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1 **Females adjust maternal hormone concentration in eggs**
2 **according to male condition in a burying beetle**

3

4

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15

16

17 ABSTRACT

18 In birds and other vertebrates, there is good evidence that females adjust the allocation of hormones in
19 their eggs in response to prenatal environmental conditions, such as food availability or male
20 phenotype, with profound consequences for life history traits of offspring. In insects, there is also
21 evidence that females deposit juvenile hormones (JH) and ecdysteroids (ESH) in their eggs, hormones
22 that play a key role in regulating offspring growth and metamorphosis. However, it is unclear whether
23 females adjust their hormonal deposition in eggs in response to prenatal environmental conditions.
24 Here we address this gap by conducting an experiment on the burying beetle *Nicrophorus*
25 *vespilloides*, in which we manipulated the presence of the male parent and the size of the carcass used
26 for breeding at the time of laying. We also tested for effects of the condition (i.e., body mass) of the
27 parents. We then recorded subsequent effects on JH and ESH concentrations in the eggs. We found no
28 evidence for an effect of these prenatal environmental conditions (male presence and carcass size) on
29 hormonal concentration in the eggs. However, we found that females reduced their deposition of JH
30 when mated with heavier males. This finding is consistent with negative differential allocation of
31 maternal hormones in response to variation in the body mass of the male parent. We encourage further
32 work to investigate the role of maternally derived hormones in insect eggs.

33

34 Keywords: differential allocation, ecdysone, eggs, juvenile hormone, maternal effect, *Nicrophorus*
35 *vespilloides*

36

37 INTRODUCTION

38 In many animals, including birds, fishes and insects, females deposit hormones, such as testosterone
39 (T), corticosterone, thyroid hormones, juvenile hormones (JH), and ecdysteroids (ESH) into their eggs
40 (De Loof et al., 2013; Gharib and de Reggi, 1983; Groothuis et al., 2005; Power et al., 2001; von
41 Engelhardt and Groothuis, 2011). Maternal hormones play an important role in shaping the
42 offspring's subsequent development, growth, survival and behaviour (Groothuis et al., 2019, 2005;
43 Groothuis and Schwabl, 2007; Power et al., 2001; Schwander et al., 2008; von Engelhardt and
44 Groothuis, 2011). Studies on several bird species and one fish species show that females adjust the
45 deposition of such hormones in response to environmental cues available to females at the time of egg
46 laying (Gasparini et al., 2007; Giesing et al., 2010; Gil et al., 1999). Studies on birds show that
47 females adjust hormone deposition in response to cues that predict variation in the amount of food
48 offspring are likely to receive after hatching, such as the quality of the male partner in species with
49 biparental care (Gil et al., 1999) and the number of care-givers in cooperatively breeding species
50 (Paquet et al., 2013). Such adjustments are often thought to be adaptive, providing females with a
51 mechanism for altering the offspring's phenotype to match the environmental conditions offspring are
52 likely to encounter after hatching (Groothuis et al., 2019; Meylan et al., 2012). In birds, maternal
53 hormones affect the offspring's begging behaviour, which in turn influences offspring growth and
54 development via the effect of offspring begging on the amount of food provisioned by male and
55 female parents (Paquet and Smiseth, 2015; Smiseth et al., 2011). Thus, prior work on birds suggests
56 that female adjustment of maternal hormone levels in eggs is associated with offspring begging and
57 biparental food provisioning. These conditions are not unique to birds, as offspring begging and
58 biparental provisioning of food for offspring also occurs in some insects, such as burying beetles of
59 the genus *Nicrophorus* (Eggert and Müller, 1997; Scott, 1998). Thus, to determine whether female
60 adjustment of maternal hormone levels in eggs is associated with biparental food provisioning and
61 offspring begging, we need to extend the study of female adjustment of maternal hormones to relevant
62 non-avian taxa, such as burying beetles.

