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Competition of insect decomposers over large vertebrate carrion: *Necrodes* beetles (Silphidae) vs. blow flies (Calliphoridae)

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Abstract

Large carrion is inhabited by highly variable and interactive communities of insects. Positive interactions in carrion insect communities have been recently the focus in carrion ecology. In contrast, competition between carrion insects is rather undervalued. Here we provide evidence that blow flies (Calliphoridae) and *Necrodes* beetles (Silphidae), dominant decomposers of large carcasses in terrestrial habitats, compete over carrion. By reanalyzing the results from 90 pig carcasses, we demonstrated that the contribution of the flies and the beetles to the decay was negatively related. The greater part of the large carrion pool was monopolized by blow flies, whereas *Necrodes* beetles abundantly colonized carcasses, on which blow flies were less effective as decomposers. In behavioral assays, we found that adult beetles killed 4 times more frequently feeding than postfeeding third instar larvae of the flies, with the large decrease in the killing frequency after the larvae reached the age of early third instar. Therefore, adult *Necrodes* beetles preferentially killed the larvae that were before or in their peak feeding. The study provides evidence that the interaction between blow flies and *Necrodes* beetles is a combination of indirect exploitative effects of the flies and direct interference effects of the beetles (the mixed competition).

Key words: carrion, competition, interspecific interactions.

Carrion, particularly of large vertebrates, is a complex microenvironment, consisting of various resources (e.g., muscles, intestines, skin, cartilage, hairs, or feathers), that differ in quality and dynamics of decomposition (Carter et al. 2007; Barton et al. 2013; Benbow et al. 2019). It has been suggested that interspecific competition between decomposers "appears minimal upon initial examination as most species utilise discrete non-overlapping resources and have different strategies of carcass utilisation" (Braack 1987). In effect, positive interactions were mainly the focus in carrion ecology recently. The facilitation has been suggested as a mechanism of succession on carrion (Connell and Slatyer 1977). The aggregation behavior of blow fly larvae was demonstrated to facilitate the assimilation of carrion resources and to optimize the development of larvae, and thus have positive intraspecific and interspecific effects (Rivers et al. 2011; Scanvion et al. 2018; Komo et al. 2021). A review of social

strategies in carrion insects indicated that positive interactions between (mostly conspecific) decomposers (e.g., parental care of burying beetles or larval aggregation of blow flies) are prevalent within the necrobiome (Charabidze et al. 2021).

Negative interactions between decomposers of large carrion were not the focus of many studies. The competition was examined mostly in relation to small carrion. Some traits of burying beetles were linked with competition over small carrion mostly with congeners and blow flies, for instance, the burial of cadavers or symbiosis with phoretic mites *Poecilochirus* (Mesostigmata: Parasitidea) that reduce or eliminate pressure from competitors (Pukowski 1933; Wilson and Knollenberg 1987; Trumbo 1990; Scott 1998; Sun and Kilner 2020; Trumbo and Sikes 2021). Interspecific competition of necrophagous flies was also investigated on small carrion or small pieces of meat, demonstrating the prevalence of exploitative competition and

examples of interference competition (Ullyett 1950; Denno and Cothran 1976; Kneidel 1984; Hanski 1987a; Goodbrod and Goff 1990; Wells and Greenberg 1994; Prinkkila and Hanski 1995; MacInnis and Higley 2020). On the other hand, large cadavers (pigs, 35–70 kg) were found to host more diverse, abundant, and long-lasting insect communities, with the representation from all important groups, whereas on small carcasses (5–30 kg) late-colonizing insects were highly underrepresented (Matuszewski et al. 2016). In addition, active decay had lower rate and advanced decay started later on large cadavers as compared to small cadavers and this effect was interpreted as a consequence of competition between different groups of insects over large carrion (Matuszewski et al. 2014). Therefore, competition may be more frequent on large cadavers and it might exert a stronger influence on its necrobiome, shaping its structure and changes during decomposition.

