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Collective exodigestion favours blow fly colonization and development on fresh carcasses

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1 **Collective exodigestion favours blowfly colonisation**
2 **and development on fresh carcasses**

3
4
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17
18 **Running head**

19 Social strategies in necrophagous blow flies

20
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23
24 **Competing interests**

25 We have no competing interests.

26 **Abstract**

27 Necrophagous flies breeding on carcasses face high selection pressures and therefore
28 provide interesting opportunities to study social adaptations. We postulated that blowfly
29 necrophagous larvae gregariousness is an adaptive response to the environmental
30 constraints of fresh carcasses. Cooperation is indeed believed to be a key to the global
31 success of social species. To test this idea, the development of *Lucilia sericata*
32 (Diptera: Calliphoridae) larvae growing on low- or high-digestibility food substrate
33 (control or trypsin-added ground beef muscle, respectively) at different larval densities
34 was monitored. Results showed that larvae developed faster and had decreased
35 mortality at high larval density compared with low larval density. Furthermore,
36 aggregation had no deleterious effect on the morphological characteristics (e.g., size)
37 of post-feeding larvae and flies. We concluded that increased density positively
38 affected population fitness, which is a conclusion consistent with the predictions of the
39 Allee effect. Compared with regular food, larvae fed on high-digestibility food had
40 reduced mortality and faster development on average. According to these results, we
41 postulated that collective exodigestion might be an adaptive response allowing
42 blowflies to colonise fresh carcasses before the arrival of other insects and the
43 multiplication of microbes. This hypothesis is consistent with the idea that cooperation
44 may enable species to expand their niches.

45

46 **Keywords:** Adaptive ecology, Aggregation, Allee effect, exodigestion, niche selection

47

48 INTRODUCTION

49 Collective behaviour is powerful adaptive strategy to cope with a harsh environment,
50 and cooperation is believed to be a key to the global success of social species
51 (Cornwallis et al., 2017). Although eusocial Hymenoptera such as bees and ants are
52 well-known insect societies, gregarious insects also provide interesting examples of
53 efficient collective strategies (Choe & Crespi, 1997; Costa, 2006). Grassé, (1946)
54 defined a part of social behaviour as a *Mass effect*, i.e., a group effect caused by a
55 modification of the medium by the population itself. A striking example of this idea is
56 provided by terrestrial crustacean woodlice, for which desiccation is a primary concern:
57 in response to this environmental stress, aggregation offers group protection against
58 drying (Broly et al., 2014). Deneubourg et al. (1990) also observed that bark beetle
59 larvae aggregate and use communal feeding as a way to overwhelm the reaction of the
60 tree. Similarly, social spiders collectively inject digesting enzymes and then suck up the
61 liquidised prey content, thereby exploiting a common resource that was jointly created
62 (Schneider & Bilde, 2008). In flies, egg aggregations allow larvae to warm and moisten
63 the organic material (Barnard & Geden, 1993; Bryant, 1977), improve larval ability to
64 dig and burrow into the food (Durisko et al., 2014), and limit the growth of competitive
65 fungi (Rohlf's et al., 2005; Zvereva, 1986).

66

67 Blowflies (Diptera: Calliphoridae) are the most widespread and abundant
68 necrophagous species and are attracted quickly after death (Smith, 1986). Female flies
69 lay eggs on fresh vertebrates carcasses on which their larvae feed until
70 metamorphosis; larvae are confined to the carrion until reaching a sufficient
71 mass/instar. Although some studies report competition among carrion flies larvae
72 (Denno & Cothran, 1975, 1976; Feinberg & Pimentel, 1966; Hanski, 1987; Ives, 1991),
73 Flores et al. (2017) observed that female flies do not preferentially oviposit on
74 carcasses without larvae, and several studies show that ovipositing gravid blowflies

75 enhance the attractiveness of a carrion (Brodie et al., 2014; Denno & Cothran, 1975;
 76 Jiang et al., 2002; Kneidel, 1984; Wertheim, 2005). Therefore, egg aggregation is
 77 clearly not avoided and is even sought by adult blow flies. Furthermore, during the
 78 three feeding instars, calliphorid larvae actively aggregate (Boulay et al., 2013, 2016):
 79 masses of several thousands of larvae are commonly observed in the field (Fenton et
 80 al., 1991; Slone & Gruner, 2007). Because females and larvae should avoid
 81 competition, whether necrophagous larval aggregation is advantageous is a
 82 reasonable question (see Table 1). When they reach a sufficient weight, late third-
 83 instar disperse to find sheltered places for pupation, a process known as postfeeding
 84 larval dispersal (Gomes et al., 2006). Consequently, postfeeding larvae and pupae are
 85 not subject to the same selection pressure than feeding instars.

86

87 **Table 1:** The benefits and costs of gregariousness necrophagous larvae of calliphorids
 88 flies.

	BENEFITS	COSTS
Temperature	The mechanisms remain hypothetical (larval metabolism, permanent friction of larvae, microbial activity), but heat production by large larval masses is clearly demonstrated (Charabidze et al., 2011; Gruner et al., 2017; Heaton et al., 2014; Slone & Gruner, 2007). The microclimate inside the mass should increase the developmental speed of maggots.	When the temperature approaches the thermal tolerance range of the larvae, self-regulation processes within the mass must be initiated, which reduce the time allocated to feeding and therefore the growth rate (Charabidze, Hedouin, & Gosset, 2013).
Food availability	Cooperative exodigestion was supposed but not experimentally	After a critical number of individuals are reached, the availability of nutrients per

	demonstrated (Rivers et al., 2011).	individual becomes insufficient, leading to decreases in the speed of development, survival, and weights and sizes (Table A1).
Physico-chemical modifications	Increasing the mass may protect larvae against evaporation and temperature decreases (Broly et al., 2014; Huntington et al., 2007; Pérez et al., 2016).	Excreta rich in ammonia accumulates (Hobson, 1931).
Predators and parasites	High population densities can reduce the risk of predation/parasitism but remains to be demonstrated for necrophagous species (Hunter, 2000; Rohlf & Hoffmeister, 2004).	Chemical signals of the larvae and metabolites increase as a result of feeding (no experimental evidence, see (Frederickx et al., 2014).
Other	Repulsion of scavengers and burying beetles: female burying beetles typically exploit carcasses without blowfly larvae (Smith, 1986). Scavengers may avoid carcasses heavily colonised by larvae and/or bacteria (DeVault et al., 2004).	Increased risk of disease: many pathogens are transmitted in a density-dependent fashion, but this phenomenon has not been demonstrated in necrophagous larvae (Pöppel et al., 2015).

89 Adapted from Rivers et al. (2011).

90

91 Necromass is by nature a discrete and ephemeral food source. Decomposition

92 processes quickly alter flesh (Clark et al., 1997), and the probability of carcass removal

93 by scavengers increase with time and decomposition odour (DeVault et al., 2004). As

94 pioneer species, blowflies strategy depends on their ability to quickly colonize carrions

95 (Denno & Cothran, 1975). Furthermore, many birds, wasps and necrophagous insects
96 prey on maggots. Voss et al. (2009) report an overall 11.8% parasitism of maggots, and
97 Frederickx et al. (2013) observed that on average, 48% of fly pupae were killed by
98 hymenopteran parasitoids (from 3.5% in May to 90% in September). When the larvae
99 develop rapidly, less time is spent on the cadaver and probability of a food shortage,
100 predation, parasitism and interspecific competition decrease (DeVault et al., 2004;
101 Rivers et al., 2012). That can noticeably be achieved by favouring high-temperatures
102 that increase their development speed (Auberon et al, 2016, Grassberger & Reiter,
103 2001). In this respect, a well-known adaptive strategy of blow flies larvae is the so-
104 called *maggot-mass effect*, a local temperature increase attributed to larval metabolism
105 (Charabidze et al., 2011; Slone & Gruner, 2007). Because additional heat gain reduces
106 developmental time, aggregated larvae benefit from this larval-mass effect with faster
107 development than that of isolated individuals (Erzinçlioglu, 1996; Heaton et al., 2014;
108 Johnson & Wallman, 2014). In the same way, aggregation can protect larvae from
109 abrupt decreases in temperature (Huntington et al., 2007; Magni et al., 2016; Rivers et
110 al., 2011). However, these thermal effects mostly occurs during late feeding instars and
111 only within large masses above a thousand larvae (Charabidze et al., 2011; Heaton et
112 al., 2014). Here, we postulated that beside maggot-mass effect, gregariousness
113 favours necrophagous larvae feeding and their early exploitation of fresh carcasses.

