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

Natalia Lis[1-3,†], Anna Mądra-Bielewicz[2,3,†], Jędrzej Wydra[2,3], Szymon Matuszewski[2,3,*]

[1]*Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland*

[2]*Laboratory of Criminalistics, Adam Mickiewicz University, Święty Marcin 90, 61-809 Poznań, Poland*

[3]*Wielkopolska Centre for Advanced Technologies, Adam Mickiewicz University, Uniwersytetu Poznańskiego 10, 61-614 Poznań, Poland*

[†] These authors contributed equally to this work.

* Corresponding author: Szymon Matuszewski, szymmat@amu.edu.pl

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

Keywords: Carrion; group-size effect; intraspecific interactions; larval societies

Introduction

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

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

Since carrion is primarily the food source for the larvae, its effective digestion is probably the major ecological cause for larval aggregation. Necrophagous larvae ingest liquefied food and need to preprocess carrion through exodigestion (Rivers *et al*., 2011; Scanvion *et al*., 2018). Collective exodigestion by aggregated larvae of *Lucilia sericata* (Diptera: Calliphoridae) has been demonstrated to shorten their development and reduce mortality (Scanvion *et al*., 2018). Shorter development times and reduced mortality have been also reported for *Necrodes* *littoralis* beetles (Staphylinidae: Silphinae) when they were reared in aggregations compared to those reared individually (Gruszka and Matuszewski, 2021). Similar differences were found in the case of *Thanatophilus sinuatus* beetles, when the development patterns for larvae kept individually (Montoya-Molina *et al*., 2021) were compared with the patterns for aggregated larvae (Lis *et al*., 2023). Although there is no direct evidence to link these benefits with the cooperative exodigestion in carrion beetles, such interpretation is plausible (Gruszka and Matuszewski, 2021; Lis *et al*., 2023). Thermal effects of aggregation are also important for carrion insect larvae, as they may largely accelerate growth. Such effects were demonstrated in carrion blow flies and *Necrodes* beetles (Slone and Gruner, 2007; Charabidze *et al*., 2011; Matuszewski and Mądra-Bielewicz, 2021). Juvenile societies on carrion can also bring protective benefits, i.e. a repulsion of vertebrate scavengers or a dilution effect for insect predators or parasites, although there is no experimental evidence in support (Mooring and Hart, 1992; Burkepile *et al*., 2006; Charabidze *et al*., 2021). Aggregations may benefit the larvae also by facilitating control of microbial communities on carrion (Pöppel *et al*., 2015; Duarte *et al*., 2018; Körner *et al*., 2023).

Due to these demonstrated and potential benefits, larval societies on carrion can be viewed as the cooperative systems (Charabidze *et al*., 2021). On the other hand, competition must play an important role in these systems (Ullyett, 1950; Hanski, 1987). Carrion is a patchy resource with all the consequences (Butterworth *et al*., 2023). Since necrophagous larvae are generally not very mobile, they are usually forced to feed on the carcass, where they began their development. Moreover, because carrion is ephemeral and it frequently depletes when insects are still feeding, larvae are faced with overcrowding and the resulting food shortages. Several experimental studies using larval blow flies demonstrated that under such conditions mortality increases and size of the flies decreases (Ullyett, 1950; Ireland and Turner, 2006; Johnson and Wallman, 2014). Similar density effects were observed in burying beetles, although in this insect group they can be attenuated through infanticide or parental care (Bartlett, 1987; Trumbo, 1990a; Smiseth *et al*., 2007; Schrader *et al*., 2015). Because each larva depletes through feeding the total pool of carrion resources, this is an example of exploitative competition that must have shaped behaviour of the larvae within the juvenile societies on carrion (Ullyett, 1950; Hanski, 1987; Matuszewski and Mądra-Bielewicz, 2022). A very high feeding rate and rapid growth of necrophagous larvae are probably major effects of the competition for food with congeners. Completion of development before the food resources on carrion are exhausted or their quality is reduced is probably the key selection pressure in this context (Hanski, 1987). Intriguingly, the feeding race of the larvae may generate positive by-products for all members of an aggregation. In order to feed at a faster rate, a larva must produce more digestive enzymes, which increases the enzyme pool on carrion and, in turn, facilitates collective exodigestion to the benefit of all larvae in the aggregation. Therefore, the benefits from the juvenile aggregations on carrion can be viewed as the by-products of the actions of individual larvae to maximize their own fitness. This would be an example of the "by-product mutualism" as specified by Clutton-Brock "…in which an individual's behavior maximizes its own immediate fitness and any positive effects on the fitness of other individuals are coincidental and do not contribute to the selection pressures maintaining the behavior" (Clutton-Brock, 2002).

