

1 This is the submitted version of the following article:

2 Lis, N., Mądra-Bielewicz, A., Wydra, J. and Matuszewski, S. (2024), Competition, cooperation, and  
3 parental effects in larval aggregations formed on carrion by communally breeding beetles *Necrodes*  
4 *littoralis* (Staphylinidae: Silphinae). Insect Science. <https://doi.org/10.1111/1744-7917.13353>,

5 which has been published in final form at:

6 <https://onlinelibrary.wiley.com/doi/10.1111/1744-7917.13353>

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8 **Competition, cooperation and parental effects in larval aggregations formed on carrion**  
9 **by communally breeding beetles *Necrodes littoralis* L. (Staphylinidae: Silphinae)**

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18 Running title: Competition in larval aggregations of *Necrodes*

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22

23 **Abstract**

24           Aggregations of juveniles are the dominant forms of social life in some insect groups.  
25 Larval societies are shaped by competitive and cooperative interactions of the larvae, in  
26 parallel with parental effects. Colonies of necrophagous larvae are excellent systems to study  
27 these relationships. *Necrodes littoralis* (Staphylinidae: Silphinae), a carrion beetle that  
28 colonizes cadavers of large vertebrates, forms massive juvenile aggregations. By spreading  
29 over carrion anal and oral exudates, the beetles form the feeding matrix, in which the heat is  
30 produced and by which adults affect the fitness of the larvae. We predict that exploitative  
31 competition shapes the behaviour of *N. littoralis* larvae in their aggregations. However,  
32 cooperative interactions may also operate in these systems due mainly to the benefits of  
33 collective exodigestion. Moreover, indirect parental effects (i.e. formation of the feeding  
34 matrix) probably modulate larval interactions within the aggregations. By manipulating  
35 parental effects (present/absent) and larval density (0.02-1.9 larvae/1 g of meat), we found a  
36 strong negative group-size effect on the fitness of *N. littoralis*, in colonies with parental  
37 effects over almost the entire density range, and in colonies without parental effects for  
38 densities larger than 0.5 larva/g. This was accompanied by positive group-size effects in terms  
39 of development time (it shortened with larval density) and thermogenesis (it increased with  
40 larval density). A pronounced positive group-size effect on juvenile fitness was found only in  
41 colonies without parental effects and only in the low density range. These results support the  
42 hypothesis that larval societies of *N. littoralis* are shaped by exploitation competition.

43

44 **Keywords:** Carrion, Larval societies, Intraspecific interactions, Group-size effect

45

## 46 **Introduction**

47       Aggregations of juveniles (e.g. societies of tent caterpillars or carrion maggots) are the  
48 dominant forms of social life in some insect groups (Costa, 2006, Charabidze et al., 2021).  
49 There is a considerable diversity of such forms, but certain collective behaviours recur with  
50 high regularity, e.g. the group building of a shelter in gregarious caterpillars (Joos et al., 1988,  
51 Ruf and Fiedler, 2002, Costa, 2006) or the group feeding in carrion insect larvae (Charabidze  
52 et al., 2021, Rivers et al., 2011). Generally, aggregating may benefit the larvae in terms of  
53 foraging or feeding, thermoregulation, nest construction or defence (Costa, 2006).

54       Most necrophagous larvae aggregate, in particular blow flies (Rivers et al., 2011), carrion  
55 beetles (Gruszka et al., 2020, Popescu et al., 2023, Bonacci et al., 2011) or skipper flies  
56 (Matuszewski et al., 2014). At one end of the spectrum there are small family groups formed  
57 by burying beetles (Staphylinidae: Silphinae: *Nicrophorus*), which comprise usually no more  
58 than 20-30 larvae, the offspring of a single female (Trumbo, 1990a, Bartlett and Ashworth,  
59 1988). At the other end there are larval masses formed by blow flies or *Necrodes* beetles  
60 (Staphylinidae: Silphinae), which may consist of thousands of larvae from hundreds of  
61 females breeding communally on one large cadaver (Gruszka et al., 2020, Rivers et al., 2011).  
62 Between there are larval aggregations of *Thanatophilus* beetles (Staphylinidae: Silphinae) or  
63 *Stearibia* flies (Piophilidae), usually of moderate size but from many females (Bonacci et al.,  
64 2011, Matuszewski et al., 2014).