114

115 According to Disney (1986), the switch to ingesting liquid instead of solid food could be
116 a fundamental revolution in the evolution and diversification of flies. Necrophagous
117 larvae are actually unable to ingest solid particles other than those that are
118 microscopic, and their buccal apparatus is not suitable for mastication (Guyénot, 1907).
119 Therefore, larvae must liquefy the food before ingestion (i.e., exodigestion). The
120 necrophagous larvae of calliphorid flies excrete/secrete various proteolytic enzymes,
121 including trypsin, lipases and amylases (Chambers et al., 2003; Hobson, 1931; Rivers

122 et al., 2011; Terra & Ferreira, 1994). The sharing of enzymes has been reported as a
123 likely benefit of calliphorid larval aggregation, but this idea has never been
124 demonstrated (Ireland & Turner, 2006; Reis et al., 1999; Rivers et al., 2011). To test
125 the hypothesis of collective benefits through exodigestion, *L. sericata* larvae were bred
126 at different larval densities. Two hypotheses were experimentally tested: (1) larval
127 development speed and survival increases with increasing larval density, and (2) larval
128 development speed and survival increases with increasing food digestibility.

129

130 **MATERIALS AND METHODS**

131 ***Ethical note***

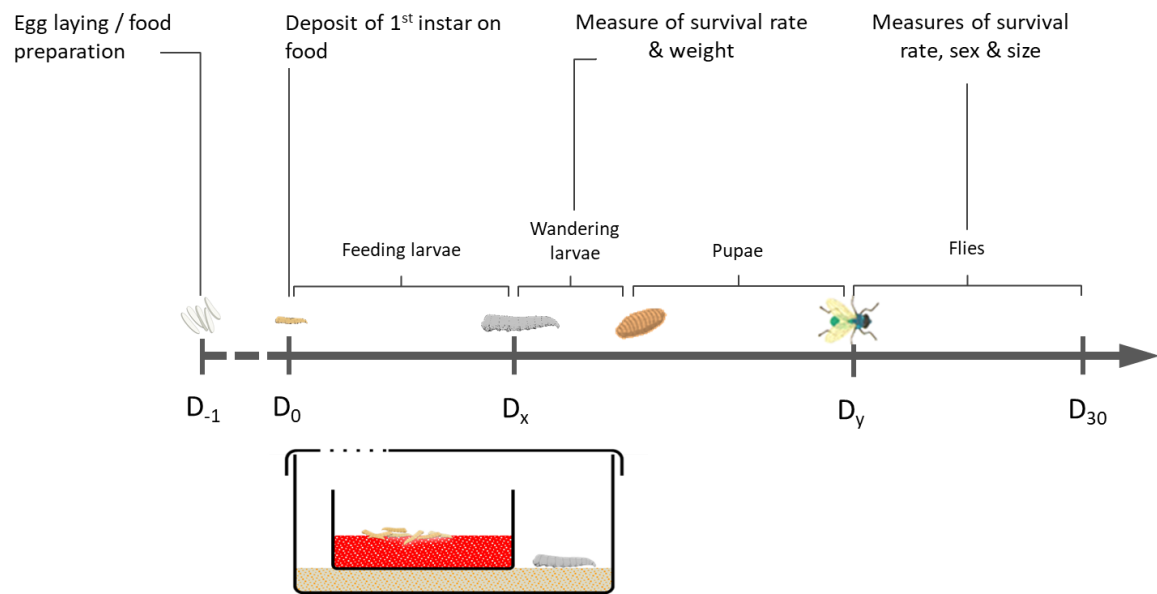
132 This research adheres to the ASAB/ABS Guidelines for the Use of Animals in
133 Research, the legal requirements of the country in which the work was conducted, and
134 all institutional guidelines. During the design of the study, a minimum of manipulation
135 with a minimum of used animals was sought.

136

137 ***Insect rearing***

138 *Lucilia sericata* larvae were obtained from adult flies bred in the laboratory and
139 maintained in tulle cages (50x50x50 cm) at room temperature (23±2°C). Wild flies, 100
140 to 200, were added every 2 months. The flies were fed caster sugar and water *ad*
141 *libitum*. After each new generation, minced beef liver was added for 7 days to provide
142 proteins for vitellogenesis and then removed. Laying of eggs was triggered with the
143 placement of 25±5 g of beef liver for 2 h (from 10 a.m. to midday) in cages. Oviposition
144 time (precision ±1 h) was noted as D -1 (see Fig. 1).

145



146

147 Figure 1: Chronological diagram of the experimental process. D_{-1} is the time of
 148 oviposition (± 1 h). Larvae were counted and placed on food on the next day (D_0). D_x is
 149 the first post-feeding larva and D_y is the first emerging fly.

150

151 **Larval development**

152 A density-dependent increase in competition is found in many blowfly developmental
 153 studies (see Table A1). In some studies, measurements were conducted during the
 154 feeding of instars, which likely disrupts aggregates and prevents detection of any social
 155 benefit (Martinez Sanchez et al., 2007; Simkiss et al., 1993). Many others suffer from a
 156 lack of statistical power (few replications, time between measures too long) (Martinez
 157 Sanchez et al., 2007; Reigada & Godoy, 2006; Saunders & Bee, 1995; Serra et al.,
 158 2011). Finally, many authors used mixed liver or synthetic medium to feed larvae
 159 (Martinez Sanchez et al., 2007; Reis et al., 1999; Simkiss et al., 1993; Smith & Wall,
 160 1997). These food sources are more digestible and less nutritious than muscular
 161 tissues and significantly affect larval development (Barnes & Gennard, 2013; Bernhardt
 162 et al., 2017; El-Moaty & Kheirallah, 2013; Pérez et al., 2016, 2016; Rueda et al., 2010;
 163 Tachibana & Numata, 2001). By contrast, our experiments were performed with
 164 relevant larval densities feeding on beef muscle, a realistic food source.

165

166 All experiments on development were conducted from January to May 2017 in a
167 thermostatic chamber set at $25\pm 0.1^{\circ}\text{C}$ with constant ventilation (ST4; POL-EKO
168 Aparatura®, Wodzisław Śląski, Poland). A 12:12 h photoperiod cycle was obtained with
169 a fluorescent tube placed inside the incubator (430 lumens, light from 9:30 a.m. to 9:30
170 p.m.).

171

172 Eggs obtained at Day -1 were incubated for 22 h, and then first instars were counted
173 and placed in boxes containing food (D0). Each box was placed in a second box
174 containing 1 cm of sand. All experiments were performed at 25°C . Two independent
175 variables were studied: the initial number of larvae (i.e., larval density) and the
176 digestibility of the food. Six replications for densities d10, d50, d100, and d250 and
177 three replications for density d500 were prepared for each food type.