63 Although there is evidence that female insects deposit hormones in their eggs (Schwander et
64 al. 2008; De Loof et al., 2013; Gharib and de Reggi, 1983), it is currently unclear whether females
65 *adjust* the deposition of maternal hormones in response to prenatal environmental cues (see below).
66 Insect hormones are different from those in birds and other vertebrates, suggesting that female
67 deposition of maternal hormones into eggs has independent evolutionary origins in these taxa. In
68 insects, the main hormones deposited in eggs are JH and ESH, which are jointly involved in the
69 regulation of numerous aspects of insect phenotype, such as metamorphosis and offspring growth and
70 development (Nijhout, 1998). There are many functional similarities between JH and T, including
71 evidence that female hormone levels vary in response to social environments (reviewed in Tibbetts et
72 al., 2019; Tibbetts and Crocker, 2014). Furthermore, hormone levels in offspring affect their growth
73 and begging behaviour in a species where both parents can provision their offspring with food after
74 hatching (Crook et al., 2008). Prior work on insects provide evidence for an association between ESH
75 levels in eggs and environmental conditions during development, such as population density in desert
76 locusts (*Schistocerca gregaria*) (Hägele et al., 2004) and day length in migratory locusts (*Locusta*
77 *migratoria*) (Tawfik et al., 2002). However, it is unclear whether these associations are caused by
78 *adjustment* of female allocation of maternal hormones in response to environmental conditions as
79 opposed to differential mortality of eggs with different ESH levels under different environmental
80 conditions, or irreversible changes in ESH levels due to exposure of different environmental
81 conditions during development. For example, Hägele et al. (2004) found marked differences in ESH
82 levels of eggs produced by female migratory locusts that had been raised in a crowded or a solitary
83 environment over several generations. However, there were no differences in ESH levels of eggs
84 produced by solitary females and solitary females temporarily maintained in a crowded environment
85 at the time of egg laying, suggesting that females did not adjust the allocation of ESH to the
86 environmental conditions they were exposed to during egg laying. A recent study on house crickets
87 (*Acheta domesticus*) found that the interaction between maternal and grand-maternal diets influenced
88 the amount of ESH in eggs suggesting that females adjust their deposition of ESH in eggs based on
89 prenatal environmental cues (Crocker and Hunter, 2018). This study investigated effects on the
90 content rather than concentrations of maternal hormones in eggs. Given that female insects often

91 adjust the size of their eggs in response to prenatal conditions (Fox et al., 1997), it is therefore unclear
92 whether the greater amount of ESH in eggs reflects that eggs had a higher concentration of ESH or
93 whether larger eggs simply contain a greater amount of ESH. Finally, given that these insect species
94 do not show parental food provisioning or offspring begging, it is unclear how these studies relate to
95 our understanding based on prior work on maternal hormone deposition in birds. Thus, there is now a
96 need for studies that investigate whether females adjust the deposition of maternal hormones in eggs
97 in insects and more particularly in species with extensive post-hatching parental care involving food
98 provisioning by both parents and offspring begging, and such studies should control for potential
99 confounding effects due to egg size.

100 Here we investigate whether females adjust hormone deposition in their eggs in response to
101 prenatal environmental conditions in the burying beetle *Nicrophorus vespilloides*. This species is well
102 suited to investigate this hypothesis as it exhibits offspring begging and biparental food provisioning
103 after hatching (Eggert and Müller, 1997; Scott, 1998). Females only mature their oocytes once they
104 encounter the carcass of a small vertebrate (Scott and Traniello, 1987), and females start laying eggs
105 3–28 hours after encountering a carcass (Ford and Smiseth, 2017). Given that egg production starts
106 after females encounter a carcass, females might adjust the deposition of hormones into their eggs
107 based on various prenatal environmental cues that may predict the amount of food available to
108 offspring after hatching. Firstly, the presence or absence of a male partner at the carcass at the start of
109 egg laying provides females with a cue for the likelihood that the male will assist in food provisioning
110 after hatching (Paquet and Smiseth, 2017). Females will store sperm from prior matings, allowing
111 them to breed on their own if no male is present (Eggert 1992). There is evidence that females adjust
112 offspring mass at hatching (Paquet and Smiseth, 2017), but not egg size (Ford, 2019) or clutch size
113 (Ford, 2019; Paquet and Smiseth, 2017), in response to the presence of the male during egg laying.
114 Secondly, the size of the vertebrate carcass used for breeding determines the total amount of resources
115 that will be available for the developing larvae. The size of the carcass used for breeding varies and
116 there is evidence that females lay more but smaller eggs when breeding on large carcasses (Botterill-
117 James et al., 2017). It is currently unclear whether females adjust the deposition of maternal hormones

118 in response to the presence of the male and/or the size of the carcass. Here, we used a 2×2 factorial
119 design where we manipulated the presence or absence of the male parent and the size of the carcass
120 (small versus large) at the time of egg laying. We then measured subsequent effects on the
121 concentration of JH and ESH in the eggs. We predicted that females would deposit more JH (and
122 possibly more ESH) in their eggs when breeding on large carcasses and in the presence of the male.
123 Prior work shows that JH stimulates larval begging in our study species (Crook et al., 2008), and that
124 male parents respond to increased larval begging by provisioning more food (Smiseth and Moore,
125 2004). There is also evidence that both the presence of the male and access to a larger carcass have
126 positive effects on larval growth (Paquet and Smiseth, 2017; Sieber et al., 2017). Prior work on birds
127 suggest that females also may adjust the deposition of hormones in eggs depending on their condition
128 (Pilz et al., 2003; Sandell et al., 2007) or the condition of their partner (Sheldon, 2000). Therefore, we
129 tested for effects of the prenatal body mass of both parents on female deposition of maternal
130 hormones in eggs, using body mass as a proxy of their condition.