65       Since carrion is primarily the food source for the larvae, its effective digestion is probably  
66 the major ecological cause for larval aggregation. Necrophagous larvae ingest liquefied food  
67 and need to preprocess carrion through exodigestion (Rivers et al., 2011, Scanvion et al.,  
68 2018). Collective exodigestion by aggregated larvae of *Lucilia sericata* (Diptera:  
69 Calliphoridae) has been demonstrated to shorten their development and reduce mortality  
70 (Scanvion et al., 2018). Shorter development times and reduced mortality have been also

71 reported for *Necrodes littoralis* beetles (Staphylinidae: Silphinae) when they were reared in  
72 aggregations compared to those reared individually (Gruszka and Matuszewski, 2021).  
73 Similar differences were found in the case of *Thanatophilus sinuatus* beetles, when the  
74 development patterns for larvae kept individually (Montoya-Molina et al., 2021) were  
75 compared with the patterns for aggregated larvae (Lis et al., 2023). Although there is no direct  
76 evidence to link these benefits with the cooperative exodigestion in carrion beetles, such  
77 interpretation is plausible (Gruszka and Matuszewski, 2021, Lis et al., 2023). Thermal effects  
78 of aggregation are also important for carrion insect larvae, as they may largely accelerate  
79 growth. Such effects were demonstrated in carrion blow flies and *Necrodes* beetles (Slone and  
80 Gruner, 2007, Charabidze et al., 2011, Matuszewski and Mađra-Bielewicz, 2021). Juvenile  
81 societies on carrion can also bring protective benefits, i.e. a repulsion of vertebrate scavengers  
82 or a dilution effect for insect predators or parasites, although there is no experimental  
83 evidence in support (Burkepile et al., 2006, Mooring and Hart, 1992, Charabidze et al., 2021).  
84 Aggregations may benefit the larvae also by facilitating control of microbial communities on  
85 carrion (Duarte et al., 2018, Pöppel et al., 2015, Körner et al., 2023).

86 Due to these demonstrated and potential benefits, larval societies on carrion can be viewed  
87 as the cooperative systems (Charabidze et al., 2021). On the other hand, competition must  
88 play an important role in these systems (Ullyett, 1950, Hanski, 1987). Carrion is a patchy  
89 resource with all the consequences (Butterworth et al., 2023). Since necrophagous larvae are  
90 generally not very mobile, they are usually forced to feed on the carcass, where they began  
91 their development. Moreover, because carrion is ephemeral and it frequently depletes when  
92 insects are still feeding, larvae are faced with overcrowding and the resulting food shortages.  
93 Several experimental studies using larval blow flies demonstrated that under such conditions  
94 mortality increases and size of the flies decreases (Ullyett, 1950, Ireland and Turner, 2006,  
95 Johnson and Wallman, 2014). Similar density effects were observed in burying beetles,

96 although in this insect group they can be attenuated through infanticide or parental care  
97 (Smiseth et al., 2007, Schrader et al., 2015, Bartlett, 1987, Trumbo, 1990a). Because each  
98 larva depletes through feeding the total pool of carrion resources, this is an example of  
99 exploitative competition that must have shaped behaviour of the larvae within the juvenile  
100 societies on carrion (Ullyett, 1950, Matuszewski and Mądra-Bielewicz, 2022, Hanski, 1987).  
101 A very high feeding rate and rapid growth of necrophagous larvae are probably major effects  
102 of the competition for food with congeners. Completion of development before the food  
103 resources on carrion are exhausted or their quality is reduced is probably the key selection  
104 pressure in this context (Hanski, 1987). Intriguingly, the feeding race of the larvae may  
105 generate positive by-products for all members of an aggregation. In order to feed at a faster  
106 rate, a larva must produce more digestive enzymes, which increases the enzyme pool on  
107 carrion and, in turn, facilitates collective exodigestion to the benefit of all larvae in the  
108 aggregation. Therefore, the benefits from the juvenile aggregations on carrion can be viewed  
109 as the by-products of the actions of individual larvae to maximize their own fitness. This  
110 would be an example of the “by-product mutualism” as specified by Clutton-Brock “...in  
111 which an individual’s behavior maximizes its own immediate fitness and any positive effects  
112 on the fitness of other individuals are coincidental and do not contribute to the selection  
113 pressures maintaining the behavior” (Clutton-Brock, 2002).

114 Parental effects are also important in carrion insect societies, particularly in the case of  
115 carrion beetle larvae. There are many examples of positive effects. Adult *Ptomascopus*  
116 (Staphylinidae: Silphinae) or *Necrodes* beetles secure the resource pool for the larvae by  
117 clearing carrion of the maggots (Suzuki and Nagano, 2006, Matuszewski and Mądra-  
118 Bielewicz, 2022). Blow flies are similarly eliminated by adult burying beetles (Pukowski,  
119 1933, Scott, 1998). Density of the larvae in the aggregation may be directly optimised by  
120 these beetles through infanticide (Bartlett, 1987, Trumbo, 1990b). Furthermore, adult beetles