178

179 Two independent variables were studied: the initial larval population (i.e., larval density)
180 and the digestibility of the food. The larval densities (d) tested were 10, 50, 100, 250
181 and 500 larvae per 50 ± 1 g of food, low enough to prevent maggot-mass effect (i.e.
182 significant local temperature increase, Charabidze et al., 2011). Two types of food were
183 used: the control food (C) was 50 ± 1 g of minced beef muscle at 15% fat mixed with 15
184 mL of 0.9% NaCl, and pre-digested food (Tr) was obtained with the same meat mixed
185 with 50 ± 0.5 mg of trypsin (Trypsin T4799, porcine pancreas; Sigma-Aldrich®) diluted in
186 15 mL of 0.9% NaCl. The addition of trypsin changes food digestibility (the size of the
187 molecules) but not the composition or nutritional value of the meat. The beef muscle
188 was thawed for 6h at ambient temperature, mixed according to the two-
189 abovementioned modalities and placed in translucent polypropylene boxes (100x75x63
190 mm). Six replications for densities d10, d50, d100, and d250 and three replications for
191 density d500 were prepared for each food type.

192

193 ***Experimental procedure***

194 The eggs obtained at D-1 were incubated at 25°C for 22 h (first instar; see Fig. 1)
195 (Grassberger & Reiter, 2001). The next day (D0), young first instars were counted and
196 placed in the boxes containing food. Each box was placed in a second translucent
197 polypropylene box (180x135x195 mm) containing 1 cm of sand. To preserve
198 aggregation and avoid disturbance of feeding larvae, no measurements were
199 performed on feeding instars. Only wandering larvae (also called post-feeding larvae,
200 i.e., third instar larvae that had finished feeding and left the food) were measured. The
201 post-feeding larvae and adult flies were checked three times a day (10:00 a.m., 4:00
202 p.m., 10:00 p.m.).

203 Post-feeding larvae found in the sand were quickly rinsed with water and dried on
204 absorbent paper. After weighing (Kern® ALT310-4, Grass Valley, USA), these post-
205 feeding larvae were placed in new boxes (100x75x63 mm) containing 1 cm of sand.
206 The few larvae that left the food before the third instar or weighed less than 25 mg
207 (below the first percentile) were not counted as post-feeding larvae. The mortality rate
208 to post-feeding and the weighted mean of larval weights per replication were
209 calculated.

210 Newly emerged flies were sampled, left in development for at least 6 h, and then
211 sacrificed by freezing. The flies were sorted by sex using cephalic morphology
212 (Erzinçlioglu, 1996). The area of the right wing was measured to estimate the sizes of
213 males and females (Saunders & Bee, 1995). Data were recorded by taking pictures of
214 dissected and flattened right wings, with the surface area determined using ImageJ
215 software (version 1.51j8, Wayne Rasband, National Institutes of Health, USA, public
216 domain). All female and male flies from densities 10 and 50 were analysed. Random
217 samples of 25 male flies and 25 female flies per replication were analysed for the
218 densities 100, 250 and 500.

219

220 **Statistical analyses**

221 Statistical tests were conducted bilaterally, with a significance threshold of $\alpha=0.05$.
222 When appropriate, 95% bilateral confidence intervals are presented. All data were
223 analysed with R software via R studio (R version 3.3.2©2016; The R foundation for
224 Statistical Computing | Version 1.0.136©2009-2016 RStudio Inc.).

225 A logistic regression model (quasibinomial with a link function cauchit) was used for the
226 statistical analysis of larval development times. The variables to explain were (i) the
227 proportion of post-feeding larvae and (ii) the flies present during the respective periods
228 from D2 to D5 and D11 to D15. A step-by-step selection technique was used to
229 improve the model. The AIC criterion (Akaike Information Criterion) was used to
230 determine the quality of a model. For this reason, the model was applied to each food
231 type separately, with time and larval density as explicative factors. Then, the model
232 was applied to compare the effect of food at each density, with time and substrate the
233 explicative factors. ANOVA was used to determine the significance of the model
234 factors.

235 The odds ratio (OR) of each explicative variable was calculated, which provides an
236 estimate (with confidence interval) for the relationship between two binary variables:
237 when the OR is greater than 1, the two variables are considered associated.

238 Chi-square tests were used to compare mortality rates by food type, and when
239 significance was detected, pairwise *post hoc* tests were applied.

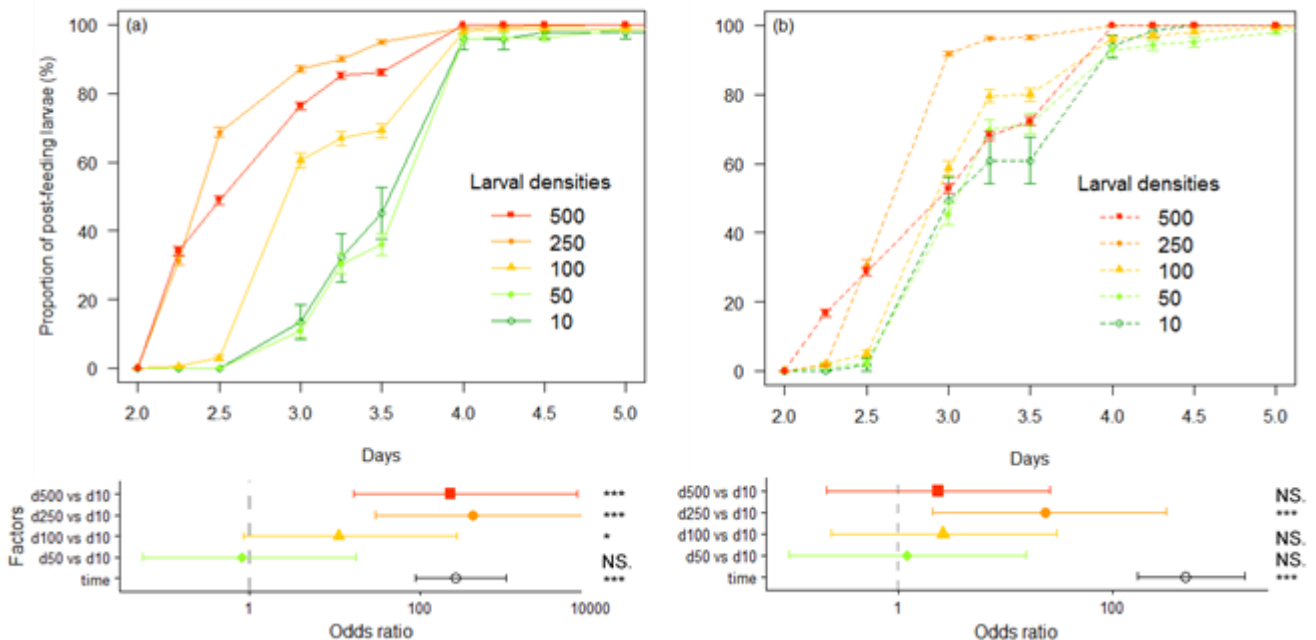
240 Two-way ANOVAs (density, food) were used to compare morphometric parameters
241 (size and mass). The data for size of flies from the few individuals of the d10 larval
242 density were insufficient (or even absent for one sex); therefore, we excluded the d10
243 data from the ANOVAs: only descriptive results are presented.