Dominant insect decomposers of large cadavers in temperate and terrestrial habitats are blow flies (Calliphoridae) and carrion beetles (Silphidae) (Matuszewski et al. 2016; Anderson et al. 2019). In Central European nonurban habitats *Lucilia caesar*, *L. sericata*, *Calliphora vicina*, and *Calliphora vomitoria* among calliphorid flies and *Necrodes littoralis* among silphid beetles were found to dominate active decay on large carcasses (Matuszewski et al. 2010, 2014). Blow flies usually colonize carrion as first insects, shortly after death, whereas *Necrodes* beetles arrive later, most frequently around the peak of carrion bloating; larvae from these groups feed on a carcass at different times in the succession (Ratcliffe 1972; Matuszewski et al. 2011; Charabidze et al. 2016). However, both groups utilize carrion similarly and are dependent on the same carrion resources. Their larvae feed on carrion soft tissues, on large cadavers they may form massive aggregations that are highly efficient in carrion decay and that may elevate temperature in their feeding environment (Slone and Gruner 2007; Rivers et al. 2011; Gruszka et al. 2020; Charabidze et al. 2021; Matuszewski and Mądra-Bielewicz 2021). Other striking similarities comprise communal breeding, with many conspecifics ovipositing on the same carrion and *r*-selected traits that characterize life histories in both groups (Charabidze et al. 2021).

Similarities in carrion utilization prompted us to hypothesize that blow flies and *Necrodes* beetles compete over large carrion. The interaction is probably asymmetric and associated with the sequence, in which they colonize carrion. Because blow fly larvae start to feed much earlier than *Necrodes* larvae, we predicted that they have indirect effects on the beetles in terms of the exploitation of carrion. In effect, we assumed that blow flies monopolize the greater part of the resource pool, whereas the colonization by *Necrodes* beetles depends on the effectiveness of carrion decay by the flies. When the fly decay is reduced, the colonization by *Necrodes* beetles increases and we predicted that seasonal, environmental, or resource-based gradients are important in this respect. These predictions were tested by reanalyzing the results of our earlier experiments with pig carcasses. Second, because *Necrodes* beetles colonize carrion later than blow flies, we assumed that they have direct effects on the flies in terms of the interfering behaviors that reduce consumption of carrion by larvae of flies. Adult *Necrodes* beetles are well-known for their voracious feeding on blow fly larvae (Ratcliffe 1996; Charabidze et al. 2016). Although a conventional view treats this behavior as the acquisition of food, observations of *Necrodes surinamensis* made by Ratcliffe revealed that adult beetles were selective while feeding on blow fly larvae, they frequently picked and dropped several larvae before killing one of them (Ratcliffe 1972). Therefore, we predicted that the beetles compete with blow flies by killing the larvae that are prior or in their peak feeding phase (i.e. late second instar larvae, hereafter L2 and early third instar larvae, hereafter L3). These predictions were tested in behavioral laboratory assays.

Materials and Methods

Field data

The dataset

To expose interactions and partitioning of the large carrion pool between blow flies and *Necrodes* beetles, we reanalyzed the results of our earlier experiments on the decomposition of pig carcasses (Table 1). The analyses were made using a dataset that comprised 90 pig carcasses. The experiments were performed at the Biedrusko military range (Western Poland, 52°31′N, 16°54′/55′E). Carcasses were inspected with similar frequency (once a day until the end of active decay, then less frequently). Pictures were taken and written on-site reports were made to document decomposition and insect fauna. Insects were sampled using pitfall traps and manually. The dataset (provided as Supplementary Material for this article) comprised the results of 3 separate research projects, so there were some differences in the methods used in terms of carcass mass, seasons, habitats, and years of carcass exposure (Table 1). Since we investigated interspecific interactions, these differences did not invalidate our main analyses, but resulted in a larger variation of the dataset and, therefore, its higher external validity. Only habitat and seasonal patterns in Necrodes Colonization Score (NCS) might have been affected by these differences. However, we did not identify any systematic difference between the projects that could weaken our findings.

Variables

Cadaver mass decayed by insect larvae. The variable was defined as the percentage of initial carcass mass (ICM) that was decayed by the larvae (separately *Necrodes* beetles and blow flies). In "2011" and "2012", experiments carcasses were regularly weighed in the field [a weighing protocol was described by Matuszewski et al. (2014)]. Using these measurements, we determined carcass mass when the first larval aggregation of a taxon was recorded and then the mass that was left after larvae of this taxon ceased feeding. The relevant days were identified based on pictures of carcasses, on-site reports, and insect occurrence matrixes. After transforming weights into percentages of initial mass, we calculated the difference to obtain the cadaver mass that was decayed by the taxon. In a "2006/2007" experiment, we had to estimate this variable, since only data on ICM were available. Relevant days were identified as above. The corresponding masses were assumed based on the average seasonal values for "2011" and "2012" experiments. When the first larval aggregation was recorded, the average carcass mass was about 95% of the initial mass in spring and about 99% in summer or autumn. Carcass mass that was left after larvae ceased feeding was on average about 20% in spring or autumn and 15% in summer. If larger amounts of carcass tissues were recorded, higher percentages were assumed. There were also a few cases when larvae of both groups participated in active decay. To obtain cadaver mass that was decayed by each group, we assessed (based on the pictures and on-site reports) the mass that was left after larvae of blow flies ceased feeding.