121 may prevent putrefaction through carrion preservation, which was demonstrated in burying  
122 beetles and is plausible in *Necrodes* beetles (Duarte et al., 2018, Shukla et al., 2018, Hoback  
123 et al., 2004, Matuszewski and Mađra-Bielewicz, 2021). Adults may also facilitate nutrition of  
124 the larvae, directly by food provisioning in burying beetles or indirectly by formation of the  
125 feeding matrix in *Necrodes* beetles or the feeding holes in carrion ball in burying beetles  
126 (Matuszewski and Mađra-Bielewicz, 2021, Shukla et al., 2018, Vogel et al., 2017, Smiseth  
127 and Moore, 2004, Trumbo, 2017, Pukowski, 1933). There are negative effects, as well.  
128 Because adult carrion beetles share the food with their offspring, they may decrease the  
129 fitness of the larvae through self-feeding (Trumbo and Xhiani, 2015, Matuszewski and  
130 Mađra-Bielewicz, 2021).

131 The interaction between parental and juvenile effects was studied in burying beetles.  
132 Under low larval density when all parental effects were removed, larvae revealed an increase  
133 in body mass with brood size (a positive group-size effect), consistent with the larval  
134 cooperation hypothesis; when posthatching effects were removed cooperation occurred only  
135 in the species with more independent larvae (Prang et al., 2022). Under high larval density  
136 when posthatching effects were removed, some authors reported negative group-size effect,  
137 consistent with larval competition hypothesis (Schrader et al., 2015), whereas others  
138 demonstrated no significant group-size effect (Smiseth et al., 2007, Magneville et al., 2018).  
139 However, when larvae were provided with full parental effects (i.e. pre and posthatching),  
140 their growth was generally enhanced, although there was a significant negative group-size  
141 effect that indicated competition for resources (Schrader et al., 2015, Smiseth et al., 2007).

142 In the current study, we analysed the interaction between parental and juvenile effects  
143 in larval colonies of communally breeding carrion beetles *Necrodes littoralis*. The species,  
144 occurring in the Palearctic, colonizes cadavers of large vertebrates (including humans), where  
145 it forms massive juvenile aggregations (Bajerlein et al., 2018, Gruszka et al., 2020,

146 Charabidze et al., 2016). In order to secure food resources for their offspring, adults clear  
147 carrion of the blow fly larvae by selectively killing maggots, which are before or in peak  
148 feeding phase (Matuszewski and Mądra-Bielewicz, 2022). Through spreading over carrion  
149 anal and oral exudates, they form feeding matrix, in which the heat is produced and by which  
150 adult beetles affect fitness of the larvae (Matuszewski and Mądra-Bielewicz, 2021). These  
151 effects may be categorized as simple forms of indirect parental care. Usually, many *Necrodes*  
152 females oviposit in the soil nearby the carcass. Larvae aggregate, exodigest carrion tissues and  
153 modify the feeding matrix, in which they feed and benefit from thermogenesis (Matuszewski  
154 and Mądra-Bielewicz, 2021). Since *Necrodes* larvae frequently face food shortages, we  
155 hypothesize that intraspecific exploitative competition shapes their behaviour in aggregations.  
156 Competitive effects occur probably under high larval density, whereas under low density  
157 cooperative effects may act due to the benefits of collective exodigestion. We also predict that  
158 parental effects (i.e. formation of the feeding matrix) modulate these interactions and in effect  
159 change the relationship between density and fitness of the larvae.

160

## 161 **Materials and methods**

### 162 **Main beetle colony**

163 We used beetles from our main colony that is maintained in Laboratory of  
164 Criminalistics at AMU (Poznań, Poland). The colony was established in June 2017 using  
165 about 50 adult beetles collected in a forest of Biedrusko military range (52°31'N, 16°54'E;  
166 Western Poland). It was replenished with about 50 beetles sampled in 2018 (52°31'N,  
167 16°54'E) and again in 2022 (52°26'N, 17°09'E).

168 Males and females were kept separately in plastic insect breeding containers (capacity:  
169 7.5 l; 30-50 beetles/container), on a humid soil (1/3 of a container) with aluminium foil as a  
170 cover. The beetles were fed with raw pork pieces and had constant access to cotton wool  
171 soaked in water. Usually, 3-5 containers were kept at a time under a fume hood (20-23 °C, 50-  
172 60 % RH).

173

### 174 **Experimental design**

175 In order to test the influence of larval density and parental effects on the fitness of  
176 juvenile *N. littoralis* beetles, we conducted two experiments, both according to the factorial  
177 block design. The factors were: the presence of adult beetles in the pre-larval phase  
178 (hereinafter 'parental effects') and the number of larvae in a juvenile colony in the larval  
179 feeding phase (hereinafter 'larval density'). 'Parental effects' were considered on two levels  
180 in both experiments: present or absent (+/-). 'Larval density' was considered on five levels in  
181 experiment 1: 5, 10, 20, 40 and 80 larvae/colony. In order to study larval colonies of very low  
182 and very high density, we conducted a follow-up experiment (experiment 2), in which 'larval  
183 density' was considered on six levels: 2, 4, 6, 8, 10 and 120 larvae/colony. Both experiments  
184 were replicated ten times (10 blocks).