244

245 **RESULTS**

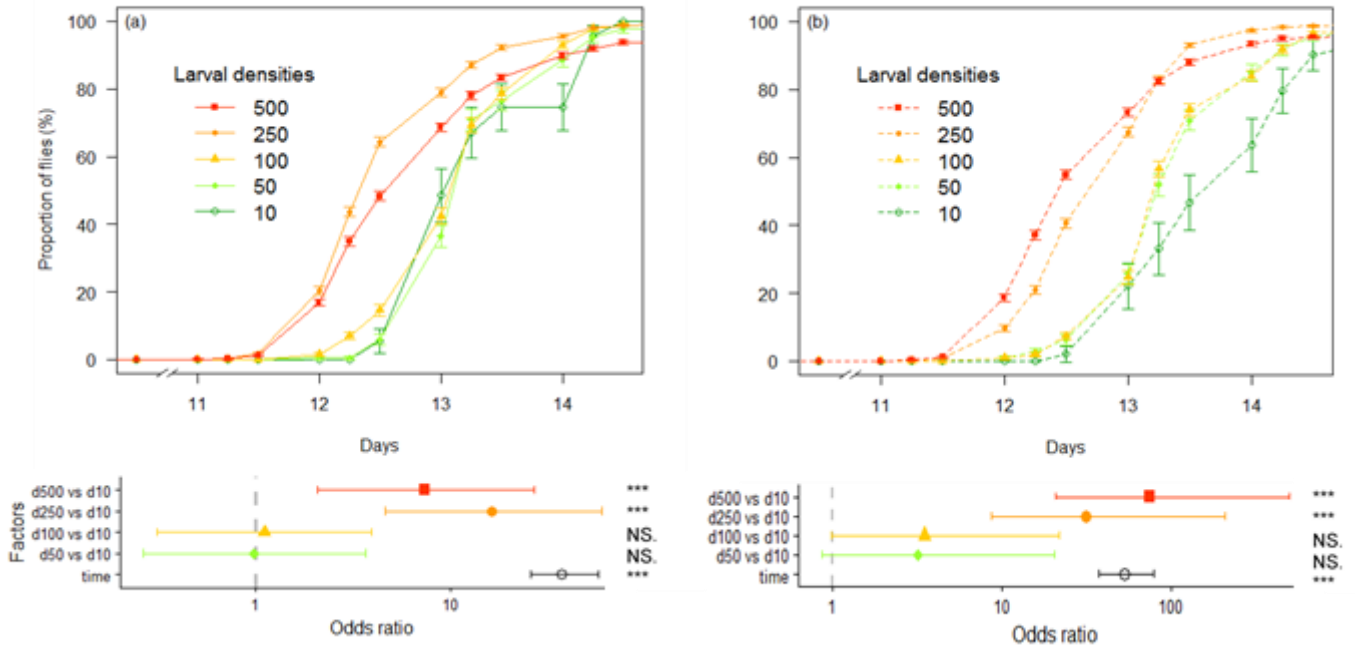
246 ***Effects of larval density***

247 The proportions of post-feeding larvae according to development time are shown in
 248 Fig. 2(a) (control food condition). The logistic regression model showed a significant
 249 effect of larval density on the proportion of post-feeding larvae ($P<0.001$). The
 250 development time decreased with larval density above 50 and up to 250 larvae and
 251 then slightly increased at d500 (see Fig. 2(a) for odds ratio of each density condition
 252 versus d10). The logistic regression model also demonstrated a significant effect of
 253 larval density on the development time until emergence, but this effect was less
 254 pronounced ($P<0.001$, see Fig. 3(a)).
 255 Early larval mortality rates (from first instar to post-feeding) were significantly affected
 256 by larval density ($X^2=101.4$, $P<0.001$). The pairwise *post hoc* test highlighted a
 257 decrease in mortality with the increase in larval density (see Fig. 4(a)). Considering
 258 overall mortality (from first instar to emergence), a significant decrease with increasing
 259 larval density was also observed ($P<0.001$; see Fig. 4(b)).

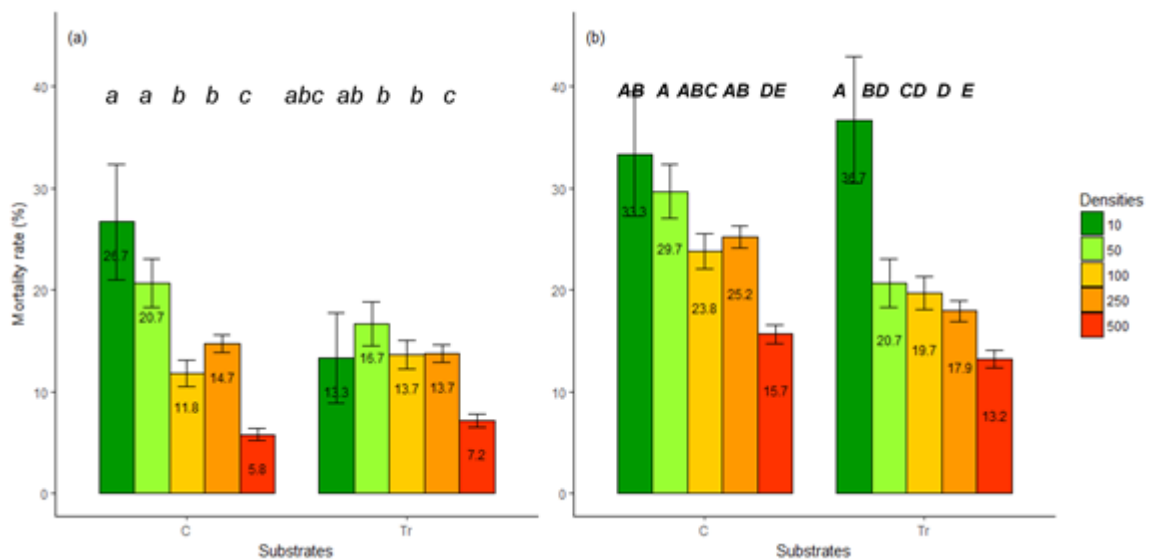


260 Figure 2: Kinetics of transition from larvae to post-feeding instars: (a). control food; (b).
 261 food with addition of trypsin. Percentages \pm SE of post-feeding larvae are given in
 262 relation to larval densities. Odds ratios with 95% confidence interval of the logistic

263 regressions are also given. d = no. of larvae / 50 g of food; NS. = not significant; *
 264 $P < 0.05$, *** $P < 0.001$.



265 Figure 3: Kinetics of imago emergence: (a) control food; (b) food with addition of
 266 trypsin. Percentages \pm SE of imagos are given in relation to larval densities. Odds
 267 ratios with 95% confidence interval of the logistic regressions are also given. d = no. of
 268 larvae / 50 g of food; NS. = not significant; *** $P < 0.001$.
 269
 270

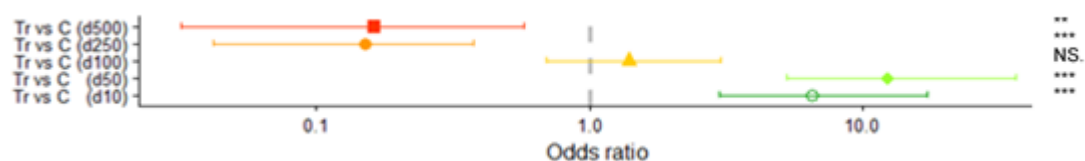


271 Figure 4: Average mortality rate (numbers in bars) \pm SE from first instar (a) to post-
 272 feeding and (b) to emergence. C = control food; Tr = trypsin-added food; densities =
 273 no. of larvae / 50 g food. Columns with the same letters are not significantly different
 274 ($P > 0.05$).
 275