131

132 METHODS

133 *Study population and animal husbandry*

134 In these experiments, we used virgin beetles that had been reared in the laboratory. The beetles
135 derived from lines originally collected in the wild in Edinburgh, UK. Non-breeding beetles were
136 housed individually in transparent, plastic containers (124 × 82 mm and 20 mm high) containing
137 moist soil and were maintained at 21±2°C under a 16:8 h light:dark cycle. We fed nonbreeding beetles
138 small pieces of raw, organic beef twice a week.

139

140 *Experimental design and procedures*

141 We used a 2 × 2 factorial design to investigate whether females adjust deposition of maternal
142 hormones in their eggs in response to whether the male partner was present or absent at the time of
143 egg laying and whether females were provided with a large or a small mouse carcass for breeding. We
144 randomly selected pairs of non-sibling males and females for use in the experiments. We paired

145 beetles at random to exclude any potential effect of assortative mating between males and females
146 (Smiseth and Moore, 2004). At the beginning of the experiment, we weighed all males and females to
147 record their pre-breeding body mass, using this as a proxy of their body condition To ensure that
148 females were able to lay fertilized eggs regardless of whether a male was present or absent at the time
149 of egg laying, we placed all pairs in plastic containers (110 × 110 mm and 30 mm high) with
150 approximately 10 mm deep moist soil for at least 24 h (range: 25.16–28.40 h) before moving females
151 to a larger plastic container (170 × 120 and 60 mm high) filled with a 10–20 mm layer of soil and
152 provided with a previously frozen mouse carcass (supplied by Livefoods Direct Ltd, Sheffield, UK) to
153 initiate breeding (Paquet and Smiseth, 2017; Steiger, 2013). We assigned all females at random to the
154 different treatment groups. We moved both parents to the new container for those females that were
155 assigned to the treatments where the male was present, while we moved the female only for those
156 females that were assigned to the treatments where the male was absent. Females assigned to the
157 treatments involving a small carcass were provided with a mouse carcass with a mean mass of 6.57 g
158 (range 4.54–9.23g), and females assigned to the treatments involving a large carcass were provided
159 with a mouse carcass with a mean mass of 23.24 g (range 19.00–27.34 g).

160 To record the time of the initiation of egg laying, we placed the boxes on flat-bed scanners
161 (Canon Canoscan 9000F Mark II, Canon Inc., Tokyo, Japan) (Ford and Smiseth, 2016). We scanned
162 the breeding boxes every hour using Vuescan professional edition software (Hamrick Software,
163 Sunny Isles Beach, FL) and recorded the time of appearance of the first laid eggs in the bottom of the
164 box. We set up 134 experimental females across the experiment. We excluded 12 experimental
165 females because they laid fewer than 5 eggs (7 from the treatment where the female only bred on a
166 small carcass, 3 for the treatment where the female only bred alone on a large carcass, and 2 for the
167 treatment where both parents bred on a large carcass). Thus, the final sample sizes for each treatment
168 were as follows: both parents breeding on a small carcass (n = 30 clutches), both parents breeding on
169 a large carcass (n = 28 clutches), female only breeding on a small carcass (n = 32), and female only
170 breeding on a large carcass (n = 32). When possible, we collected 10 eggs within a day from laying
171 initiation to limit potential effects due to egg development (mean: 11.35 hours since start of laying,
172 range: 5.50–25.25 h). We collected 2 × 5 eggs (5 for each hormone analysis) that were gently

173 collected with forceps, weighted by five in an Eppendorf tube (in order to later calculate hormonal
174 concentrations per gram of eggs) and kept frozen until further analyses. When there were fewer than
175 10 eggs for a given female (N=16 clutches), we collected 5 eggs that were randomly assigned to the
176 analysis for each of the two hormones.

177

178 *Hormones assay*

179 *Juvenile hormone radio-immunoassay:* We assigned five eggs from each clutch at random for the
180 analyses of JH. The eggs were crushed in glass tubes with 500 μ L of distilled water. We extracted JH
181 by adding 3 mL of diethyl-ether to the tubes and by vortexing the mixture. The solvent and the
182 aqueous phases were separated by centrifuging the tubes for 5 min at 2000 rpm (4°C). The aqueous
183 phase contained water, eggshells and proteins, while JH, which is a lipidic hormone, remains in the
184 solvent. We then placed the tubes in a cold bath to freeze the water. The diethyl-ether phase
185 containing the hormone was decanted and poured off in new glass tubes. This step was performed
186 twice for each sample and the resultant was then evaporated at 37°C. We dissolved the dried extracts
187 in 400 μ L of phosphate buffer and JH concentrations were assayed in duplicates. Specifically, 100 μ L
188 of extract or JHIII standard (Sigma Aldrich, US) were incubated overnight with 4000 cpm of the 3 H-
189 juvenile hormone III (Perkin Elmer, US) and polyclonal antiserum (provided by Prof. Walter
190 Goodman, Wisconsin-Madison University). The bound fraction was then separated from the free
191 fraction by addition of dextran-coated charcoal and activity was counted on a tri-carb 2810 TR
192 scintillation counter (Perkin Elmer, US). Inter- and intra-assay variation in JH concentrations were
193 19.47% and 15.86%, respectively. Intra-assay measurements were highly repeatable (Pearson
194 correlation coefficient = 0.82, 95%CI = 0.75–0.87). The JH lowest detectable concentration was
195 57.84 pg/100 μ L of extract. Sample dilution displacement curves were parallel to the standard curve
196 showing that the sample hormone is recognized in the same way as the JHIII standard.