Parental effects are also important in carrion insect societies, particularly in the case of carrion beetle larvae. There are many examples of positive effects. Adult *Ptomascopus* (Staphylinidae: Silphinae) or *Necrodes* beetles secure the resource pool for the larvae by clearing carrion of the maggots (Suzuki and Nagano, 2006; Matuszewski and Mądra-Bielewicz, 2022). Blow flies are similarly eliminated by adult burying beetles (Pukowski, 1933; Scott, 1998). Density of the larvae in the aggregation may be directly optimised by these beetles through infanticide (Bartlett, 1987; Trumbo, 1990b). Furthermore, adult beetles may prevent putrefaction through carrion preservation, which was demonstrated in burying beetles and is plausible in *Necrodes* beetles (Hoback *et al*., 2004; Duarte *et al*., 2018; Shukla *et al*., 2018; Matuszewski and Mądra-Bielewicz, 2021). Adults may also facilitate nutrition of the larvae, directly by food provisioning in burying beetles or indirectly by formation of the feeding matrix in *Necrodes* beetles or the feeding holes in carrion ball in burying beetles (Pukowski, 1933; Smiseth and Moore, 2004; Trumbo, 2017; Vogel *et al*., 2017; Shukla *et al*., 2018; Matuszewski and Mądra-Bielewicz, 2021). There are negative effects, as well. Because adult carrion beetles share the food with their offspring, they may decrease the fitness of the larvae through self-feeding (Trumbo and Xhihani, 2015; Matuszewski and Mądra-Bielewicz, 2021).

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

In the current study, we analysed the interaction between parental and juvenile effects in larval colonies of communally breeding carrion beetles *Necrodes littoralis*. The species, occurring in the Palearctic, colonizes cadavers of large vertebrates (including humans), where it forms massive juvenile aggregations (Charabidze *et al*., 2016; Bajerlein *et al*., 2018; Gruszka *et al*., 2020). In order to secure food resources for their offspring, adults clear carrion of the blow fly larvae by selectively killing maggots, which are before or in peak feeding phase (Matuszewski and Mądra-Bielewicz, 2022). Through spreading over carrion anal and oral exudates, they form feeding matrix, in which the heat is produced and by which adult beetles affect fitness of the larvae (Matuszewski and Mądra-Bielewicz, 2021). These effects may be categorized as simple forms of indirect parental care. Usually, many *Necrodes* females oviposit in the soil nearby the carcass. Larvae aggregate, exodigest carrion tissues and modify the feeding matrix, in which they feed and benefit from thermogenesis (Matuszewski and Mądra-Bielewicz, 2021). As a rule *Necrodes* larvae feed on carrion tissues (Ratcliffe, 1972; Charabidze *et al*., 2016). They colonize cadavers late in decomposition, usually when the fly larvae are no longer present (killed by adult beetles or migrated); therefore, under natural conditions, maggots are not their food source (Matuszewski *et al*., 2011; Matuszewski and Mądra-Bielewicz, 2022). *Necrodes* larvae largely contribute to the formation of the feeding matrix, by increasing surface area of the matrix and modifying its physical characteristics and composition (Matuszewski and Mądra-Bielewicz, 2021). Although there is no published data on densities of *Necrodes* larvae on carrion in the wild, we reanalysed results of our previous experiments on decomposition of pig carcasses and found densities up to about 2.3 larvae per gram of carrion. When the beetles monopolized carrion, densities were usually between 0.6 and 1.6 larvae per gram of carrion (Matuszewski, unpublished data).

Since *Necrodes* larvae frequently face food shortages, we hypothesize that intraspecific exploitative competition shapes their behaviour in aggregations. Competitive effects occur probably under high larval density, whereas under low density cooperative effects may act due to the benefits of collective exodigestion. We also predict that parental effects (i.e. formation of the feeding matrix) modulate these interactions and in effect change the relationship between density and fitness of the larvae. To test these hypotheses, we manipulated larval density and the presence of adult beetles in the pre-larval phase and measured their effects on fitness components of *N. littoralis* larvae.