276 **Effects of food digestibility (addition of trypsin)**

277 The addition of trypsin reduced larval development time at low densities, but did not
278 eliminated the benefits of gregariousness at highest densities ($P<0.001$; see Fig. 2(b)).
279 Compared with the control food, the average larval development time on trypsin-added
280 food was shorter at d10 and d50 ($P<0.001$), was similar at d100 ($P=0.27$) and was
281 longer at d250 and d500 ($P<0.001$ and $P=0.01$, respectively) (see Fig. 5). Development
282 time was significantly reduced at d250 versus d10 (OR=23.4[2.1–309.8], $P<0.01$); but
283 with the other increases in larval number, larval development time did not significantly
284 decrease. Regarding development time to fly emergence, a significant effect of density
285 was observed at d250 and d500 ($P<0.001$, OR = 31.4 [8.7–207.4] and 74.9 [20.7–
286 499.8], respectively; see Fig. 3(b) and Fig. A1).
287 Larval mortality rates on trypsin-added food were also influenced by larval density
288 ($X^2=45.5$, $P<0.001$), with the mortality at d500 significantly lower than that at other
289 densities ($P<0.001$, pairwise *post hoc* tests, see Fig. 4). No significant effect of trypsin
290 on the total development time was detected, with the following p-values: d10: $P=0.08$;
291 d50: $P=0.30$; d100: $P=0.91$; d250: $P=0.97$; and d500, $P=0.17$).

292



293 Figure 5: Odds ratios with 95% confidence interval of the effect of trypsin-added food
294 versus control food for larval development time (first instar to post-feeding). d = no. of
295 larvae / 50 g of food; Tr = trypsin-food; C = control food; NS. = not significant; **
296 $P<0.01$, *** $P<0.001$.

297

298 **Morphometrics**

299 The mean weight (\pm standard error) of post-feeding larvae was 55 ± 0.4 mg (all
300 conditions combined). Larval density ($F=1.41$, $P=0.24$) or food type ($F=1.93$, $P=0.17$)
301 did not affect the weight (see Fig .A2).

302 The male to female sex ratio was 0.99 ± 0.003 and was not modified by experimental
303 conditions ($X^2=5.84$, $P=0.76$). Wing surface area of the flies (see Fig A3) was not
304 affected by larval density or by food type for either females ($F=1.81$, $P=0.14$; $F=0.40$,
305 $P=0.53$, respectively) or males ($F=2.24$, $P=0.08$; $F=1.63$, $P=0.20$, respectively). The
306 mean right wing surface area was 15.7 ± 0.1 mm² for females and 13.1 ± 0.1 mm² for
307 males. As expected, males were smaller than females ($F=922.2$, $P<0.001$).

308

309 **DISCUSSION**

310 Experiments demonstrated (1) a decrease in development time and mortality with
311 increasing larval density and (2) a decrease in development time and mortality on high-
312 digestible food compared with low-digestible food (at low densities of larvae). These
313 results provide evidence of the benefits of collective exodigestion and aggregation and
314 possibly of much larger consequences at the community level such as niche
315 partitioning.

316

317 ***Aggregation of necrophagous larvae is consistent with the Allee effect***

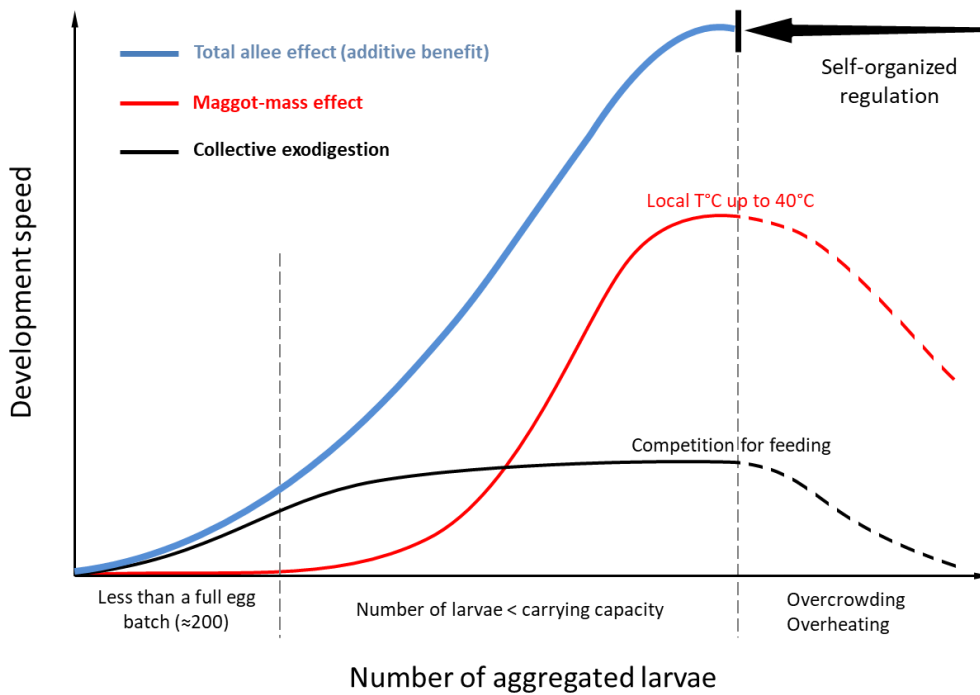
318 Increasing larval density resulted in better development of *L. sericata* larvae on the
319 control fresh meat. With the increase in larval density, (i) *L. sericata* development time
320 was reduced, and (ii) mortality decreased. Due to the selection pressure on the feeding
321 maggots (competition with scavengers, food shortage, parasitism and
322 interspecific/interkingdom competition), their survival notably depends on development
323 time (Benbow et al., 2015, DeVault et al., 2004; Frederickx et al., 2013, Hanski, 1987;
324 Jordan & Tomberlin, 2017). Thus, the observed difference in developmental time with
325 density might have even more dramatic impact on larval survival in the field.
326 Furthermore, larval density did not affect adult fly morphometrics: larvae growing in the
327 large groups assimilated sufficient food fast enough not to affect their metamorphosis

328 and final size/weight (Charabidze et al., 2013). As the fertility of a female depends on
329 its size (Saunders & Bee, 1995), we reasonably concluded that increased density had
330 no deleterious effect and positively affected population fitness, which is a result
331 consistent with the predictions of the Allee effect (i.e., a positive relationship between
332 fitness and population size) (Allee, 1927; Courchamp et al., 2008).