185

### 186 **Experimental beetle colonies and laboratory protocols**



187 Experiment 1 started in March 2022, the last block was completed in October 2022.  
188 Experiment 2 started in January 2023, the last block was completed in May 2023. One to  
189 three blocks were conducted at a time. The same protocols were used in experiment 1 and 2.

190 Experimental beetle colonies were kept in plastic insect breeding containers (capacity:  
191 3.5 l), 1/4 filled with a humid soil. To increase humidity and provide constant access of the  
192 beetles to water, we used cotton wool that was regularly soaked with water. The aluminium  
193 foil was placed over the colony to maintain high humidity. Raw pork pieces were used as  
194 food, one piece per colony. Meat was weighed at the beginning of the experiment, at the end  
195 of the pre-larval phase (in ‘parental effects (+)’ treatment) and at the end of the larval feeding  
196 phase. In ‘parental effects (+)’ treatment about 100 g/colony was used at the beginning. After  
197 weighing the meat at the end of the pre-larval phase, we calculated the average (per block)  
198 mass of the remaining meat. The same average mass of fresh meat was used in ‘parental  
199 effects (-)’ treatment (60-90 g/colony depending on the block).

200 In the ‘parental effects (+)’ treatment, ten adult beetles (5♀;5♂), chosen at random  
201 from the main colony, were used per container. The beetles stayed in the containers for four  
202 days (this was the pre-larval phase). Then, adults were returned to the main colony, and the  
203 meat they had prepared was weighed and transferred to new experimental containers. At this  
204 time, containers for ‘parental effects (-)’ treatment were also prepared using the fresh pork  
205 pieces. Freshly hatched first instar larvae were used to establish larval colonies. Larvae were  
206 chosen at random from the egg rearing containers that were established using eggs from the  
207 auxiliary and experimental colonies. Larvae stayed in the containers until majority of them  
208 ceased feeding and started to bury themselves for pupation. At this moment, we counted and  
209 weighed them using laboratory scale (AS 82/220.R2, Radwag, Poland). To ease monitoring of  
210 further development (pupal chamber formation, pupation and eclosion), larvae were  
211 transferred to Petri dishes (9x1.4 cm; 5 larvae/dish), filled with humid soil. Adults were

212 weighed after they became fully coloured (1-2 days after the eclosion) and were preserved in  
213 75% ethanol for further measurements. Experimental containers and Petri dishes were kept in  
214 23 °C in temperature chambers (ST 1/1 BASIC or +, POL EKO, Poland). During the pre-  
215 larval phase we used photoperiod: 12:12 (L:D). Larvae and pupae were kept all time in the  
216 dark. Experimental colonies were monitored daily to supplement water and take thermal  
217 images. We used Testo 885-2 (Testo, Germany) thermal imager on a tripod with 30° x 23°  
218 lens (emissivity: 0.8, reflected temperature: 17°C). Images were taken at room temperature  
219 and humidity, containers were taken out of the chamber for 1-2 m.

220

## 221 **Data preparation and analyses**

222 'Parental effects' and 'larval density' (i.e. the number of larvae per 1 g. of meat) were  
223 independent variables. As dependent variables we used: beetle body mass and mortality at  
224 eclosion, the relative colony mass at eclosion, larval, pupal and total premature development  
225 times and the average thermogenesis in the larval feeding phase.

226 All eclosed adult beetles were weighed and their average per colony body mass [mg]  
227 was used in the analyses. The total beetle mortality at eclosion was defined as the total  
228 number of beetles that died during rearing, expressed in percentages of the initial colony size.  
229 Development times [in days] were defined as follows: larval development, from colony  
230 establishment to pupation; pupal development, from pupation to eclosion and total premature  
231 development, from colony establishment to eclosion.

232 The relative colony mass at eclosion was defined as the total mass of eclosed adult  
233 beetles per colony, expressed in percentages of the maximum possible colony mass. The  
234 maximum possible colony mass was calculated by multiplying the highest average adult  
235 beetle body mass (in experiment 1: 357.9 mg, in experiment 2: 329 mg) by the initial colony  
236 size (assuming 100% survival).

