	0.246
Water*Day 3	-0.336	0.256	-1.31	0.191
Water*Day 4	0.738	0.200	3.68	<0.001
Male number (1 male)*Day 3	-0.101	0.276	-0.37	0.714
Male number (4 males)*Day 3	-0.089	0.275	-0.32	0.747
Male number (1 male)*Day 4	0.236	0.217	1.09	0.278
Male number (4 males)*Day 4	-0.053	0.102	-0.52	0.605
Water*Male number (1 male)*Day 3	0.557	0.349	1.59	0.111
Water*Male number (4 males)*Day 3	0.939	0.347	2.42	0.016
Water*Male number (1 male)*Day 4	0.247	0.281	0.88	0.379
Water*Male number (4 males)*Day 4	0.959	0.279	3.44	0.001

584

585 Table A4. Effect of water availability, exposure to males, and days since mating on offspring
586 development time. GLMM model outputs from full (including interaction terms) and reduced
587 (main effects only) models, and statistical comparison of model fits (see text). Significant
588 values are in bold.

Egg development time (days)

	Estimate	Std. Error	z-value	p	
Full model					
(Intercept)	3.237	0.028	114.3	<0.001	
Water (yes)	0.003	0.04	0.08		
Mating (1 male)	0.006	0.04	0.14		
Mating (4 males)	0.002	0.041	0.05		
Day 3	-0.001	0.040	-0.02		
Day 4	-0.002	0.040	-0.06		
Water*Male number (1 male)	-0.004	0.058	-0.07	0.948	
Water*Male number (4 males)	0.011	0.058	0.19	0.85	
Water*Day 3	0.003	0.057	0.06	0.952	
Water*Day 4	-0.017	0.057	-0.29	0.769	
Male number (1 male)*Day 3	-0.004	0.057	-0.07	0.947	
Male number (4 males)*Day 3	-0.007	0.058	-0.12	0.901	
Male number (1 male)*Day 4	-0.018	0.057	-0.32	0.747	
Male number (4 males)*Day 4	-0.013	0.058	-0.22	0.826	
Water*Male number (1 male)*Day 3	0.006	0.083	0.07	0.945	
Water*Male number (4 males)*Day 3	-0.009	0.083	-0.12	0.907	
Water* Male number (1 male)*Day 4	0.019	0.083	0.24	0.813	
Water*Male number (4 males)*Day 4	0.009	0.083	0.11	0.909	
Reduced model					
(Intercept)	3.239	0.017	193.94	<0.001	
Water (yes)	0.004	0.014	0.27	0.784	
Mating (1 male)	0.001	0.017	0.03	0.974	
Mating (4 males)	0.001	0.017	0.04	0.965	
Day 3	-0.003	0.017	-0.2	0.839	
Day4	-0.162	0.017	-0.96	0.338	
Comparison of fit					
	df	Log-likelihood	Deviance	χ^2	p
Model					
Full	7	-2079.1	4158.1		
Reduced	19	-2078.8	4157.7	0.4516	1

589

590 Table A5. Effect of water availability and exposure to males on female survival. Cox
 591 proportional hazard model outputs from full (including interaction terms) and reduced (main
 592 effects only) models and statistical comparison of model fits (see text). Significant values are in
 593 bold.

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Female survival (days)

	Coefficient	Std.Error (coef)	z-value	p
Full model				
Water (yes)	-0.296	0.069	-4.31	< 0.001

Male number (1 male)	0.007	0.063	0.11	0.91
Male number (4 males)	0.094	0.062	1.51	0.13
Water*Male number (1 male)	0.067	0.097	0.68	0.49
Water*Male number (4 males)	0.016	0.096	0.17	0.87
Reduced model				
Water (yes)	-0.269	0.039	-6.82	< 0.001
Male number (2 males)	0.035	0.048	0.72	0.469
Male number (4 males)	0.1	0.047	2.11	0.035
Comparison of fit				
Model	loglik	χ^2	df	p
Full	-18201			
Reduced	-18201	0.51	2	0.775

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601 Figure 1. a) Total number of eggs, and b) number of eggs laid by females on each of the two
602 days after copulation in relation to the exposure to males (0, 1, 4 males) and the water
603 treatment (dry, wet) (D0: dry, no males; W0: wet, no males; D1: dry, one male; W1: wet, one
604 male; D4: dry, four males; W4: wet, four males) (Mean \pm SE).

605

606 Figure 2. Percentage of emerging adults (egg-adult survival) in each of the three days after
607 female copulation in relation to the mating and water treatments (Mean \pm SE).

608

609 Figure 3. Time for offspring development for each of the four days after female copulation in
610 relation to the mating and water treatments (Mean \pm SE).

611

612 Figure 4. Kaplan-Meier survival plots for females maintained in dry or wet conditions. There
613 are differences in female survival in relation to water availability, but not of male number nor
614 any interaction between the two treatments (see text).