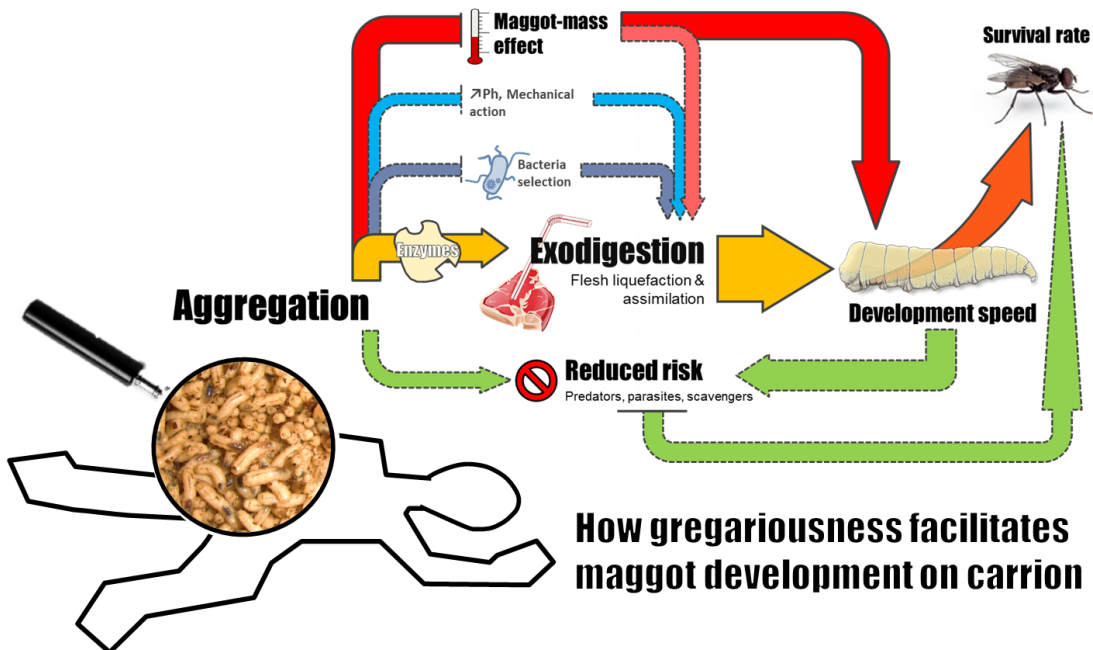
333 Inside larval masses, two collective strategies likely act synergistically. Maggot-mass
334 effect, which is a local temperature increase inside large aggregates, was formerly
335 postulated as one of the primary benefits of larval pre-social behaviour (Rivers et al.,
336 2011, 2012). However, significant local warming requires many more larvae than those
337 used for this study (see Fig. A4). As an example, a 24-h decrease in development time
338 as we observed with 250 larvae groups in the present work would require a local
339 temperature shift from 25°C to 34°C (Grassberger & Reiter, 2001), which is warming
340 that is only reached above 5690 aggregated larvae (Charabidze et al., 2011). By
341 contrast, exodigestion occurs at the scale of a single egg-batch (circa 200 larvae) and
342 is fully efficient as soon as a few hundred larvae aggregate and feed together. This
343 initial benefit can be reinforced by the aggregation of additional larvae, resulting in
344 maggot masses sufficiently large to increase local temperature (see Fig. 6 and 7).

345

346 This aggregation strategy of necrophagous blowflies may rely on the genetic proximity
347 of feeding larvae, a result previously described in caterpillars (Tsubaki & Shiotzu,
348 1982). Indeed, the genetic affinity within aggregated larvae is inherently close: each
349 female fly lays egg batches of circa 200 eggs, resulting in hundreds of aggregated
350 offspring (Wall, 1993). Picard & Wells (2010) also found that gravid females ready to
351 oviposit on the same carcass showed a pattern of relatively high relatedness.



352 Figure 6: Schema of the benefit to development speed (i.e. gain) of increasing numbers
 353 of aggregated larvae. .
 354
 355
 356
 357
 358



359 Figure 7: Summary of demonstrated (solid arrows) or hypothetical (dotted arrows)
 360 benefits of necrophagous larvae gregariousness.
 361

362 ***Gregariousness may be a blowfly adaptive strategy to breed on fresh carcasses***

363 The results obtained with trypsin-added food also highlight the benefits of aggregation.
364 Compared with those fed fresh control food, larvae fed on high-digestibility food had
365 reduced average mortality and slightly faster development. Therefore, we assumed that
366 the increased development and reduced mortality of aggregated larvae resulted in part
367 from chemical degradation (pooling of enzymes). Consistent with this idea, Wilson et
368 al. (2016) demonstrated that the addition of a trypsin inhibitor drastically reduces
369 exodigestion and slows *L. sericata* larval growth. Green et al. (2002) also showed that
370 feeding activity of groups of *Phormia regina* larvae has a greater effect on the chemical
371 nature of the diet surfaces than the feeding of isolated larvae. However, while trypsin-
372 added food significantly decreased mortality rates at d10, this increased survival was
373 offset by a higher mortality during post-feeding instars (see Fig. 4). This "catching up"
374 effect resulted in a similar global (i.e. first instar to emergence) mortality on both food
375 types. One likely hypothesis is that the addition of trypsin facilitates the ingestion and
376 digestion of the food, and thus allows the weakest larvae (those whom died on control
377 experiments) to survive artificially (Barnard & Geden, 1993). However, many of these
378 larvae were living on borrowed time and they died during post-feeding or pupal
379 development. Furthermore, the addition of trypsin did not completely eliminate the
380 benefits of gregariousness in terms of development time (electronic Table A1). Two
381 cofactors of exodigestion might explain why trypsin does not fully imitate the properties
382 of aggregated larvae. First, larval alkalisation of the medium can increase the pH-
383 dependent enzymatic degradation (Hobson, 1931). Second, a density-dependent
384 selection of competing bacteria may be an important consequence of larval
385 aggregation (Arce, Smiseth, & Rozen, 2013). Indeed, Janzen's "spoiling theory"
386 (Janzen, 1977) hypothesises that bacterial decomposers should produce compounds
387 that cause rich and ephemeral resources to rot, rendering them unpalatable, whereas
388 animals should evolve adaptive strategies of avoidance or detoxification. Thus, carrion

389 is the site of an intense interkingdom competition between insects and microbial
390 decomposers (Burkepile et al., 2006; Jordan & Tomberlin, 2017; Young et al., 2015). In
391 this context, burying beetles can cooperate to bury a carcass and limit microbial
392 proliferation (Eggert & Müller, 2011; Sun et al., 2014; Trumbo, 2017), and blowfly
393 larvae secrete/excrete efficient antimicrobial peptides, which is a property used in larval
394 debridement therapies (Teh et al., 2017). Therefore, larval aggregation may be an
395 adaptive response that locally limits bacterial proliferation and spoiling (see Fig. 7).

396

397 **Conclusions**

398 The present work demonstrates that calliphorid larvae pre-social behaviour not only
399 results in significant benefits throughout larval development but also may act as a
400 niche-separation mechanism. It has been demonstrated that fly egg-laying strategies
401 can facilitate coexistence in carrion fly communities (Hanski, 1977; Ives, 1989, 1991;
402 Kouki & Hanski, 1995; Kuusela & Hanski, 1982; Prinkkilá & Hanski, 1995; Spindola et
403 al., 2017). Progressing through the stages of decomposition, a carcass becomes
404 attractive to a relatively predictable sequence of insects (Matuszewski et al., 2011;
405 Megnin, 1894). Although highly nutritional, fresh meat is more difficult to ingest and
406 digest than decayed meat (Benbow et al., 2015). By mutualising exodigestion,
407 calliphorid larvae (i.e., pioneer species) possibly make a food source avoided by other
408 necrophagous species more edible. This hypothesis is consistent with the idea that
409 cooperation may enable species to expand their niches (the so-called 'social conquest
410 hypothesis') (Brooks et al., 2017).