237           The average thermogenesis in the larval feeding phase was calculated by averaging  
238 daily thermogenesis, which was quantified from thermal images of colonies in the larval  
239 feeding phase. Thermal images were analysed using R programming environment. The area  
240 of meat or the area covered by the feeding matrix (final days) were delineated in images.  
241 Then, we averaged temperatures in these areas. The 75% of pixels with the highest heat were  
242 used to avoid lowering thermogenesis by cold spots from soil particles that obscured the true  
243 temperature of the meat or the matrix. As a background temperature we used the average  
244 temperature of meat that was the closest to 23°C (temperature set in the chambers). Usually, it  
245 was the temperature from the first day. To get the daily thermogenesis, we subtracted the  
246 background temperature from the meat/matrix temperature.

247           Statistical analyses were conducted using the R programming language (version 4.3.0)  
248 with the tidyverse (Wickham et al., 2019) and cowplot (Wilke, 2019) libraries. The nonlinear  
249 models were fitted using the gradient descent algorithm (Ruder, 2016). Performance of the  
250 models was evaluated with the root mean square errors, which were estimated using 16-fold  
251 cross-validation. Applying the same 16-fold scheme, we sampled model coefficients to test  
252 their significance with the Welch's t-test. This test was also used to assess significance of the  
253 'larval density' factor and significance of differences between groups determined by the  
254 'parental effects' factor.

255

256 **Results**

257           When larvae were reared without parental effects, the relative colony mass at eclosion  
258 increased with larval density in the low density range (0-0.48 larvae/g) and decreased at high  
259 densities. When parental effects were present, the relationship was similar, however the  
260 colony mass increased only in the very low density range (0-0.07 larvae/g) and it started to  
261 decrease at lower densities as compared to the colonies without parental effects (Fig. 1, Table  
262 1 and I in SOM). Up to about 0.26 density, beetle fitness was better when parental effects  
263 were present, and above 0.26 density these differences reversed (Fig. 1). The decrease of  
264 beetle fitness in the moderate density range resulted mainly from the decrease in beetle body  
265 mass, whereas in the high density range it was mainly affected by the sharp increase in  
266 mortality (Figs 1 and I-II in SOM, Tables I-III in SOM).

267           In both types of colonies, a significant shortening of the total development time was  
268 found with increasing larval density. Beetles reared in colonies with parental effects had  
269 shorter development times over the entire density range compared to the beetles reared  
270 without parental effects (Fig 2; Table 2 and I in SOM). Larval development time revealed  
271 similar pattern, whereas pupal development time linearly decreased with density at a low rate,  
272 with small differences between beetles reared with and without parental effects (Figs III and  
273 IV and Tables I, IV and V in SOM).

274           Average thermogenesis in the larval feeding phase increased logarithmically with  
275 larval density in both types of colonies. Thermogenesis was generally higher in colonies with  
276 parental effects, this difference decreased in the high density range (Fig 3; Table 3).

277

278

## 279 **Discussion**

280           In general, we found a strong negative group-size effect on the colony mass of *N.*  
281 *littoralis* at eclosion, over almost the entire density range in colonies with parental effects, and  
282 for densities larger than 0.5 larva per gram of meat in colonies without parental effects. This  
283 was accompanied by strong positive group-size effects in terms of development time (it  
284 shortened with larval density) and thermogenesis (it increased with larval density). The steady  
285 increase in thermogenesis and shortening of development with larval density indicate that  
286 larvae of *N. littoralis* feed at a higher rate in larger groups, whereas the steady decline in  
287 colony mass indicates that the fitness of the beetles gradually deteriorates as the colonies  
288 became denser. Initially, the decline in beetle fitness resulted from the decrease in their body  
289 mass, while at higher densities it depended mainly on the increase in mortality. These results  
290 support the hypothesis that larvae of *N. littoralis* exploitatively compete over carrion  
291 resources (Matuszewski and Mądra-Bielewicz, 2022). When the density of larvae increases,  
292 they feed faster, but their fitness finally deteriorates, due to the proportionally smaller amount  
293 of carrion resources per larva. Similar decrease of insect fitness with larval density was  
294 reported for juveniles of blow flies [reviewed in (Scanvion et al., 2018)] and burying beetles  
295 (Schrader et al., 2015, Trumbo, 1990a). These similarities suggest that behavior of  
296 necrophagous larvae is generally shaped by exploitation competition.

297           Interestingly, beetle fitness started to decline at lower larval density in colonies with  
298 parental effects as compared to the colonies without such effects. This finding indicates that  
299 there is a conflict between nutrition of adult *N. littoralis* and their offspring. The feeding by  
300 adult beetles directly decreases the pool of meat for the larvae. Moreover, by predigesting  
301 meat adults enhance the feeding rate of the juveniles. This had a clear impact on the fitness of  
302 the larvae in our experiments. Similar negative parental effects were demonstrated in food  
303 restriction experiments with burying beetles (Trumbo and Xhahani, 2015) or earwigs