Materials and methods

Main beetle colony

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

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

Experimental design

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

Experimental beetle colonies and laboratory protocols

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

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

In the 'parental effects (+)' treatment, ten adult beetles (5♀;5♂), chosen at random from the main colony, were used per container. The beetles stayed in the containers for four days (this was the pre-larval phase). Then, adults were returned to the main colony, and the meat with the feeding matrix they formed was weighed and transferred to new experimental containers. At this time, containers for 'parental effects (–)' treatment were also prepared using the fresh pork pieces. Freshly hatched first instar larvae were used to establish larval colonies. Larvae were chosen at random from the egg rearing containers that were established using eggs from the auxiliary and experimental colonies. Larvae stayed in the containers until majority of them ceased feeding and started to bury themselves for pupation. At this moment, we counted and weighed them using laboratory scale (AS 82/220.R2, Radwag, Poland). To ease monitoring of further development (pupal chamber formation, pupation and eclosion), larvae were transferred to Petri dishes (9x1.4 cm; 5 larvae/dish), filled with humid soil. Adults were weighed after they became fully coloured (1-2 days after the eclosion) and were preserved in 75% ethanol for further measurements. Experimental containers and Petri dishes were kept in 23 °C in temperature chambers (ST 1/1 BASIC or +, POL EKO, Poland). During the pre-larval phase we used photoperiod: 12:12 (L:D). Larvae and pupae were kept all time in the dark. Experimental colonies were monitored daily to supplement water and take thermal images. We used Testo 885-2 (Testo, Germany) thermal imager on a tripod with 30° x 23° lens (emissivity: 0.8, reflected temperature: 17°C). Images were taken at room temperature and humidity, containers were taken out of the chamber for 1-2 m.

Data preparation and analyses

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

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

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

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

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

Results

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

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

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

Discussion

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

Interestingly, beetle fitness started to decline at lower larval density in colonies with parental effects as compared to the colonies without such effects. This finding indicates that there is a conflict between nutrition of adult *N. littoralis* and their offspring. The feeding by adult beetles directly decreases the pool of meat for the larvae. This had a clear impact on the fitness of the larvae in our experiments. Similar negative parental effects were demonstrated in food restriction experiments with burying beetles (Trumbo and Xhihani, 2015) or earwigs (Meunier and Kölliker, 2012). However, in the current study such effects were already present at moderate densities, in case of which the amount of meat did not restrict larval growth in colonies without parents. These findings indicate that detrimental effects of food sharing by adults and larvae are important for *N. littoralis* beetles. The avoidance of such negative effects partially explains the short residencies of adult *N. littoralis* on carrion in the field conditions, which were frequently reported in forensic experiments using pig carcasses (Matuszewski *et al*., 2010; Jarmusz *et al*., 2020).

Despite the decline in the juvenile fitness, it was still better in the low larval density range when parental effects were present than when they were absent. Adult beetles probably facilitate feeding of the early instar larvae by forming the feeding matrix and predigesting the meat (Matuszewski and Mądra-Bielewicz, 2021). Although such positive effects should occur whenever adult beetles are present on meat in the pre-larval phase, the noticeable improvement of juvenile fitness was recorded only in small larval colonies (up to 10 larvae). These findings indicate that parental effects may be particularly important for the effective processing of carrion by small aggregations of *N. littoralis* larvae. There are also positive parental effects, which could not be revealed in this study, i.e. the clearing carrion of blow fly maggots (Matuszewski and Mądra-Bielewicz, 2022). Additionally, this study did not analyze some positive effects that have been demonstrated in other carrion beetles and are likely also relevant for *N. littoralis* beetles, such as facilitating microbial control (Rozen *et al*., 2008).

Although negative parental effects predominated in the current experiments, we suspect that under natural conditions positive effects may outweigh negative ones. However, further experiments are necessary to accurately assess the importance of positive and negative parental effects on the fitness of *N. littoralis* larvae in the wild.