197 *ESH immuno-assay:* We assigned the remaining five eggs from each clutch for the analyses
198 of ESH. Given the lipidic nature of this hormone, specific solvents were used to extract it
199 from the eggs. First, we crushed the eggs in glass tubes with 5 mL of methanol. The mixture

200 was then sonicated for 30 min and incubated overnight at 42°C. After agitation and
201 centrifugation (10 minutes, 4000 rpm, RT), we filtered the methanol containing the hormone
202 with a specific syringe-filter (membrane PTFE, 0.45 µm) in new glass tubes. This step was
203 then done twice with 2 mL of methanol. The methanol was then evaporated at 50°C under
204 nitrogen. The dried extracts were dissolved in 250 µL of assay buffer (1M phosphate with
205 BSA, NaCl, EDTA). We then assayed the ESH in duplicates with a commercial Enzyme
206 Immunoassay (SpiBio, Bertin Pharma, France) and a microplate reader (Berthold, France).
207 This assay is more specific to 20-hydroxy-ecdysone and ecdysone but the antibody can cross-
208 reacts with other ecdysteroids: 20-hydroxy-ecdysone 100%, ecdysone 100%, 2-deoxy-20-
209 hydroxy-ecdysone 88%, polypodine B 70%, 2-deoxy-ecdysone 63%, ponasterone A 43%,
210 Cyasterone 5%, podecdysone C 4.5%, makisterone A 4%, 26-hydroxy-ecdysone 1.4%,
211 muristerone A 1.2%, kaladasterone 1%, 22-epi-ecdysone <0.1%, posterone <0.1%. Inter- and
212 intra-assay variation in ESH concentrations were 16.16% and 12.70%, respectively. Intra-
213 assay measurements were highly repeatable (Pearson correlation coefficient = 0.97, 95%CI =
214 0.96–0.98). ESH lowest detectable concentration was 31 pg/100µL of extract. Samples
215 dilution displacement curves were parallel to the standard curve showing that the sample
216 hormone is recognized in the same way as the standard.

217

218 *Statistical analyses*

219 We conducted all statistical analyses in a Bayesian framework using JAGS, version 4.2.0, via the
220 ‘rjags’ package (Plummer, 2013) in R version 3.3 (R Core Team, 2013). To investigate whether
221 females adjust the deposition of JH and ESH in response to the presence or absence of the male and
222 carcass size (large or small), we built linear mixed models with treatment as a four-level fixed effect.
223 We did this to test for the main effects of carcass size and male presence, as well as for effects of the
224 interaction between them. We also added the female’s own weight, as well as the weight of the male

225 partner as fixed effects (scaled) in all models. In addition, we included time from laying until egg
226 collection as a fixed effect (scaled), hereafter termed ‘time since the onset of laying’ as a fixed effect.
227 This variable reflects the age of the first-laid eggs in a given clutch and we included this to control for
228 potential confounding effects due to the age of the eggs caused by differences in egg laying times
229 between females (Ford, 2019). There was no significant correlation between male size and the age of
230 the first-laid egg in the clutch (Pearson product moment correlation: -0.02 [-0.20,0.15], p-value=0.79)
231 and between male size and the time interval between mating and egg laying (Pearson product moment
232 correlation: -0.06 [-0.24,0.12], p-value=0.53). We included clutch ID as a random effect given that we
233 obtained 2 measures per clutch per hormone (except for 4 clutches where only one measure of ESH
234 could be taken). These two measures acted as two observations of the underlying hormonal
235 concentration of the sample and the fixed effects were applied on these estimated concentrations of
236 the samples. As male weight may be an indicator of his parental quality, we also initially investigated
237 whether female adjustment of maternal hormone deposition in response to male weight is conditional
238 upon his presence at egg laying. We did this by including an interaction between male weight and
239 male presence or absence. Given that we found no evidence for such interaction effects (12.60 ng/g [-
240 22.82–48.31], $P(>0) = 0.76$ and -0.42 ng/g [-4.10–3.23], $P(>0) = 0.41$ for JH and ESH respectively),
241 we removed this interaction from the final models. There was no indication that egg mass varied in
242 response to male presence, carcass size or their interaction (all credible intervals largely overlapped
243 zero), and we therefore excluded information on egg mass from the final models. Additionally, we
244 found no evidence for an effect of the interaction between male and female body size on
245 concentrations of JH and ESH (-0.44 ng/g [-21.99–20.98], $P(>0) = 0.47$ and -0.12 ng/g [-2.12–1.88],
246 $P(>0) = 0.46$ for JH and ESH, respectively).

