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## Single and multiple mating reduces longevity of female dumpling squid (*Euprymna tasmanica*)

### Abstract

For many species, mating is a necessary yet costly activity. The costs involved can have an important influence on the evolution of life histories and senescence. Females of many species mate multiply and this behaviour can inflict a longevity cost. Most studies investigating the effects of multiple mating on female survival have been conducted on insects and the effects in other taxa are largely unknown. We investigate the effects of both a single mating and a second mating on longevity in female dumpling squid (*Euprymna tasmanica*), a species in which both sexes mate multiply. Through comparing the longevity of virgin, once-mated and twice-mated females, we found that a single mating reduced female lifespan by 15 days on average. A second mating resulted in an additional 8 day (on average) longevity cost, despite no difference in total clutch mass, number of clutches, single egg mass or number of eggs per clutch between once-mated and twice-mated females. This demonstrates a cost to multiple mating which may be independent of the cost of egg production. Furthermore, total clutch mass and female lifespan were positively correlated, whilst female lifespan decreased with increasing average water temperature. The presence of an additive effect of reproduction on longevity suggests that multiple mating in cephalopods may have benefits that outweigh these costs, or that there is a conflict in optimal mating frequency between males and females.

**Keywords:** mating, cost, cephalopod, lifespan, survival, trade-off

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## 33 1. Introduction

34

35 Organisms allocate resources to both survival and reproduction throughout their lifetime to  
36 maximise fitness (Barnes & Partridge, 2003, Partridge & Harvey, 1988, Williams, 1957). However,  
37 there is generally a trade-off between survival and reproduction such that it is not possible to  
38 maximise both (Reznick, 1992). Assessing this trade-off is crucial to understand the evolution of life  
39 history strategies, ageing and reproductive behaviours. Many studies demonstrate that lifespan is  
40 decreased when individuals invest in activities related to reproduction (Golet et al., 1998, Andrade,  
41 1996, Chapman et al., 1995, Gems & Riddle, 1996). A classic example is Clutton-Brock et al's (1989)  
42 research demonstrating that lactation has a negative effect on overwinter survival of female red  
43 deer (Golet et al., 1998). In addition to parental care, other reproductive behaviours can decrease  
44 lifespan, including mate search (Andrade, 2003), courtship (Cordts & Partridge, 1996, Clutton-Brock  
45 & Langley, 1997) and mating (Kimura & Chiba, 2015). Currently, the majority of research into some  
46 of these costs, for example mating costs, has been conducted in insects (Chapman et al., 1998,  
47 Vahed, 1998, Martin & Hosken, 2004). This limits our ability to make generalisations regarding the  
48 evolution of life history strategies.

49 Mating is an essential component of reproduction for many species, yet there are a variety  
50 of costs associated with mating for both males and females. These costs could be inflicted through  
51 disease transfer (Knell & Webberley, 2004), energy allocation (Franklin et al., 2012), and injury (Stutt  
52 & Siva-Jothy, 2001). In extreme cases, one individual in the pair may even die (Andrade, 1996). These  
53 costs can differ between the sexes and cause a conflict in optimal mating frequency. There are  
54 several examples of mating costs differentially affecting males and females: female praying mantids  
55 (*Pseudomantis albofimbriata*) can cannibalise males (Barry et al., 2008), male bed bugs (*Cimex*  
56 *lectularius*) traumatically inseminate females (Morrow & Arnqvist, 2003) and male crickets (*Gryllus*  
57 *campestris*) are more likely to be predated upon than females (Rodríguez-Muñoz et al., 2011). In  
58 many cases, it is necessary for the female to live longer after copulation so that she has time to  
59 produce offspring. Thus, mating costs may be particularly important in shaping the optimal  
60 reproductive strategy for females and could help us to understand conflicts between the sexes in  
61 mating frequency and the evolution of polygamy.

62 Mating costs in females can be difficult to assess because mating is generally tied to egg or  
63 offspring production. As such, there are only a handful of studies investigating the cost of mating  
64 independently of the costs of egg/offspring production (Gems & Riddle, 1996, Bateman et al., 2006,  
65 Chapman et al., 1998, Schrepf et al., 2005). These studies tend to assess the cost of mating by  
66 comparing single-mated and multiple-mated females. The results vary, from a decrease in longevity

67 (Chapman et al., 1995, Gems & Riddle, 1996, Bateman et al., 2006), to no effect detected (Martin &  
68 Hosken, 2004, Kotiaho & Simmons, 2003) or even an increase in longevity (Vahed, 1998, Wagner &  
69 Harper, 2003, Schrempf et al., 2005). Whilst there can be many benefits of multiple mating for  
70 females (Arnqvist & Nilsson, 2000, Squires et al., 2012, Svärd & McNeil, 1994), the optimal  
71 reproductive strategy is likely to be a balance of these benefits against costs, such as a longevity  
72 cost.