304 (Meunier and Kölliker, 2012). However, in the current study such effects were already present  
305 at moderate densities, in case of which the amount of meat did not restrict larval growth in  
306 colonies without parents. Therefore, detrimental effects of food sharing by adults and larvae  
307 should be considered important for *N. littoralis* beetles. These effects partially explain the  
308 short residencies of adult *N. littoralis* on carrion in the field conditions, which were frequently  
309 reported in forensic experiments using pig carcasses (Matuszewski et al., 2010, Jarmusz et al.,  
310 2020). Despite the decline in the juvenile fitness, it was still better in the low larval density  
311 range when parental effects were present than when they were absent. Adult beetles probably  
312 facilitate feeding of the larvae by forming the feeding matrix and predigesting the meat  
313 (Matuszewski and Mađra-Bielewicz, 2021). Although such positive effects should occur  
314 whenever adult beetles are present on meat in the pre-larval phase, the noticeable  
315 improvement of juvenile fitness was recorded only in small larval colonies (up to 10 larvae).  
316 These findings indicate that parental effects may be particularly important for the effective  
317 processing of carrion by small aggregations of *N. littoralis* larvae. There are also positive  
318 parental effects, which could not be revealed in this study, i.e. the clearing carrion of blow fly  
319 maggots (Matuszewski and Mađra-Bielewicz, 2022). Additionally, this study did not analyze  
320 some positive effects that have been demonstrated in other carrion beetles and are likely also  
321 relevant for *N. littoralis* beetles, such as facilitating microbial control (Rozen et al., 2008).  
322 Therefore, positive parental effects probably outweigh negative ones under natural conditions.

323         A pronounced positive group-size effect on the juvenile fitness was currently found  
324 only in colonies without parental effects and only in the low larval density range. When we  
325 analyzed fitness factors in isolation, mortality decreased up to a density of about 1 larva per  
326 gram of meat, whereas body mass increased only up to about 0.25 larva per gram of meat.  
327 Positive effects of conspecifics in terms of mortality occur supposedly in larger larval  
328 aggregations of *N. littoralis* than positive effects in terms of the beetle body mass.

329 Interestingly, parental effects cancelled this positive group-size effect. The presence of adult  
330 beetles in the pre-larval phase and the presence of other larvae in the larval feeding phase  
331 apparently bring similar benefits. Most likely, these effects can be reduced mainly to the  
332 facilitation of feeding, by adults towards the larvae and mutually in the case of the larvae.  
333 Parental and larval facilitation of feeding are presumably not additive, and their small amount  
334 is probably sufficient for the larvae to benefit. Positive group-size effect is generally frequent  
335 in societies of juvenile insects (Costa, 2006, Dornhaus et al., 2012). As for carrion insects, it  
336 was recorded in burying beetles under conditions of no or low parental care (Prang et al.,  
337 2022, Schrader et al., 2015) and in some larval blow fly studies (Scanvion et al., 2018). The  
338 effect is usually interpreted as the consequence of cooperation between the larvae. Currently  
339 reported effects are consistent with the cooperation hypothesis but also with the by-product  
340 mutualism hypothesis (Clutton-Brock, 2002). The latter is more plausible. According to this  
341 hypothesis the benefits of larval aggregations are the by-products of the competitive actions of  
342 individual *N. littoralis* larvae to maximize their own fitness. First, competition shapes carrion  
343 insect communities, with the rapid larval feeding and development as its key evolutionary  
344 consequences (Matuszewski and Mądra-Bielewicz, 2022, Hanski, 1987). Second, we are not  
345 aware of any mechanism of larval communication to induce actions of individual larvae for  
346 the benefit of other members of an aggregation. Therefore, in line with the parsimony  
347 principle we interpret current positive group-size effect as the by-product mutualism.

348 Under natural conditions *N. littoralis* colonizes large cadavers and aggregations of its  
349 larvae are usually formed in an advanced state of carrion decomposition (Charabidze et al.,  
350 2016, Gruszka et al., 2020). In the present experiments the beetles were investigated in small  
351 colonies and on small pieces of fresh meat. Although experimental conditions differed in  
352 several respects from the natural carrion environment, we think that results reported in this  
353 study accurately represent natural patterns. Both adult and larval beetles behaved normally in

354 experimental colonies. Under optimal densities, a total mortality at eclosion was about 30%.  
355 Therefore, conditions in the colonies were not detrimental for the beetles. Phenomena  
356 inherent to this study (i.e. larval aggregations, feeding matrix, thermogenesis etc.) occur  
357 naturally, only on a larger scale. In effect, the small size of the food substrate and beetle  
358 colonies resulted in the proportional decrease in the size of some effects reported in this paper  
359 (e.g. thermogenesis), whereas the patterns of differences between the treatments in our  
360 opinion nicely reflect the natural patterns. As for the use of the fresh meat, compared to  
361 natural carrion in an advanced decomposition, this difference may be important and may  
362 reduce external validity of the results. However, in all colonies the fresh meat was used,  
363 therefore this difference had no effect on internal validity of our results and most importantly  
364 it did not influence pattern of differences between the treatments.