247 We estimated parameters using vague priors (that is, prior distributions allowing for a wide
248 range of values, see script in supplementary material for more details). Posterior samples from three
249 Markov Chain Monte Carlo (MCMC) chains were based on 3000 iterations after an adaptation period
250 of 5000, burn-in of 5000 and thinning interval of 3 for each model. Model convergence was
251 confirmed both visually and by using the ‘R hat’ Gelman–Rubin statistic (Gelman and Rubin, 1992).
252 To assess the goodness of fit of our models, we performed post predictive checks using the χ^2

253 discrepancy metric (Gelman et al., 1996). We found no evidence for lack of fit (Bayesian p values:
254 0.492 and 0.498, values close to 0 or 1 would indicate lack of fit). We present the means [and 95%
255 Credibility Intervals] from the posterior distributions of interest, as well as $P(>0)$ the proportion of the
256 posterior distribution that was higher than zero (all posterior distributions are symmetrical). We
257 interpret effects as ‘statistically clear when 95% CI did not overlap zero and we report estimates for
258 all parameters of interest regardless of their statistical clarity (Dushoff et al., 2019). We estimated
259 effect sizes of continuous fixed effect variables by dividing their effect (for each posterior sample) by
260 the standard deviation of the estimated true underlying hormone concentrations (mean and 95%
261 Credibility Intervals of the estimated standard deviations 89.68 ng/g [83.09–96.84] for JH and 9.05
262 ng/g [8.63–9.65] for ESH). To estimate the proportion of variation in the concentration of JH and
263 ESH explained by our models, we computed R^2 following Gelman and Pardoe (2006). We note that
264 negative values of R^2 are possible when the model has a poor ability to predict the response variable
265 (Gelman and Pardoe, 2006).

266

267 RESULTS

268 There was no evidence that females adjusted the concentrations of either JH or ESH in their eggs in
269 response to the presence or absence of a male partner, the size of the carcass (small or large), or the
270 interaction between them (Table 1, **Fig.1**). However, females deposited less JH in eggs when they
271 were mated with heavier males (effect size: -0.21 [-0.40–0.02], Table 1; **Fig.2**). There were also
272 some indication that heavier females deposited less JH in eggs, although this evidence was
273 inconclusive as the 95% credibility intervals overlapped zero (effect size: -0.16 [-0.35–0.04], Table
274 1; **Fig.2**). There was no evidence that females adjusted the concentration of ESH in the eggs in
275 response to either their body mass or the body mass of their male partner (effect sizes: 0.01 [-0.19–
276 0.21] and -0.03 [-0.24–0.16], respectively Table 1; **Fig.3**). There were some indications that
277 concentration of JH in eggs increased with time since the onset of laying, although this evidence was
278 inconclusive as the 95% credibility intervals overlapped zero (effect size: 0.16 [-0.06–0.37], Table 1).
279 There was no evidence that the concentration of ESH increased or decreased with time since the onset
280 of laying (effect size: -0.11 [-0.32–0.11], Table 1). Our estimated R^2 values suggested that the fixed

281 effects included in our models explained 6.2% of the variation in JH concentration in the eggs (R^2
282 =0.062), while the fixed effects failed to explain any variation in ESH concentration (R^2 =-0.02).

283

284 DISCUSSION

285 Here we found no evidence that females adjusted the concentration of maternal hormones in response
286 to either the presence or absence of the male partner at the time of egg laying or the size of the carcass
287 used for breeding in *N. vespilloides*. However, we found that females deposited less JH when they
288 were mated with heavier males. We also found some weak indication that heavier females laid eggs
289 with lower JH concentrations. Our study provides evidence for female adjustment of maternal
290 hormone concentrations in an insect. Our results suggest that female adjustment of maternal hormones
291 in response to environmental cues is not unique to birds but may be more generally associated with
292 offspring begging and biparental provisioning of food for offspring after hatching. Below, we provide
293 a more detailed discussion of the wider implications of our results for our understanding of female
294 adjustment of maternal hormones in eggs.

295 We found that females deposited *more* JH when they were mated to lighter males. Given that
296 lighter males are likely to be in poorer condition than heavier males, our results suggest that females
297 compensate for the potential detrimental effects of poor male condition by depositing more JH in
298 eggs. Thus, our study provides evidence of reproductive compensation or negative differential
299 allocation in *N. vespilloides*; that is, a reduction in female allocation to reproduction in response to
300 their male partner being in better condition (Groothuis et al., 2005; Haaland et al., 2017). We note that
301 our results derive from an experimental design where we paired males and females at random. This
302 aspect of our design is important because it allowed us exclude any potential effects due to assortative
303 mating, such as females depositing more hormones mating assortatively with heavier males. Thus, our
304 results provide evidence that females facultatively adjust hormone levels in their eggs in response to
305 prenatal cues about the condition of their male partner. We note that our study provides no
306 information on the potential adaptive value of female adjustment of maternal hormones in eggs given
307 that we collected the eggs for use in the hormone assays. Thus, there is now a need for studies that
308 investigate potential fitness consequences of maternal hormone levels for parents and offspring.