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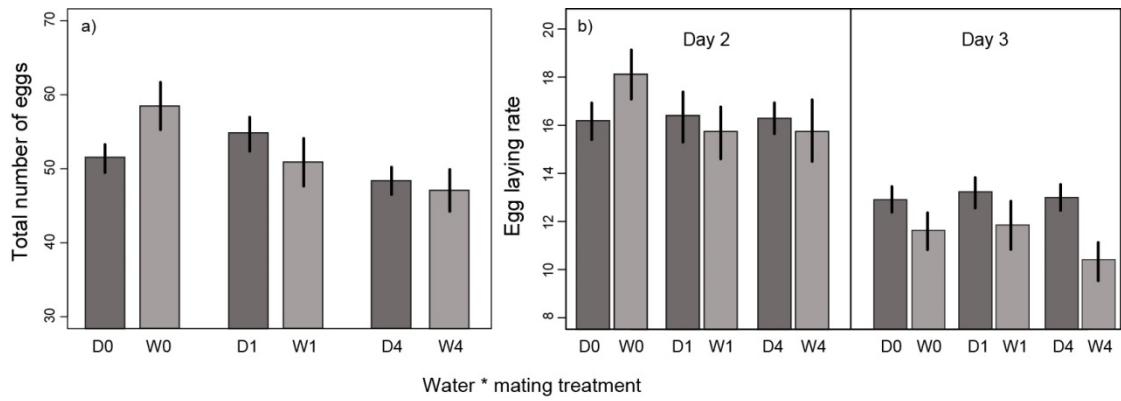
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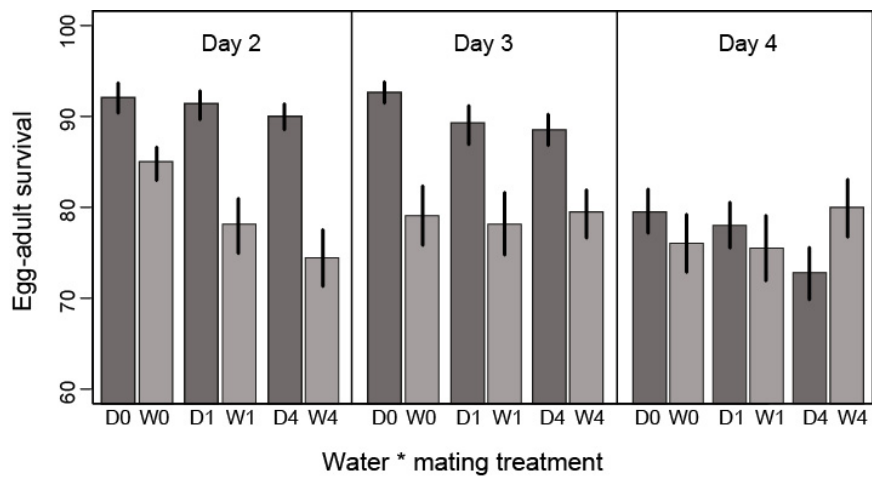
623 Fig 1



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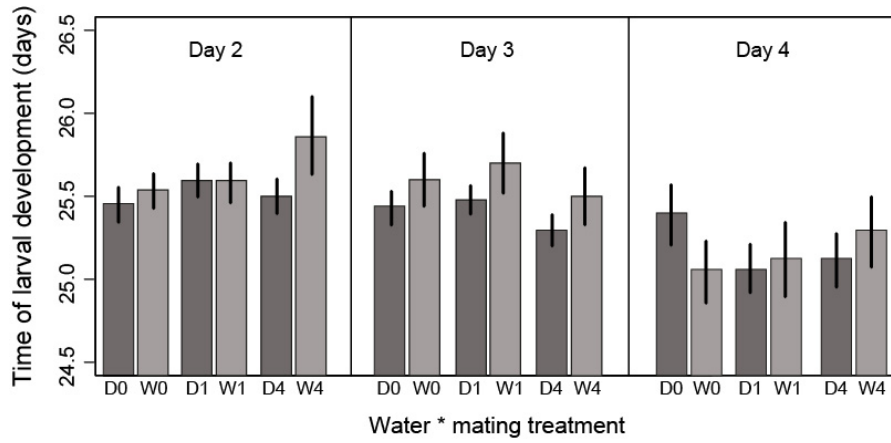
625 Fig 2

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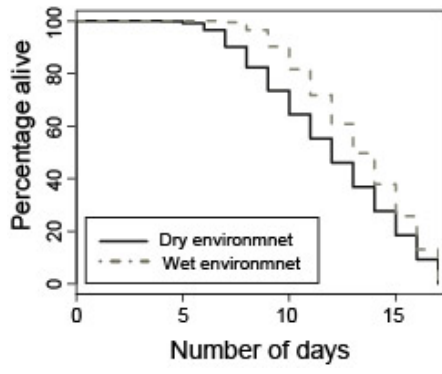
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628 Fig 3



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630 Fig 4



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