411

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413

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416

417 **References**

418

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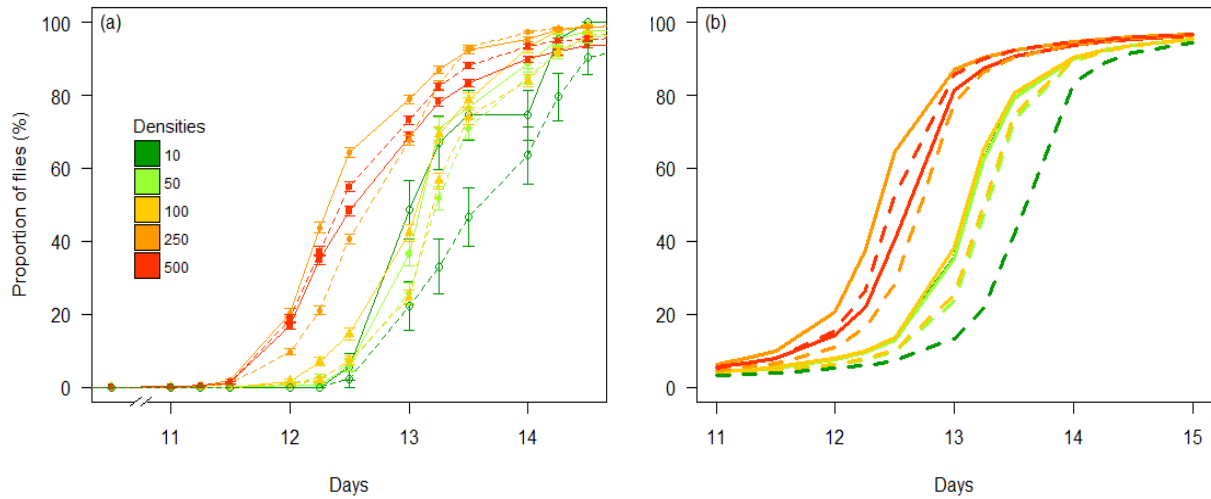
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736 APPENDIX

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738 Figure A1

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741 Figure A1: Kinetics of imago emergence for the control (solid lines) and trypsin (dashed

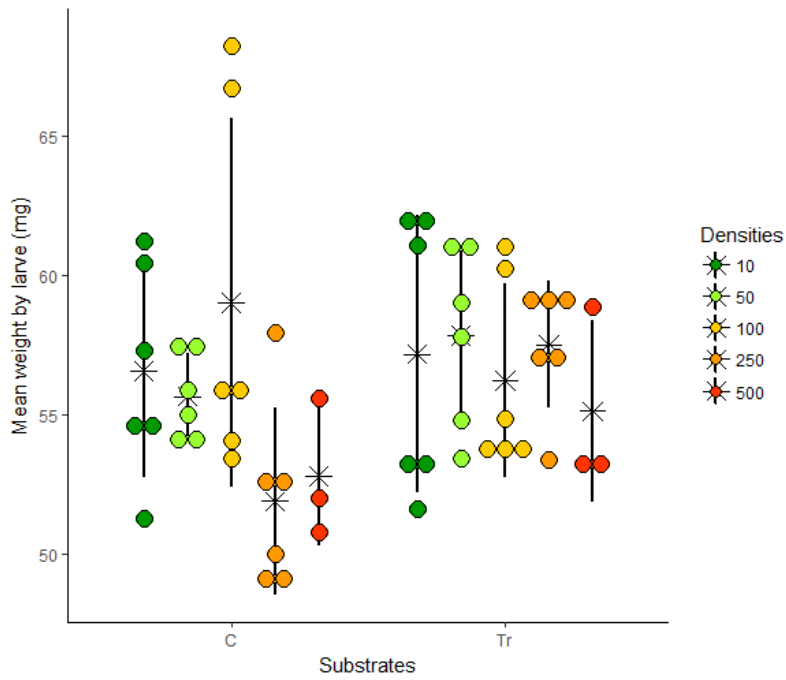
742 lines) conditions. (a) Percentages \pm SEs of imagos in relation to larval densities. (b)

743 Logistic regressions.

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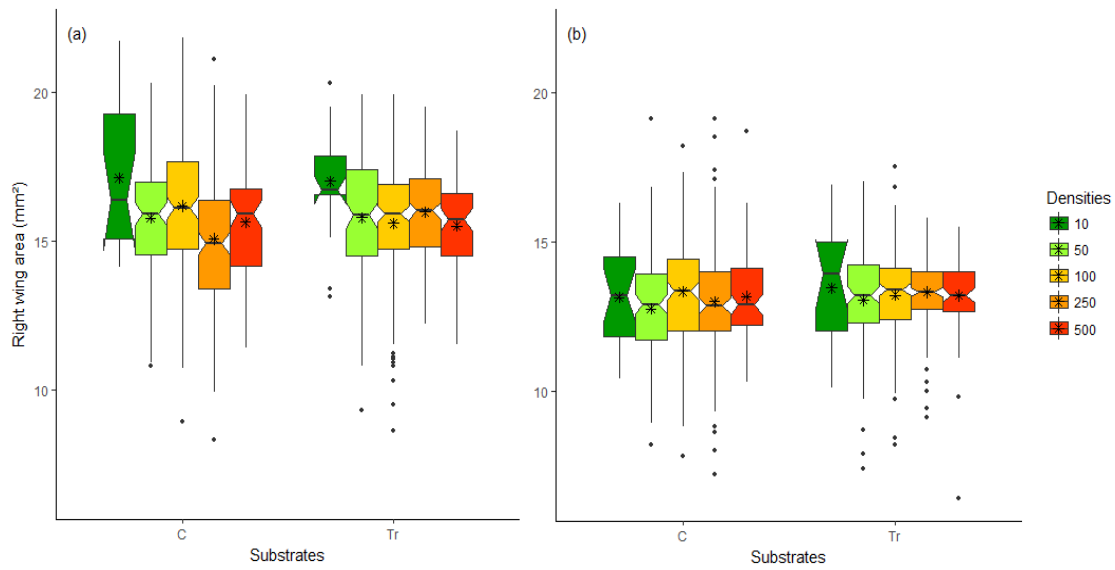
746 **Figure A2**
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Figure A2: Dot plot of the average weight (\pm SD) of post-feeding larvae per condition. C = control food; Tr = trypsin-added food; densities = no. of larvae / 50 g of food.

754 **Figure A3**



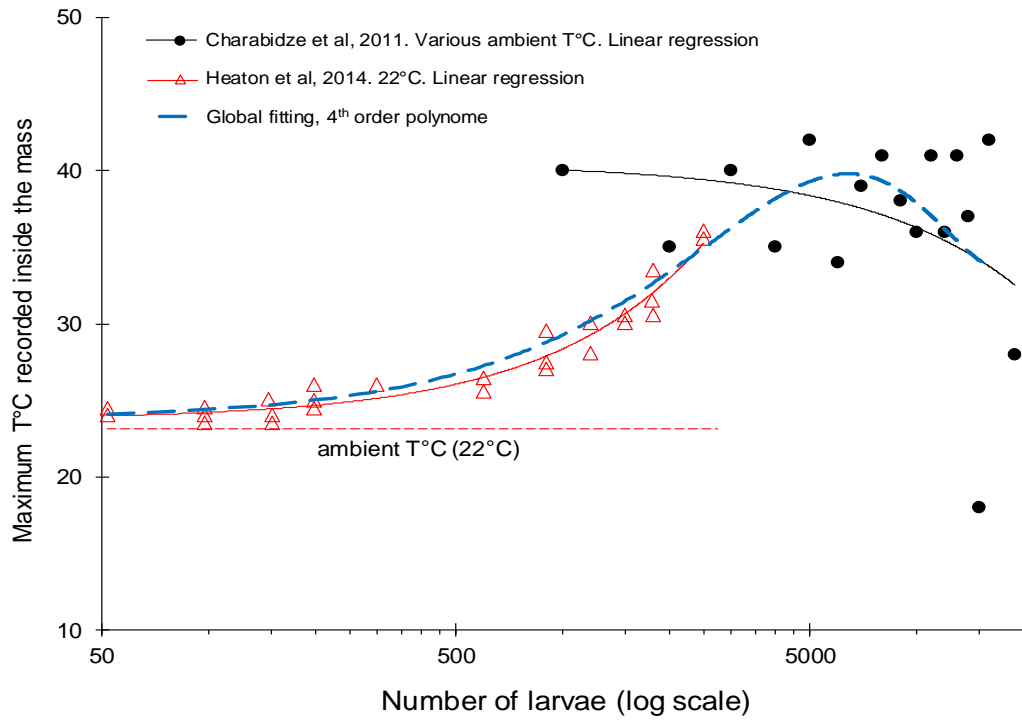
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756 Figure A3: Box plot of the size of the flies, evaluated by the area of the right wing,
757 according to sex: (a) females; (b) males. * = mean area; C = control food; Tr = trypsin-
758 added food; densities = no. of larvae / 50 g of food. The box plots show the median and
759 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the
760 interquartile range and the circles are outliers