365         In conclusion, by manipulating the size of larval aggregations, we found strong  
366 negative group-size effect on the fitness of *N. littoralis* beetles. This finding indicates that  
367 exploitation competition is the key interaction in juvenile societies formed on carrion by these  
368 beetles. Moreover, this negative effect was exacerbated by the presence of adult beetles in the  
369 pre-larval phase that demonstrates detrimental consequences of food sharing between adults  
370 and larvae. We also provide evidence for a positive group-size effect on the beetle fitness, but  
371 only in small larval colonies that were reared without parental effects, which is in line with  
372 the by-product mutualism hypothesis.

373



374 **Acknowledgments**

375 The study was funded by the National Science Centre of Poland (grant no.  
376 2021/41/B/NZ8/00474).

377

378 **Ethics statement**

379 The manuscript describes laboratory experiments using insect species *Nicrodes littoralis*  
380 (Linnaeus, 1758) (Coleoptera: Staphylinidae: Silphinae). The species is not under protection.

381

382 **Data accessibility statement**

383 Datasets supporting this article are provided on a reasonably request by the corresponding  
384 author.

385

386 **Competing interests' statement**

387 We have no competing interests to declare.

388

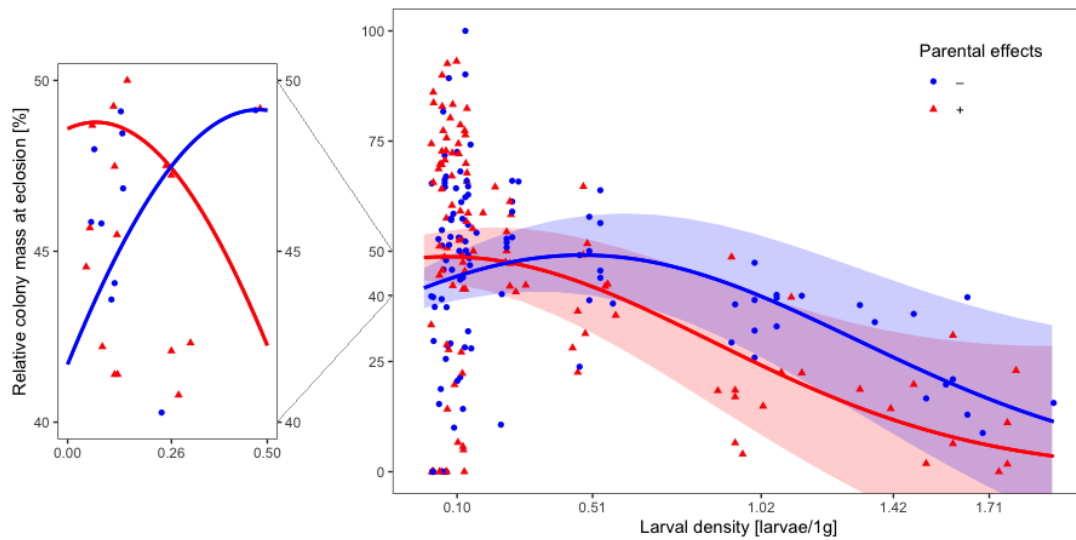
389 **Authors' contributions statement**

390 S.M. developed the concept for the study and the article, performed experiments, prepared  
391 raw data for the analyses, performed analyses, discussed the results, prepared figures and  
392 wrote the manuscript. N.L. and A.M.B. performed experiments, prepared raw data for the  
393 analyses, discussed the results and reviewed the manuscript. J.W. prepared raw data for the  
394 analyses, performed analyses, discussed the results, prepared figures and reviewed the  
395 manuscript.

396

397 **Figures**

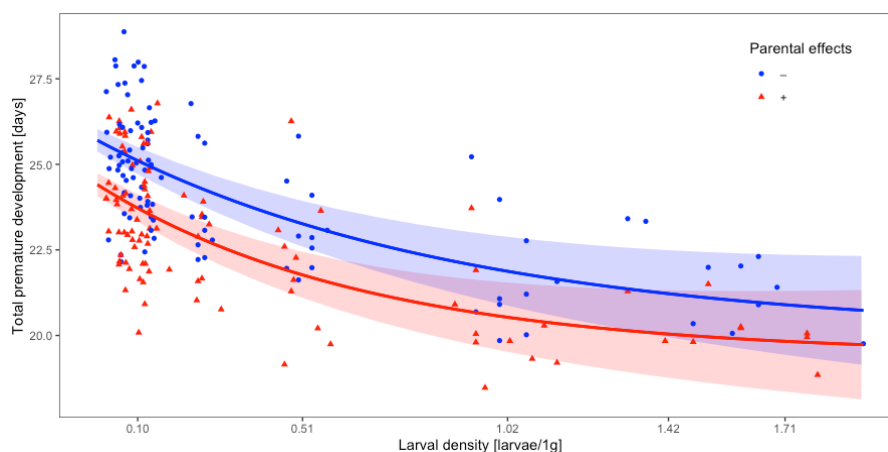
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399