A pronounced positive group-size effect on the juvenile fitness was currently found only in colonies without parental effects and only in the low larval density range. When we analyzed fitness factors in isolation, mortality decreased up to a density of about 1 larva per gram of meat, whereas body mass increased only up to about 0.25 larva per gram of meat. Positive effects of conspecifics in terms of mortality occur in larger larval aggregations of *N. littoralis* than positive effects in terms of the beetle body mass. Interestingly, when parental effects were added we could not detect the positive larval group-size effect. The presence of adult beetles in the pre-larval phase and the presence of other larvae in the larval feeding phase apparently bring similar benefits. Most likely, these effects can be attributed mainly to the facilitation of feeding, by adults towards the larvae and mutually in the case of the larvae. A small amount of feeding facilitation is probably sufficient for the larvae to benefit, regardless of whether the adult beetles or other larvae provide these benefits. Positive group-size effect is generally frequent in societies of juvenile insects (Costa, 2006; Dornhaus *et al.*, 2012). As for carrion insects, it was recorded in burying beetles under conditions of no or low parental care (Schrader *et al.*, 2015; Prang *et al.*, 2022) and in some larval blow fly studies (Scanvion *et al.*, 2018). The effect is usually interpreted as the consequence of cooperation between the larvae. Currently reported effects are consistent with the cooperation hypothesis but also with the by-product mutualism hypothesis (Clutton-Brock, 2002). The latter is more plausible. According to this hypothesis the benefits of larval aggregations are the by-products of the competitive actions of individual *N. littoralis* larvae to maximize their own fitness. First, competition shapes carrion insect communities, with the rapid larval feeding and development as its key evolutionary consequences (Hanski, 1987; Matuszewski and Mądra-Bielewicz, 2022). Second, we are not aware of any mechanism of larval communication to induce actions of individual larvae for the benefit of other members of an aggregation. Therefore, in line with the parsimony principle we interpret current positive group-size effect as the by-product mutualism.

Under natural conditions *N. littoralis* colonizes large cadavers and aggregations of its larvae are usually formed in an advanced state of carrion decomposition (Charabidze *et al.*, 2016; Gruszka *et al.*, 2020). In the present experiments the beetles were investigated in small colonies and on small pieces of fresh meat. Although experimental conditions differed in several respects from the natural carrion environment, we think that results reported in this study accurately represent natural patterns. Both adult and larval beetles behaved normally in experimental colonies. Under optimal densities, a total mortality at eclosion was about 30%. Therefore, conditions in the colonies were not detrimental for the beetles. Phenomena inherent to this study (i.e. larval aggregations, feeding matrix, thermogenesis etc.) occur naturally, only on a larger scale. In effect, the small size of the food substrate and beetle colonies resulted in the proportional decrease in the size of some effects reported in this paper (e.g. thermogenesis), whereas the patterns of differences between the treatments in our opinion nicely reflect the natural patterns. As for the use of the fresh meat, compared to natural carrion in an advanced decomposition, this difference may be important and may reduce external validity of the results. However, in all colonies the fresh meat was used, therefore this difference had no effect on internal validity of our results and most importantly it did not influence pattern of differences between the treatments.

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

Acknowledgments

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Ethics statement

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

Data accessibility statement

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

Competing interests' statement

We have no competing interests to declare.