73 Cephalopods are an ideal group to research mating costs because they mate multiply  
74 (Hanlon et al., 1999, Squires et al., 2014, Emery et al., 2001), are generally short lived and perform  
75 copulatory behaviours which may be costly to females. These behaviours include traumatic  
76 insemination in argonauts (Wells & Wells, 1977), prolonged copulations in dumpling squid (Franklin  
77 et al., 2012) and continuation of copulation in the presence of predators (Franklin et al., 2014). Here,  
78 we use dumpling squid, *Euprymna tasmanica* (Pfeffer, 1884), to assess the effects of single and  
79 multiple mating on female longevity. Dumpling squid are a short lived (5 – 8 months; Sinn &  
80 Moltschaniwskyj, 2005), small (4 – 7cm) squid species found in shallow waters off the southern coast  
81 of Australia. Mating is energetically costly (Franklin et al., 2012) and can last up to 3 hours (mean  $\pm$   
82 SEM:  $86.1 \pm 7.6$  min; range: 48.3 – 184.2 min; Franklin et al., 2012). Females tend to lay multiple  
83 clutches of eggs for several days after mating (Squires et al., 2013), but generally do not provide care  
84 for the offspring. Both sexes mate multiply; in the wild a single clutch of eggs can have up to four  
85 sires (Squires et al., 2014). Females that mate multiply produce larger hatchlings relative to egg mass  
86 and lay clutches at a faster rate (although they lay the same total number of eggs; Squires et al.,  
87 2012). By comparing the adult lifespan of virgin, once-mated and twice-mated female dumpling  
88 squid, we investigated the hypotheses that once-mated females will experience a longevity cost and  
89 that twice-mated females will experience an additional reduction in longevity.

90

## 91 **2. Methods**

92

### 93 *2.1 Squid culture*

94

95 We collected juvenile female and adult male *Euprymna tasmanica* from December 2010  
96 until February 2011 during multiple shallow (< 5m) night SCUBA dives at Clifton Springs (38°09'18S,  
97 144°34'03E) and St Leonards (38°10'13S, 144°43'11E) in Port Phillip Bay, Victoria, Australia. Upon  
98 capture, squid were transferred to facilities at the Victorian Marine Science Consortium (VMSC) in  
99 Queenscliff. Here, we housed juvenile females individually in round plastic buckets (diameter x  
100 height: 20 x 19 cm, volume = 6.0 L) until they reached sexual maturity (see below) and were then

101 relocated to larger glass tanks (length x width x height: 24 x 24 x 24 cm, volume = 13.8 L). Males  
102 were housed individually in glass tanks. Each holding aquarium contained a layer of sand substrate  
103 and a short (diameter x length: 5.5 x 6.5 cm) length of PVC pipe for shelter and in which females  
104 could lay eggs. Aquarium lights provided a reverse 12:12 h day/night cycle and all aquaria received a  
105 constant flow of aerated, ambient temperature (13-21°C) seawater pumped directly from Port  
106 Phillip Bay. We fed squid *Palaemon shrimp ad libitum*, checked them and recorded the water  
107 temperature every second day.

108

## 109 2.2 Experimental protocols

110

111 We investigated the effect of single and multiple mating on adult lifespan (sexual maturity  
112 until death) because all female squid were captured as juveniles. To determine the onset of sexual  
113 maturity, juvenile females were monitored every day for the appearance of the accessory  
114 nidamental gland. This gland forms part of the female reproductive tract and is responsible for  
115 providing a protective outer coating to the egg prior to laying (Norman & Lu, 1997). During sexual  
116 maturity the gland develops a coral red colour (Bloodgood, 1977) that can be observed through the  
117 translucent ventral wall (Figure 1). The day the gland was first visible was recorded as day one of the  
118 squid's adult lifespan. On this day, squid were blotted and weighed and moved to a larger tank. To  
119 ensure females had completed sexual maturation, we allowed enough time for them to reach adult  
120 size (45 d) before administering a mating treatment.

121 On day 45, females were randomly allocated to one of three treatment groups; virgin ( $n =$   
122 11), once-mated ( $n = 10$ ) or twice-mated ( $n = 10$ ). Each female was blotted and weighed before  
123 being placed in the mating chamber (10 x 10 x 11.5 cm, volume = 1.2 L) and allowed 10 minutes to  
124 acclimate. This chamber had constant flowing seawater. For the once-mated and twice-mated  
125 treatments, a wild caught, adult, male squid was added to the mating chamber and squid were left  
126 to mate. If mating had not commenced after 10 minutes, the female was gently disturbed to allow  
127 the male to initiate copulation. On 15 occasions, squid would not mate for unknown reasons. If this  
128 occurred, we removed the male and 10 minutes later added a different male. Squid were monitored  
129 every 15 minutes until the conclusion of mating and then moved back to their holding tanks. Two  
130 hours after this, they were returned to the mating chamber and the same procedure was followed.  
131 However, this time, only females in the twice-mated treatment were paired with a male. All females  
132 allocated to the once-mated treatment mated with a male and all those allocated to the twice-  
133 mated treatment mated with the second male. Mating durations were similar to previous  
134 experiments (range: 48.3 – 184.2 min; Franklin et al., 2012). Males were not mated more than once

135 per fortnight to allow for spermatophore replenishment. After treatment, we monitored squid every  
136 second day for eggs and to check survival. Every clutch was weighed and clutch masses were  
137 summed for each squid to calculate total clutch mass across lifespan. We also counted number of  
138 eggs in each clutch and weighed ten randomly selected eggs to calculate average single egg mass.  
139 Eggs from virgins were kept in brooding chambers to confirm they were infertile. Two 'virgin'  
140 females laid multiple fertile clutches indicating they had mated in the wild before sexual maturity  
141 and stored the sperm. These females were excluded from the analysis. Final sample sizes were virgin  
142 = 9, once-mated = 10 and twice-mated = 10.