309 There are several potential explanations for why females deposited *more* JH when mated to
310 lighter males in *N. vespilloides*. First, females may do so to speed up larval development, thereby
311 compensating for the detrimental effects of poor male condition. For example, there is evidence that
312 larger males are better at protecting the brood against conspecific intruders that would kill the brood if
313 they succeed in taking over the carcass (Otronen, 1988). However, this explanation seems unlikely
314 given that females were mated before they were given a carcass for breeding and that there was no
315 evidence that the effect of male prenatal mass was conditional on whether the male was present or
316 absent when females were provided with a carcass. Second, females may deposit more JH in their
317 eggs to compensate for the effects of poor male condition if male condition serves as an indicator of
318 the offspring's subsequent growth and development. Differential allocation of JH could be mediated
319 by different sperm quality or quantity from males of different sizes. For example, there is growing
320 evidence that males can affect offspring phenotype via sperm or seminal fluids (see e.g. Simmons and
321 Lovegrove, 2019). Finally, males could alter female condition and hormonal levels through their
322 behaviour during mating if for example heavier males have higher copulation rates (Pitnick and
323 García-González, 2002). Future work is needed to understand the underlying mechanism of the effect
324 of male weight on JH levels in eggs (e.g. whether due to genetic differences between males or due to
325 paternal effects due to the male's phenotype), as well as its adaptive value for parents and offspring.
326 Such studies could manipulate the body mass of parents (Steiger, 2013) and measure subsequent
327 consequences on maternal hormone levels in eggs and the fitness consequences for parents and
328 offspring.

329 Contrary to what we predicted, we found no evidence that females adjusted the deposition of
330 maternal hormones in response to the presence or absence of the male at the time of egg laying or the
331 size of the carcass used for breeding. This is surprising given that these two factors are major
332 determinants of food availability for offspring after hatching in this species (Paquet and Smiseth,
333 2017; Sieber et al., 2017). Previous work shows that larvae were smaller at hatching but nevertheless
334 compensated for their initial lower mass during growth (at the expense of male weight gain) when
335 females laid the eggs in the presence rather than the absence of a male parent (Paquet and Smiseth,
336 2017). Our results show that differential allocation of JH or ESH is unlikely to be the mechanism

337 responsible for this maternal effect. Future studies could assess whether females insects alter their
338 allocation in other egg compounds such as proteins (vitellin) and lipids in response to male presence
339 and carcass size.

340 An alternative explanation for why we found no evidence for differential hormonal deposition
341 in eggs in response to male presence and carcass size is that females may adjust their allocation in
342 response to other key factors indicating the conditions experienced by offspring after hatching, such
343 as temperature (Grew et al., 2019) or carcass decomposition (Ford and Smiseth, 2017). This
344 suggestion is supported by the observation that most of the estimated variation in JH and ESH
345 concentrations remains unexplained in our study. Finally, we cannot exclude the possibility that
346 females may adjust the allocation of maternal hormones for later-laid eggs given that we only
347 collected eggs laid within 26 hours after the onset of egg laying to limit potential effect of embryo
348 development. Such within-clutch variation may arise as a consequence of physiological constraints or
349 they may represent an adaptive strategy as suggested in prior studies on birds (Groothuis and
350 Schwabl, 2002; Love et al., 2008).. In our study species, females lay their eggs asynchronously over a
351 period of more than 60 hours (Ford and Smiseth, 2017). Currently, there is little (if any) evidence
352 from any taxa that females differentially adjust hormone deposition in early and late eggs in response
353 to environmental cues (Schmaltz et al., 2008; van Dijk et al., 2013; Verboven et al., 2005; Verboven
354 Nanette et al., 2003). We encourage future work to investigate the presence and fitness consequences
355 of such patterns in invertebrates.

356 Our study was motivated by prior work on birds suggesting that female adjustment of
357 maternal hormone levels evolved in the context of biparental food provisioning and offspring begging
358 (Groothuis et al., 2019). We found evidence for female adjustment of maternal hormone levels in *N.*
359 *vespilloides*; an insect with biparental food provisioning and offspring begging. However, we urge
360 caution in interpreting our results as evidence that female adjustment of maternal hormone levels is
361 causally associated with biparental food provisioning and offspring begging. The main reason for this
362 is that there are alternative adaptive and non-adaptive explanations for why females appear to adjust
363 maternal hormone levels in response to environmental conditions. For example female hormonal
364 deposition may influence how dispersing offspring respond to the prenatal environment as reported

365 for common lizards (*Zootoca vivipara*) where experimentally manipulated maternal corticosterone
366 levels increased offspring philopatry (De Fraipont et al., 2000). Furthermore, maternal hormones may
367 be passively transferred to the eggs with deleterious consequences for offspring. For example, a study
368 on the tropical damselfish *Pomacentrus amboinensis* shows that maternal cortisol reduces the body
369 size of fry at hatching (McCormick, 1998). Concurring with this possibility, prior work on our study
370 species shows that an experimental increase in larval levels of methoprene (a JH analogue) induced
371 reduced larval growth (Crook et al., 2008). Thus, there is now a need for more work to determine
372 whether female adjustment of maternal hormones is a general phenomenon across insect species
373 either with or without parental care.