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763 **Figure A4**
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Table A1: Table of literature review

<u>Species</u>	<u>Larval density</u> (larvae per <i>n</i> gram of substrate)	<u>Substrate</u>	<u>Outcomes</u>	<u>Effects</u>	<u>References</u>
<i>Lucilia sericata</i>	100 ; 200 ; 500 ; 1000 ; 2000 ; 3000 ; 16000 /140g	Beef	Development time Larval weight Weight and size of pupae Fecundity of flies Mortality rate	Increases with increased larval density Decreases with increased larval density Decreases with increased larval density Decreases with increased larval density Increases with increased larval density	Ullyett <i>et al.</i> , 1950
<i>Chrysomya albiceps</i>					
<i>Chrysomya chloropyga</i>					
<i>Chrysomya megacephala</i>	25 ; 50 ; 100 ; 200 ; 250 ; 500 / 25g	Beef liver	Development time Weight and size of pupae and flies Mortality rate of larvae and pupae	Decreases for the intermediate density from 100 to 500/25g Decreases with increased larval density Decreases for the intermediate density from 100 to 200/25g	Goodbrod <i>et al.</i> , 1990
<i>Chrysomya rufifacies</i>					
<i>Lucilia sericata</i>	8 / 4g ; 8g ; 16g ; 32g	Agar + 20 % blood horse meat + 5 % yeast	Weight of pupae Proportion of emergences Weight of flies	Decreases with increased larval density Decreases with increased larval density Decreases with increased larval density	Simkiss <i>et al.</i> , 1992
<i>Lucilia sericata</i>	2 ; 4 ; 8 ; 16 ; 32 ; 64 ; 128 ; 256 ; 512 / 1g	Beef	Mortality rate until emergence Size, fecundity and longevity of flies	Decreases with increased larval density Decreases with increased larval density	Prinkkilä <i>et al.</i> , 1995
<i>Lucilia illustris</i>			Mortality rate until emergence Size, fecundity and longevity of flies	Increases for the intermediate density ~32/1g then strongly decreases Decreases with increased larval density	
<i>Lucialia silvarum</i>			Mortality rate until emergence Size, fecundity and longevity of flies	Decreases with increased larval density Decreases with increased larval density	
<i>Lucilia caesar</i>			Mortality rate until emergence Size, fecundity and longevity of flies	Increases for the intermediate density ~16/1g then strongly decreases Decreases with increased larval density	
<i>Calliphora vicina</i>	1 ; 5 ; 10 ; 20 ; 50 ; 100 ; 150 ; 200 ; 300 / 50g	Beef	Weight of post-feeding larvae Development time Mortality rate Size of flies	Decreases with increased larval density Increases with increased larval density Increases with increased larval density Decreases with increased larval density	Saunders <i>et al.</i> , 1995
<i>Chrysomya putoria</i>	From 100 to 2000 (by 100) / 50g	Whole dried milk and whole dried egg	Weight of pupae Mortality rate	Decreases with increased larval density Decreases with increased larval density	Godoy <i>et al.</i> , 1996
<i>Lucilia sericata</i>	10 ; 20 ; 30 ; 50 ; 100 ; 150 ; 250 ; 300 ; 500 ; 750 ; 1000 ; 1500 / 15g	Lamb liver	Size of flies Proportion of emergences	Decreases with increased larval density Decreases with increased larval density	Smith <i>et al.</i> , 1997
<i>Calliphora vicina</i>	10 ; 20 ; 30 ; 50 ; 100 ; 150 ; 250 ; 300 ; 500 ; 750 ; 1000 ; 1500 / 15g		Size of flies Proportion of emergences	Decreases with increased larval density Decreases with increased larval density	

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<i>Cochliomyia macellaria</i>	200 ; 400 ; 800 ; 1600 / 50g	Whole dried milk and whole dried egg	Mortality rate	Increases with increased larval density	Dos Reis <i>et al.</i> , 1999
<i>Chrysomya putoria</i>					
<i>Lucilia sericata</i>	10 ; 50 ; 100 ; 200 ; 350 ; 500 / (20g renewed every 48 hours)	Agar + 20 % blood horse meat + 5 % yeast	Mortality rate from first instar to fly Weights of pupae and flies Larval development time Longevity of flies Fecundity of flies	Decreases slightly with density from 10 to 100 then increase, particularly from first to third instar. The proportion of emergences decrease with the density. Decreases with increased larval density Increases with increased larval density Decreases with increased larval density Decreases with increased larval density	Moe <i>et al.</i> , 2002
<i>Chrysomya megacephala</i>	200 ; 1000 / 50g	Beef	Mortality rate Fecundity of flies Size of flies	No significant difference Decreases with increased larval density Decreases with increased larval density	Reigada <i>et al.</i> , 2004
<i>Calliphora vomitoria</i>	1 ; 5 ; 10 ; 20 ; 50 ; 75 ; 100 / 10g	Prok liver, brain or meat	Development time from first instar to emergence Mortality rate Size of flies	Decreases with increased larval density Increases with increased larval density Decreases with increased larval density	Ireland <i>et al.</i> , 2005
<i>Lucilia sericata</i>	20-30 ; 50 ; 150 ; 300 ; 650-750 / 15g	Pork liver	Mortality rate Size of flies Development time until emergence	Increases with increased larval density Decreases with increased larval density No significant difference	Martinez-Sánchez <i>et al.</i> , 2007
<i>Protophormia terraenovae</i>	25 ; 50 ; 100 ; 200 ; 500 ; 1000 ; 2000 / 150g	Beef liver	Development time from larvae to pupariation Time for emergence Weight of pupae Proportion of emergence	Decreases with increased larval density No significant difference Decreases with increased larval density Decreases with increased larval density	Rivers <i>et al.</i> , 2010
<i>Lucilia eximia</i>	100 ; 200 ; 400 ; 600 ; 800 / 50g	Whole dried milk and whole dried egg	Mortality rate Fécondity of flies	Increases with increased larval density Decreases with increased larval density	Serra <i>et al.</i> , 2011
<i>Chrysomya albiceps</i>	100 ; 200 ; 400 ; 600 ; 800 ; 1000 /50g				
<i>Cochliomyia macellaria</i>	from 200 to 2000 by 200 / 50g				
<i>Chrysomya megacephala</i>					
<i>Chrysomya albiceps</i>					
<i>Chrysomya rufifacies</i>	10mg ; 50mg 100mg of first instar larvae /150g	Kangaroo meat	Development time Weight and size of post-feeding larvae	Decreases with increased larval density Decreases with increased larval density	Johnson <i>et al.</i> , 2014
<i>Calliphora vicina</i>			Development time Weight and size of post-feeding larvae	No significant difference Decreases with increased larval density	
<i>Lucilia sericata</i>	0 ; 2,5 ; 5 ; 7,5 ; 10 ; 12,5 ; 15 (Biobag) / 20g	Pork liver	Mass of tissues digested after 48 h Average mass by larvae after 48 h Larval mortality rate after 48 hours	Increases until 7,5 Decreases from 5 No significant difference	Wilson <i>et al.</i> , 2016