143

### 144 2.3 Statistical analysis

145

146 All statistical analyses were conducted in R v. 3.1.1 (R Core Team, 2014). We used a negative  
147 binomial generalised linear model (GLM; MASS package; Venables & Ripley, 2002) to assess the  
148 effect of treatment (virgin, once-mated, twice-mated) on adult lifespan. To investigate factors  
149 affecting adult lifespan we included treatment, total clutch mass and average water temperature in  
150 the lab (*i.e.* throughout adult lifespan) as fixed effects. We also investigated if the proportion of  
151 dumpling squid that laid eggs differed between treatment groups, and, for the dumpling squid that  
152 laid eggs, we determined if total clutch mass, number of clutches, number of eggs per clutch or  
153 single egg mass differed between treatment groups. To investigate if proportion of dumpling squid  
154 laying eggs, total clutch mass or number of eggs per clutch varied between the treatment groups, we  
155 ran GLMs with treatment as the independent variable. Generalised linear mixed models (GLMM)  
156 were used to assess the effect of treatment on number of clutches and single egg mass. Squid ID was  
157 included as a random effect for 'number of clutches' to account for overdispersion, and for 'single  
158 egg mass' because single egg mass was calculated for each clutch and 13 squid laid more than one  
159 clutch. Probability error distributions used were binomial (logit link) for proportion of squid laying  
160 eggs, gamma (log link) for total clutch mass and single egg mass, Gaussian (identity link) for number  
161 of eggs per clutch, and Poisson (log link) for number of clutches. We also ran a Gaussian GLM  
162 (identity link) to determine if weight at maturity differed between treatment groups. For all  
163 statistical analyses, we assessed the significance of each term in the models using Wald chi-squared  
164 tests (R: Anova). Significant terms were investigated further using z-tests (adult lifespan, proportion  
165 that laid eggs, number of clutches) or t-tests (single egg mass) to determine which groups differed  
166 from one another. Final model fit was checked using residual plots.

167

168 This study was carried out with approval from the University of Melbourne Animal Ethics  
Committee (ID: 0810874.3) and followed recommendations for the ethical use of cephalopods in

169 scientific experiments outlined in Moltschaniwskyj et al. (2007). All animals were collected under  
170 Fisheries Victoria collecting permits (permit number: RP962).

171

### 172 3. Results

173

174 Female lifespan was significantly affected by treatment (virgin, once-mated or twice-mated;  
175  $\chi^2 = 24.5$ ,  $df = 2$ ,  $p < 0.001$ ), total clutch mass ( $\chi^2 = 47.8$ ,  $df = 1$ ,  $p < 0.001$ ) and average temperature  
176 ( $\chi^2 = 6.7$ ,  $df = 1$ ,  $p = 0.01$ ). Further investigation revealed that virgin females lived for 15 days longer  
177 on average than once-mated females ( $z_{24} = 3.12$ ,  $p = 0.002$ ), and once-mated females lived 8 days  
178 longer on average than twice-mated females ( $z_{24} = 2.06$ ,  $p = 0.040$ ; Figure 2). Female lifespan  
179 increased with total clutch mass (Figure 3a), whilst average temperature was weakly negatively  
180 correlated with lifespan (Figure 3b).

181 There was a difference in the proportion of squid that laid eggs across treatment groups ( $\chi^2$   
182  $= 6.69$ ,  $df = 2$ ,  $p = 0.035$ ); fewer virgin females laid eggs than once-mated females ( $z_{26} = 1.94$ ,  $p =$   
183  $0.05$ ) and there was no difference between once-mated and twice-mated females ( $z_{26} = 0.00$ ,  $p =$   
184  $1.00$ ). For the squid that laid eggs, we detected a difference between the treatment groups in  
185 number of clutches laid ( $\chi^2 = 13.60$ ,  $df = 2$ ,  $p = 0.001$ ), and single egg mass ( $\chi^2 = 14.36$ ,  $df = 2$ ,  $p <$   
186  $0.001$ ). Virgins laid fewer clutches and had lower single egg mass than once-mated females (number  
187 of clutches:  $z_{25} = 3.68$ ,  $p < 0.001$ ; single egg mass:  $t_{67} = 2.70$ ,  $p = 0.007$ ;). However, there was no  
188 significant difference in number of clutches or single egg mass between once-mated and twice-  
189 mated females (number of clutches:  $z_{25} = 1.01$ ,  $p = 0.31$ ; single egg mass:  $t_{67} = 1.63$ ,  $p = 0.10$ ). There  
190 was no effect of treatment on total clutch mass ( $\chi^2 = 4.51$ ,  $df = 2$ ,  $p = 0.10$ ), number of eggs per  
191 clutch ( $\chi^2 = 4.71$ ,  $df = 2$ ,  $p = 0.09$ ) and no difference between the treatment groups in weight at  
192 maturity ( $\chi^2 = 0.53$ ,  $df = 2$ ,  $p = 0.60$ ; Table1).