374 To conclude, we provide the first clear evidence for female adjustment of maternal hormone
375 levels in an insect species. Given the independent evolutionary origins of both biparental care and
376 hormones in insects and birds, our results suggest that this is a case of convergence based on
377 similarities in ecology and/or life histories. More work is clearly needed to understand the generality
378 of such patterns across different insect species with and without parental care, as well as its
379 underlying mechanisms and fitness consequences. Insects represent formidable systems to
380 experimentally investigate the causes and consequences of hormonal allocation in eggs under diverse
381 ecological conditions.

382

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391

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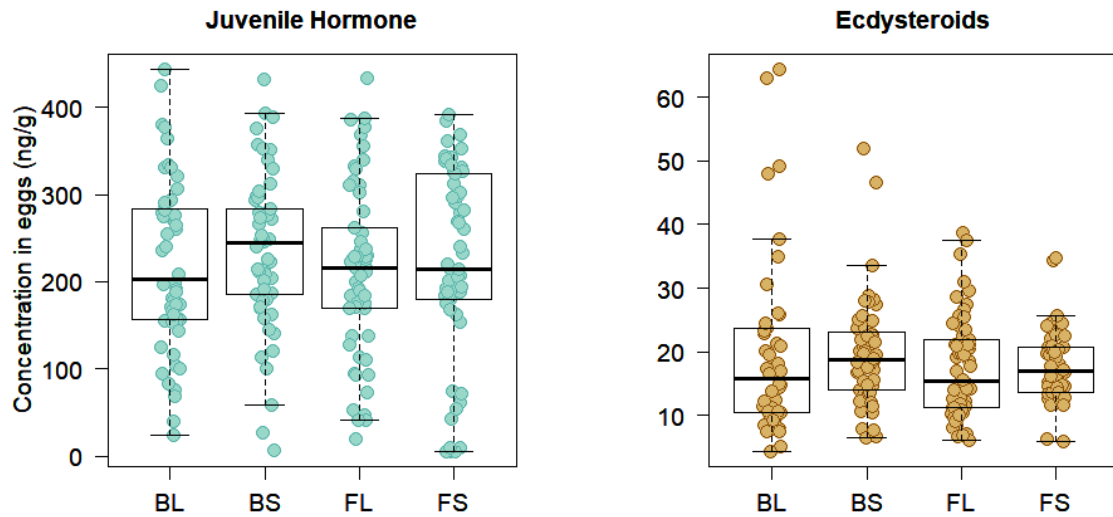
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525 **Table 1**

526 Estimated effects of male presence, carcass size and parents’ weight on hormonal concentrations in
527 the eggs. B represents treatments where both parents were present at egg laying, F when only
528 females were present, L represent treatments provided with Large carcass and S with small
529 carcasses.

Response variable	Explanatory variable	Mean estimate [95%CRI]	P(>0)
JH concentration	Male presence	B-F= 21.74 [-33.42–77.56]	0.78
	Carcass size	L-S= -16.58 [-68.54–36.83]	0.27
	Interaction	(B-F)-(L-S)= 37.46 [-12.49–90.34]	0.93
	Male weight (scaled)	-18.95 [-37.08– -1.95]	0.013
	Female weight (scaled)	-13.95 [-32.14–3.94]	0.064
	Time since onset of laying (scaled)	14.36 [-4.66–32.72]	0.93
ESH concentration	Male presence	B-F= 1.19 [-4.10–6.60]	0.67
	Carcass size	L-S= -0.13 [-5.29–5.08]	0.48
	Interaction	(B-F)-(L-S)= 1.32 [-3.95–6.54]	0.69
	Male weight (scaled)	-0.29 [-2.17–1.56]	0.38
	Female weight (scaled)	0.10 [-1.72–1.98]	0.54
	Time since onset of laying (scaled)	-0.98 [-2.95–1.06]	0.17