400 Fig. 1. The relationship between larval density (larvae per 1 g of meat) and relative colony mass at eclosion (%)  
401 in the ‘parental effects (+)’ (in red) and ‘parental effects (-)’ (in blue) treatments. The relative colony mass at  
402 eclosion is the total mass of eclosed adult beetles per colony, expressed in percentages of the maximum possible  
403 colony mass. The maximum possible colony mass was calculated by multiplying the maximum average (per  
404 colony) adult beetle mass (it was 357.9 mg in experiment 1 and 329 mg in experiment 2) by the initial colony  
405 size (assuming 100% survival). Lines are exponential regression models, bands represent 95% confidence  
406 intervals.

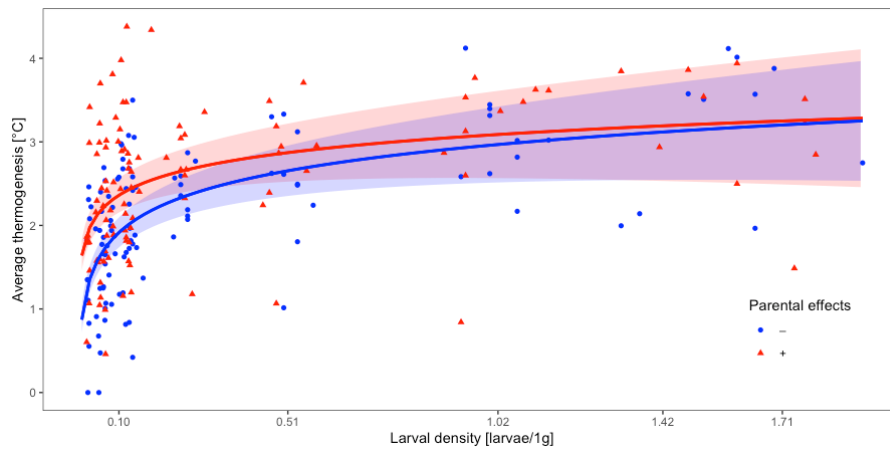
407



408

409 Fig. 2. The relationship between larval density (larvae per 1 g of meat) and the average (per colony) total  
410 premature development (in days, without the egg stage) in the ‘parental effects (+)’ (in red) and ‘parental effects  
411 (-)’ (in blue) treatments. Lines are exponential regression models, bands represent 95% confidence intervals.

412



413

414 Fig. 3. The relationship between larval density (larvae per 1 g of meat) and the average thermogenesis in the  
415 larval feeding phase (°C) in the ‘parental effects (+)’ (in red) and ‘parental effects (-)’ (in blue) treatments. Lines  
416 are logarithmic regression models, bands represent 95% confidence intervals.

417

418 **Tables**

419

420 Table 1. Exponential regression models [ $f(x) = ae^{b(x-c)^2}$ ] of the relationship between larval density and  
 421 relative colony mass at eclosion for the ‘parental effects (+/-)’ treatments (RMSE – root mean square error).

Parental effects	<i>a</i> (scale)	<i>b</i> (steepness)	<i>c</i> (location)	RMSE
+	48.77644897	-0.78056565	0.07081838	13.235
-	49.1426704	-0.7194162	0.4780374	11.307

422

423 Table 2. Exponential regression models [ $f(x) = ae^{b(x-c)} + d$ ] of the relationship between larval density and  
 424 total premature development for the ‘parental effects (+/-)’ treatments (RMSE – root mean square error).

Parental effects	<i>a</i> (scale)	<i>b</i> (steepness)	<i>c</i> (location)	<i>d</i> (limit time)	RMSE
+	4.77747164	-1.48107507	0.02660938	19.43476635	1.488
-	4.9458112	-1.1058782	0.1212268	20.0450982	1.505

425

426 Table 3. Logarithmic regression models [ $f(x) = a \ln(bx) + c$ ] of the relationship between larval density and  
 427 average thermogenesis in the larval feeding phase for the ‘parental effects (+/-)’ treatments (RMSE – root mean  
 428 square error).

Parental effects	<i>a</i>	<i>b</i>	<i>c</i>	RMSE
+	0.313058	1.029880	3.072657	0.7653613
-	0.4532117	0.9843815	2.9662310	0.6804185

429

430

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