193

### 194 4. Discussion

195

196 Our results demonstrate that there is a longevity cost for once-mated females and an  
197 additional longevity cost for twice-mated females. There was no increase in total clutch mass,  
198 number of clutches, number of eggs per clutch or single egg mass between once-mated and twice-  
199 mated females, suggesting increased investment in eggs is not responsible for the additional  
200 longevity cost. We also detected a positive correlation between female lifespan and total clutch  
201 mass and a weak negative correlation between female lifespan and average water temperature.  
202 Single and multiple mating may have similar longevity costs for females in other cephalopod species

203 because many cephalopods mate multiply (Hanlon et al., 1999, Squires et al., 2014, Emery et al.,  
204 2001) and perform costly copulatory behaviours (Wells & Wells, 1977, Franklin et al., 2012, Franklin  
205 et al., 2014). Although mating multiply could provide benefits to females (Squires et al 2012), a  
206 longevity cost could lead to a conflict between males and females in optimal mating frequency.

207 Singly mated females lived, on average, 15 days less than virgin females. This reduction in  
208 longevity could be related to energy expenditure, disease, injury, physiological changes or egg  
209 production costs. Dumping squid experience an energetic cost of copulation (Franklin et al., 2012);  
210 but they recover from this cost within 30 minutes. Therefore, it seems unlikely that this energetic  
211 cost would cause a 15 day decrease in longevity. Injuries have been reported in other cephalopods  
212 and may occur in *E. tasmanica*. Males insert their hectocotylus into the female's mantle cavity and  
213 leave it there for the duration of mating. Upon conclusion, males roughly remove the hectocotylus  
214 with several forceful movements (Squires et al., 2013). These could cause internal damage to the  
215 females, particularly because the suckers on the distal portion of the hectocotylus have a toothed  
216 sucker ring (Norman & Lu, 1997). Disease is also possible because the females were raised  
217 individually in the lab, albeit from wild-caught juvenile stage, whereas the males were wild caught as  
218 adults. Males may have carried pathogens into the laboratory that females had not encountered  
219 before (Padros et al., 2001, Kik et al., 2011). Investment into immune function could also be affected  
220 and may increase or decrease with reproduction (Nunn et al., 2000, Rolff & Siva-Jothy, 2002). This  
221 could increase metabolic costs or susceptibility to diseases, respectively. Mating can cause other  
222 physiological changes, such as endocrine changes (Fernández-Guasti et al., 2010, Shi & Murphy,  
223 2014) and alterations to gene expression (McGraw et al., 2008, Dalton et al., 2010). These changes  
224 are likely to redirect investment from survival and growth to processes relating to reproduction.  
225 Physiological changes related to egg production may occur after a single mating in dumping squid.  
226 We observed more single-mated dumping squid laid eggs than virgins and single egg mass was  
227 greater in once-mated females compared to virgin females. Whilst we did take total clutch mass into  
228 account in our statistical model, egg production may still influence longevity. This could occur if non-  
229 fertilised and fertilised eggs inflict differing costs on females (David, 1963) or if egg production has a  
230 greater effect than accounted for in our model (Reznick, 1992). In our experiment virgin females  
231 produced eggs of smaller mass, which may suggest differing costs between fertilised and non-  
232 fertilised eggs. Further investigation into physiological costs of egg production would provide insight  
233 into the longevity cost of a single mating.

234 Multiple mating also negatively influences longevity in dumping squid. Once-mated females  
235 lived longer, on average, than twice-mated females, despite no difference in total clutch mass. This  
236 result aligns with preliminary trials where females that were mated three times (in three days)

237 would often die after the third mating. However, studies conducted in the laboratory may  
238 exacerbate costs associated with multiple mating because females do not have the ability to escape  
239 males. Even so, our results indicate that one or several factors can have an additive impact on  
240 female survival. Factors that could have a cumulative effect with multiple copulations include injury,  
241 seminal toxins, disease, metabolic costs or egg production. Chapman *et al.* (1995) demonstrated in  
242 *Drosophila melanogaster* that male seminal fluid is responsible for a reduction in female lifespan and  
243 that increasing exposure had an additive effect. In cephalopods, males transfer large bundles of  
244 spermatophores to females. These may contain products that are detrimental to female lifespan;  
245 however, this has never been assessed. As mentioned above, injuries are possible in *E. tasmanica*.  
246 Injury could have a cumulative effect if additional damage is caused during the second mating  
247 (Blanckenhorn *et al.*, 2002). Disease could also have a cumulative effect on lifespan if not all males in  
248 the population carry a pathogen. In this case, mating more than once increases the likelihood that a  
249 female will mate with an infected male or be infected with more than one type of pathogen (Roberts  
250 *et al.*, 2015). It is possible that egg production costs differ between once-mated and twice-mated  
251 females. However, we found no difference in total clutch mass, number of clutches, single egg mass  
252 or number of eggs per clutch between once-mated and twice-mated females. Additionally, a  
253 previous study in dumpling squid found no difference in proportion of eggs hatched between once-  
254 mated and twice-mated females, suggesting similar fertilisation success (Squires *et al.*, 2012).  
255 Therefore, we suggest that the reduction in longevity associated with a second mating is unlikely due  
256 to egg production.