530



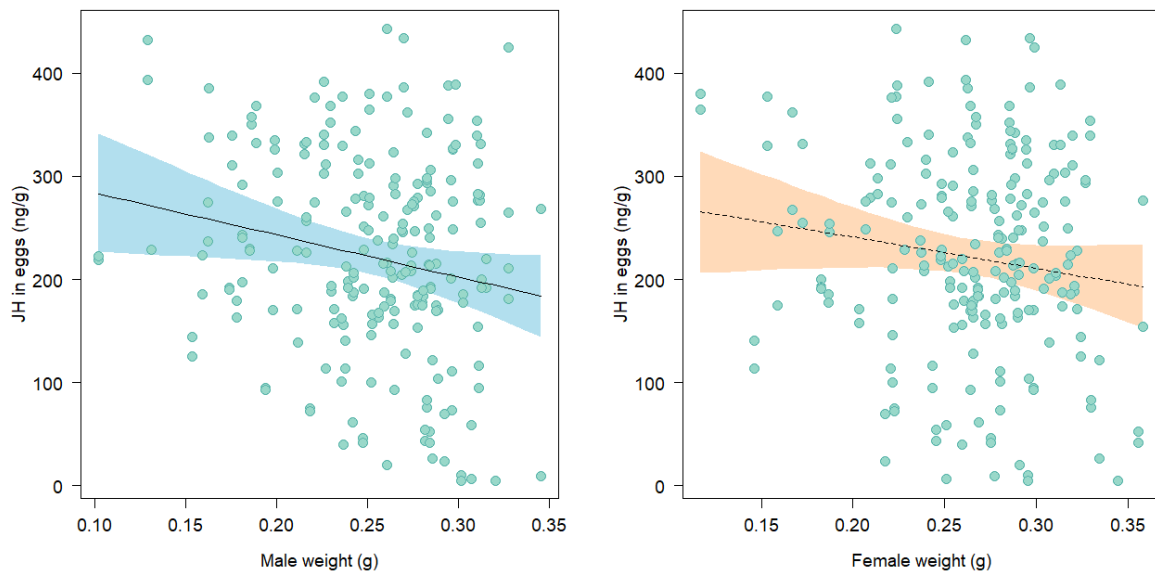
531

532 **Figure 1**

533 No clear evidence for effects of the experimental treatments on hormonal concentrations in the eggs.

534 B represents treatments where both parents were present at egg laying, F when only females were

535 present, L represent treatments provided with Large carcass and S with small carcasses.



536

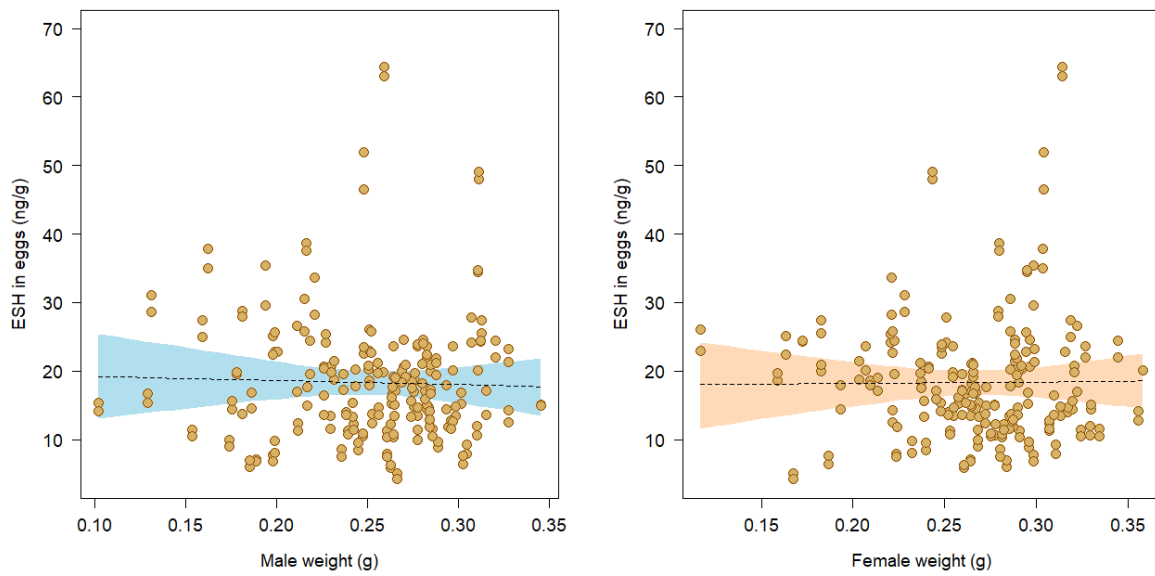
537 **Figure 2**

538 Relationship between male weight (left panel) and female weight (right panel) and JH concentrations

539 in the eggs. Lines show predicted means and shaded areas the 95% Credibility Intervals. The solid

540 line represents effects for which the 95% C.I. of the slope did not span zero whereas the dashed line

541 represents effects for which the 95% C.I. of the slope included zero.



542
543

Figure 3

544 Relationship between male weight (left panel) and female weight (right panel) and ESH
 545 concentrations in the eggs. Lines show predicted means and shaded areas the 95% Credibility
 546 Intervals. The dashed lines represents effect for which the 95% C.I. of the slope included zero.