Authors' contributions statement

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

Figures

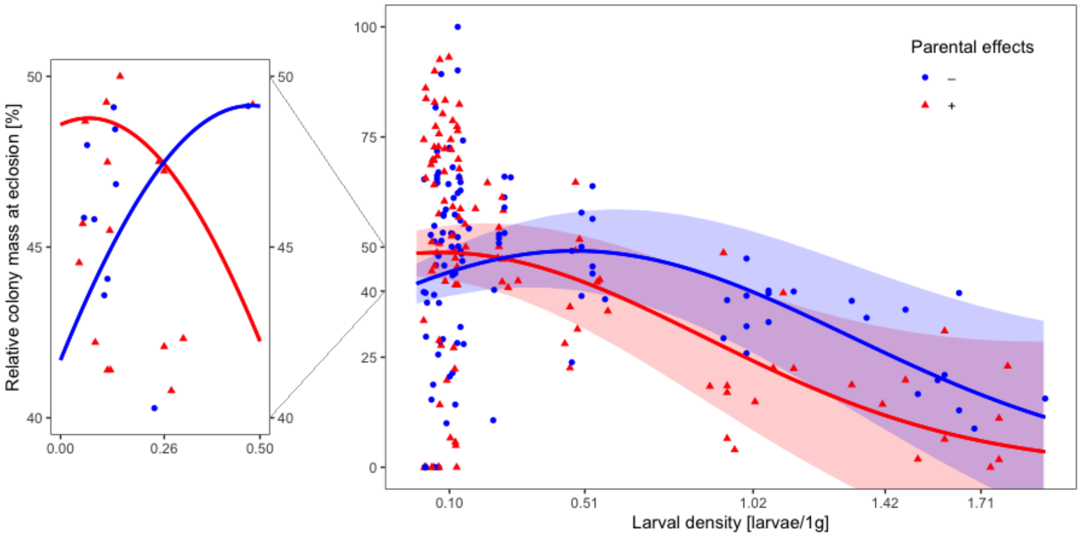

Fig. 1. The relationship between larval density (larvae per 1 g of meat) and relative colony mass at eclosion (%) in the ‘parental effects (+)’ (in red) and ‘parental effects (–)’ (in blue) treatments. The relative colony mass at eclosion is the total mass of eclosed adult beetles per colony, expressed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

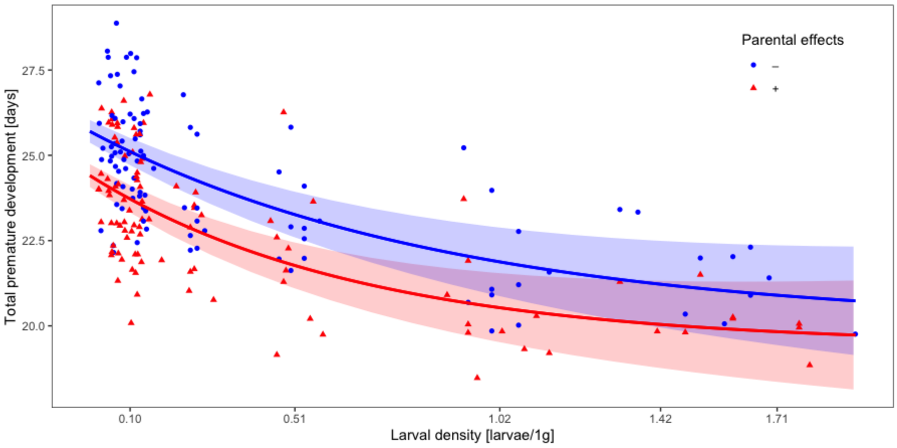

Fig. 2. The relationship between larval density (larvae per 1 g of meat) and the average (per colony) total premature development (in days, without the egg stage) in the ‘parental effects (+)’ (in red) and ‘parental effects (–)’ (in blue) treatments. Lines are exponential regression models, bands represent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

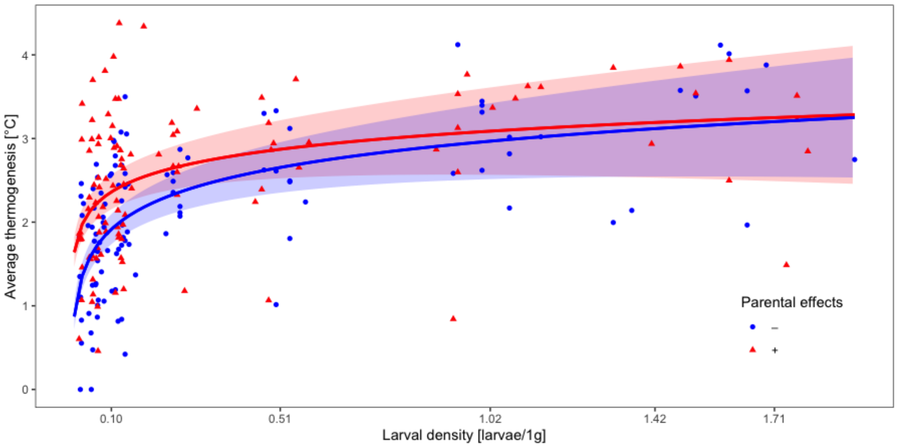

Fig. 3. The relationship between larval density (larvae per 1 g of meat) and the average thermogenesis in the larval feeding phase (°C) in the ‘parental effects (+)’ (in red) and ‘parental effects (–)’ (in blue) treatments. Lines are logarithmic regression models, bands represent 95% confidence intervals.

Tables

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

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

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

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

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

Parental effects	a	b	c	RMSE
+	0.313058	1.029880	3.072657	0.7653613
–	0.4532117	0.9843815	2.9662310	0.6804185

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