257 In our study, female longevity was related to other factors besides mating. Longer female  
258 lifespan was weakly associated with cooler average water temperatures over their adult life, and  
259 increased total clutch mass. Cooler temperatures can reduce metabolic rate (Brown *et al.*, 2004),  
260 thus reducing growth and delaying the onset of sexual maturity (Sudo, 2003). This decrease in  
261 metabolism can prolong lifespan, which could explain why female dumpling squid raised in cooler  
262 temperatures may live longer. However, this relationship was quite weak and should be interpreted  
263 carefully. The positive correlation between longevity and total clutch mass was stronger. This  
264 association may be because females that live longer lay eggs over a longer period or it may indicate  
265 that higher quality females live longer and lay more eggs.

266 The major finding of this experiment, that both once-mated and twice-mated females  
267 experience a reduction in longevity, suggests that multiple mating is costly for female dumpling  
268 squid. This could indicate a conflict between males and females in optimal mating frequency. It is  
269 likely that a female obtains enough sperm from a single copulation to fertilise all her eggs because  
270 there is no difference in hatching success, the number of clutches or total number of eggs produced

271 by dumping squid females mated once or twice in the lab (Squires et al., 2012). However, females  
272 that mate multiply produce eggs faster and have larger hatchlings relative to egg mass (Squires et  
273 al., 2012). Larger hatchling size could increase offspring survival, improving fitness of multiply mated  
274 females (Tamada & Iwata, 2005, Fox & Czesak, 2000), despite the longevity cost. The mechanisms  
275 responsible for the potential fitness benefits of multiple mating in *E. tasmanica* remain unclear. For  
276 example, fertilisation may be biased towards males with compatible genes ('genetic compatibility'  
277 hypothesis; for reviews see Simmons, 2005, Tregenza & Wedell, 2000), intrinsically good genes  
278 ('good genes' hypothesis; for reviews see Jennions & Petrie, 2000, Yasui, 1997) or both. Further  
279 investigation into dumping squid survival in the wild is needed to determine if laying eggs faster and  
280 producing larger hatchlings translates into increased fitness. Nonetheless, the results reported here,  
281 and previous research demonstrating an energetic cost to copulation (Franklin et al 2012), clearly  
282 demonstrate that single and multiple mating is costly for female dumping squid.

283

## 284 **References**

285

286 Andrade, M. C. B. 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science*  
287 **271**: 70-72.

288 Andrade, M. C. B. 2003. Risky mate search and male self-sacrifice in redback spiders. *Behav. Ecol.* **14**:  
289 531-538.

290 Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in  
291 insects. *Anim. Behav.* **60**: 145-164.

292 Barnes, A. I. & Partridge, L. 2003. Costing reproduction. *Anim. Behav.* **66**: 199-204.

293 Barry, K. L., Holwell, G. I. & Herberstein, M. E. 2008. Female praying mantids use sexual cannibalism  
294 as a foraging strategy to increase fecundity. *Behav. Ecol.* **19**: 710-715.

295 Bateman, P. W., Ferguson, J. W. H. & Yetman, C. A. 2006. Courtship and copulation, but not  
296 ejaculates, reduce the longevity of female field crickets (*Gryllus bimaculatus*). *J. Zool.* **268**:  
297 341-346.

298 Blanckenhorn, W. U., Hosken, D. J., Martin, O. Y., Reim, C., Teuschl, Y. & Ward, P. I. 2002. The costs  
299 of copulating in the dung fly *Sepsis cynipsea*. *Behav. Ecol.* **13**: 353-358.

300 Bloodgood, R. A. 1977. The squid accessory nidamental gland: Ultrastructure and association with  
301 bacteria. *Tissue Cell* **9**: 197-208.

302 Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. & West, G. B. 2004. Toward a metabolic theory  
303 of ecology. *Ecology* **85**: 1771-1789.

- 304 Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. 1995. Cost of mating in  
305 *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*  
306 **373**: 241-244.
- 307 Chapman, T., Miyatake, T., Smith, H. K. & Partridge, L. 1998. Interactions of mating, egg production  
308 and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. *Proc. Roy. Soc.*  
309 *Lond. B.* **265**: 1879-1894.
- 310 Clutton-Brock, T. & Langley, P. 1997. Persistent courtship reduces male and female longevity in  
311 captive tsetse flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). *Behav.*  
312 *Ecol.* **8**: 392-395.
- 313 Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. 1989. Fitness costs of gestation and lactation in  
314 wild mammals. *Nature* **337**: 260-262.
- 315 Cordts, R. & Partridge, L. 1996. Courtship reduces longevity of male *Drosophila melanogaster*. *Anim.*  
316 *Behav.* **52**: 269-278.
- 317 Dalton, J., Kacheria, T., Knott, S., Lebo, M., Nishitani, A., Sanders, L., Stirling, E., Winbush, A. &  
318 Arbeitman, M. 2010. Dynamic, mating-induced gene expression changes in female head and  
319 brain tissues of *Drosophila melanogaster*. *BMC Genomics* **11**: 541.
- 320 David, J. 1963. Influence de la fécondation de la femelle sur le nombre et la taille des œufs pondus. *J.*  
321 *Insect Physiol.* **9**: 13-24.
- 322 Emery, A. M., Wilson, I. J., Craig, S., Boyle, P. R. & Noble, L. R. 2001. Assignment of paternity groups  
323 without access to parental genotypes: multiple mating and developmental plasticity in squid.  
324 *Mol. Ecol.* **10**: 1265-1278.
- 325 Fernández-Guasti, A., Arteaga-López, P. & Antonio-Cabrera, E. 2010. Copulation modifies AR and ER $\alpha$   
326 mRNA expression in the male rat brain. *Physiol. Behav.* **101**: 738-745.
- 327 Fox, C. W. & Czesak, M. E. 2000. Evolutionary ecology of progeny size in arthropods. *Annu. Rev.*  
328 *Entomol.* **45**: 341-369.
- 329 Franklin, A. M., Squires, Z. E. & Stuart-Fox, D. 2012. The energetic cost of mating in a promiscuous  
330 cephalopod. *Biol. Letters* **8**: 754-756.
- 331 Franklin, A. M., Squires, Z. E. & Stuart-Fox, D. 2014. Does predation risk affect mating behavior? An  
332 experimental test in dumpling squid (*Euprymna tasmanica*). *PLoS ONE* **9**: e115027.
- 333 Gems, D. & Riddle, D. L. 1996. Longevity in *Caenorhabditis elegans* reduced by mating but not by  
334 gamete production. *Nature* **379**: 723-725.
- 335 Golet, G. H., Irons, D. B. & Estes, J. A. 1998. Survival costs of chick rearing in black-legged kittiwakes.  
336 *J. Anim. Ecol.* **67**: 827-841.

- 337 Hanlon, R. T., Ament, S. A. & Gabr, H. 1999. Behavioural aspects of sperm competition in cuttlefish,  
338 *Sepia officinalis* (Sepioidea: Cephalopoda). *Mar. Biol.* **134**: 719-728.
- 339 Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits.  
340 *Biol. Rev.* **75**: 21-64.
- 341 Kik, M. J. L., Janse, M. & Benz, G. W. 2011. The sea louse *Lepeophtheirus acutus* (Caligidae,  
342 Siphonostomatoida, Copepoda) as a pathogen of aquarium-held elasmobranchs. *J. Fish Dis.*  
343 **34**: 793-799.
- 344 Kimura, K. & Chiba, S. 2015. The direct cost of traumatic secretion transfer in hermaphroditic land  
345 snails: individuals stabbed with a love dart decrease lifetime fecundity. *Proc. Roy. Soc. Lond.*  
346 *B.* **282**.
- 347 Knell, R. J. & Webberley, K. M. 2004. Sexually transmitted diseases of insects: distribution, evolution,  
348 ecology and host behaviour. *Biol. Rev.* **79**: 557-581.
- 349 Kotiaho, J. S. & Simmons, L. W. 2003. Longevity cost of reproduction for males but no longevity cost  
350 of mating or courtship for females in the male-dimorphic dung beetle *Onthophagus binodis*.  
351 *J. Insect Physiol.* **49**: 817-822.
- 352 Martin, O. Y. & Hosken, D. J. 2004. Copulation reduces male but not female longevity in *Saltella*  
353 *sphondylli* (Diptera: Sepsidae). *J. Evol. Biol.* **17**: 357-362.
- 354 McGraw, L. A., Clark, A. G. & Wolfner, M. F. 2008. Post-mating gene expression profiles of female  
355 *Drosophila melanogaster* in response to time and to four male accessory gland proteins.  
356 *Genetics* **179**: 1395-1408.
- 357 Moltschaniwskyj, N., Hall, K., Lipinski, M., Marian, J., Nishiguchi, M., Sakai, M., Shulman, D., Sinclair,  
358 B., Sinn, D., Staudinger, M., Van Gelderen, R., Villanueva, R. & Warnke, K. 2007. Ethical and  
359 welfare considerations when using cephalopods as experimental animals. *Rev. Fish Biol.*  
360 *Fisher.* **17**: 455-476.
- 361 Morrow, E. H. & Arnqvist, G. 2003. Costly traumatic insemination and a female counter-adaptation  
362 in bed bugs. *Proc. Roy. Soc. Lond. B.* **270**: 2377-2381.
- 363 Norman, M. D. & Lu, C. C. 1997. Redescription of the southern dumpling squid *Euprymna tasmanica*  
364 and a revision of the genus *Euprymna* (Cephalopod: Sepioidae). *J. Mar. Biol. Assoc. UK* **77**:  
365 1109-1137.
- 366 Nunn, C. L., Gittleman, J. L. & Antonovics, J. 2000. Promiscuity and the primate immune system.  
367 *Science* **290**: 1168-1170.
- 368 Padros, F., Palenzuela, O., Hispano, C., Tosas, O., Zarza, C., Crespo, S. & Alvarez-Pellitero, P. 2001.  
369 *Myxidium leei* (Myxozoa) infections in aquarium-reared Mediterranean fish species. *Dis.*  
370 *Aquat. Organ.* **47**: 57-62.

- 371 Partridge, L. & Harvey, P. H. 1988. The ecological context of life history evolution. *Science* **241**: 1449-  
372 1455.
- 373 Pfeffer, G. 1884. Die cephalopoden des Hamburger Natshistorischen Museums [The cephalopods of  
374 the Hamburg Natural History Museum]. *Abhandlungen des Naturwissen Vereins in Hamburg*  
375 **8**: 63-90.
- 376 R Core Team (2014) R: A Language and Environment for Statistical Computing. R Foundation for  
377 Statistical Computing, Vienna, Austria.
- 378 Reznick, D. 1992. Measuring the costs of reproduction. *TREE* **7**: 42-45.
- 379 Roberts, K., Evison, S., Baer, B. & Hughes, W. 2015. The cost of promiscuity: sexual transmission of  
380 *Nosema* microsporidian parasites in polyandrous honey bees. *Sci. Rep.* **5**.
- 381 Rodríguez-Muñoz, R., Bretman, A. & Tregenza, T. 2011. Guarding males protect females from  
382 predation in a wild insect. *Curr. Biol.* **21**: 1716-1719.
- 383 Rolff, J. & Siva-Jothy, M. T. 2002. Copulation corrupts immunity: A mechanism for a cost of mating in  
384 insects. *P. Natl. Acad. Sci.* **99**: 9916-9918.
- 385 Schrempf, A., Heinze, J. & Cremer, S. 2005. Sexual cooperation: Mating increases longevity in ant  
386 queens. *Curr. Biol.* **15**: 267-270.
- 387 Shi, C. & Murphy, C. T. 2014. Mating induces shrinking and death in *Caenorhabditis* mothers. *Science*  
388 **343**: 536-540.
- 389 Simmons, L. W. 2005. The evolution of polyandry: sperm competition, sperm selection, and offspring  
390 viability. *Annu. Rev. Ecol. Evo. S.* **36**: 125-146.
- 391 Sinn, D. L. & Moltschaniwskyj, N. A. 2005. Personality traits in dumpling squid (*Euprymna*  
392 *tasmanica*): Context-specific traits and their correlation with biological characteristics. *J.*  
393 *Comp. Psychol.* **119**: 99-110.
- 394 Squires, Z., Wong, B., Norman, M. & Stuart-Fox, D. 2014. Multiple paternity but no evidence of  
395 biased sperm use in female dumpling squid *Euprymna tasmanica*. *Mar. Ecol. Prog. Ser.* **511**:  
396 93-103.
- 397 Squires, Z. E., Norman, M. D. & Stuart-Fox, D. 2013. Mating behaviour and general spawning  
398 patterns of the southern dumpling squid *Euprymna tasmanica* (Sepiolidae): a laboratory  
399 study. *J. Mollus. Stud.* **79**: 263-269.
- 400 Squires, Z. E., Wong, B. B. M., Norman, M. D. & Stuart-Fox, D. 2012. Multiple fitness benefits of  
401 polyandry in a cephalopod. *PLoS ONE* **7**: e37074.
- 402 Stutt, A. D. & Siva-Jothy, M. T. 2001. Traumatic insemination and sexual conflict in the bed bug  
403 *Cimex lectularius*. *Proc. Natl. Acad. Sci.* **98**: 5683-5687.

404 Sudo, H. 2003. Effect of temperature on growth, sexual maturity and reproduction of *Acanthomysis*  
405 *robusta* (Crustacea: Mysidacea) reared in the laboratory. *Mar. Biol.* **143**: 1095-1107.

406 Svärd, L. & McNeil, J. N. 1994. Female benefit, male risk: polyandry in the true armyworm  
407 *Pseudaletia unipuncta*. *Behav. Ecol. Sociobiol.* **35**: 319-326.

408 Tamada, K. & Iwata, K. 2005. Intra-specific variations of egg size, clutch size and larval survival  
409 related to maternal size in amphidromous Rhinogobius goby. *Environ. Biol. Fish.* **73**: 379-389.

410 Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage:  
411 Invited review. *Mol. Ecol.* **9**: 1013-1027.

412 Vahed, K. 1998. The function of nuptial feeding in insects: a review of empirical studies. *Biol. Rev.* **73**:  
413 43-78.

414 Venables, W. N. & Ripley, B. D. 2002. *Modern Applied Statistics with S*. Fourth edition. Springer.

415 Wagner, W. E., Jr. & Harper, C. J. 2003. Female life span and fertility are increased by the ejaculates  
416 of preferred males. *Evolution* **57**: 2054-2066.

417 Wells, M. J. & Wells, J. 1977. *Molluscs: Gastropods and Cephalopods*. New York: Academic Press.

418 Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**:  
419 398-411.

420 Yasui, Y. 1997. A "good-sperm" model can explain the evolution of costly multiple mating by  
421 females. *Am. Nat.* **149**: 573-584.

422

423 **Table 1: Mean ( $\pm$  SE) parameters for each treatment group.**

Parameter	Virgin	Once-mated	Twice-mated	Statistical test
Weight at maturity (g)	2.46 ( $\pm$ 0.22)	2.38 ( $\pm$ 0.18)	2.67 ( $\pm$ 0.23)	$\chi^2 = 0.53$ , df = 2, $p = 0.60$
Proportion that laid eggs	0.44	0.90	0.90	$\chi^2 = 6.69$ , df = 2, $p = 0.035$
Total clutch mass (g)*	5.65 ( $\pm$ 2.45)	13.73 ( $\pm$ 2.10)	13.04 ( $\pm$ 3.30)	$\chi^2 = 4.51$ , df = 2, $p = 0.10$
Number of clutches*	1.5 ( $\pm$ 0.3)	5.0 ( $\pm$ 1.0)	3.6 (0.9)	$\chi^2 = 13.60$ , df = 2, $p = 0.001$
Eggs per clutch*	96.3 ( $\pm$ 9.6)	52.9 ( $\pm$ 4.1)	60.8 ( $\pm$ 4.2)	$\chi^2 = 4.71$ , df = 2, $p = 0.09$
Single egg mass (mg)*	34.3 ( $\pm$ 0.9)	51.5 ( $\pm$ 1.4)	59.8 ( $\pm$ 1.3)	$\chi^2 = 14.36$ , df = 2, $p < 0.001$

424 \*Only for squid that laid clutches.

425 **Figure Captions**

426

427 **Figure 1: Ventral image of adult female *Euprymna tasmanica* depicting the coral red accessory**  
428 **nidamental gland.** Initial appearance of this gland was used as a marker for sexual maturity.

429

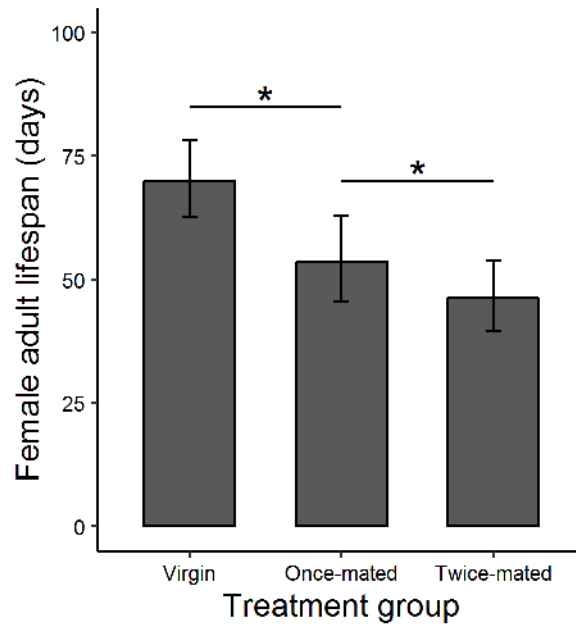
430 **Figure 2: The effect of mating treatment (virgin, once-mated or twice-mated) on female *Euprymna***  
431 ***tasmanica* adult lifespan.** Graph shows expected values (MLE, 95% CIs) when total clutch mass and  
432 average adult temperature are held constant (total clutch mass = 0 g; average temperature = 17.2 °C  
433 (average value across all squid)). Adult lifespan was calculated from sexual maturity until death.  
434 Asterisks signify significantly different groups.

435  
436 **Figure 3: The correlations between female *Euprymna tasmanica* adult lifespan and (a) total clutch**  
437 **mass (grams); (b) average water temperature across a female's lifespan (°C).** (a) Average water  
438 temperature was held constant (at 17.2 °C, average value across all squid) and (b) total clutch mass  
439 was held constant (at 9.1 g, average value across all squid). Adult lifespan was calculated from sexual  
440 maturity until death. Slopes were significantly different from zero.

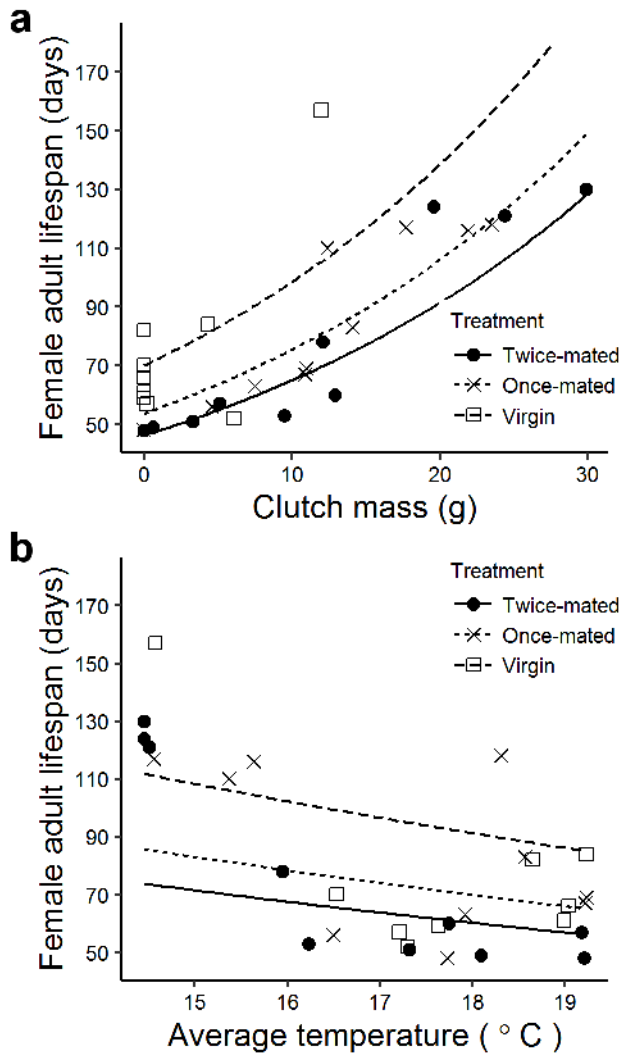
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