NCS. To quantify carrion colonization by an insect species, we created a Colonization Score. This measure combines completeness with the abundance of colonization. Colonization of carrion by insect decomposers involves oviposition (or larviposition) and

Table 1. Differences in experimental design between studies that were used to analyze interactions between blow flies and *Necrodes* beetles

Study	N	Years	Seasons	Habitats	Carcass mass (kg)		References with detailed methods
					Mean	Range	
I	36	2006 2007	Spring Summer Autumn	Alder forest Hornbeam-oak forest Pine-oak forest	26.2	14–43	Matuszewski et al. (2010)
II	30	2011	Spring Summer	Grassland Grassland at the edge of a forest Birches in grassland Alder forest Hornbeam-oak forest Birch forest	23	6–57	Matuszewski and Szafałowicz (2013)
III	24	2012	Spring Summer	Grassland	31.8	7–64	Matuszewski et al. (2014)

subsequent transition through several larval instars on carrion. Nevertheless, sometimes colonization stops at a certain life stage, for instance, when adult insects visit carrion but do not lay eggs or when first instar larvae leave carrion due to food depletion. To capture this aspect of colonization by *Necrodes* beetles, we used the colonization completeness scale, as follows: 1—adult beetles; 3—first instar larvae; 5—second instar larvae; and 7—third instar larvae. A double-step scale was used to highlight the differences between particular stages of colonization. The abundance of colonization was a total number of specimens of a given life stage that were trapped at the carcass. There were 2 traps of the same kind buried next to each of the carcasses. By multiplying completeness and abundance, we got NCS. For instance, if 50 adult beetles were trapped and no larvae were recorded, the Score would be 50 (1 × 50), and if 500 L3 were trapped, the Score would be 3,500 (7 × 500).

Combined Colonization Score (CCS). To investigate the combined effect of colonization by *Necrodes* beetles and blow flies on the rate of decomposition, we used CCS. Since we had no detailed data on the abundance of blow fly larvae (we had to assess the abundance based on pictures of carcasses and written on-site reports), a simplified scale for the abundance of colonization was used (1, 1–10; 2, 11–100; 3, 101–500; 4, 501–1000; 5, 1,001–5,000; 6, 5,001–10,000; 7, above 10,000 specimens trapped at a carcass, a one-step scale was used to ensure that the effect of abundance on the score is balanced between blow flies and *Necrodes* beetles). Completeness of colonization was captured using the scale described in the previous paragraph. Colonization Scores for *Necrodes* beetles and blow flies were calculated by multiplying completeness and abundance, and then they were added to obtain the CCS.

Duration and average rate of active decay by blow fly larvae. Active decay is the removal of carrion by insect larvae feeding in aggregation (Matuszewski et al. 2010). Days with aggregations of blow fly larvae were counted and used as the duration of active decay by blow flies. Average rate of active decay was defined as the percentage of ICM removed per day by the larvae. It was calculated by dividing the cadaver mass decayed by blow flies (see above) by the duration of active decay driven by the flies.

Average rate of combined active decay. It is the percentage of ICM removed per day by blow fly and *Necrodes* larvae. First, we added cadaver mass decayed by blow flies and *Necrodes* beetles. Then, it was divided by the total duration of active decay by both insect groups.

Duration of carcass bloating. Bloating is a by-product of putrefaction that manifests itself in the distension of carrion. We counted days with bloating using pictures and written reports.

Residency of insects. This is the number of days, in which a given life stage of a species was recorded on a carcass. We used residencies of adult *Necrodes* beetles and third instar larvae of *L. caesar*, a dominant blow fly in the studies.

Data analyses

Most of the analyses were descriptive and exploratory, thus results are presented using scatterplots and boxplots. To investigate relations between NCS and several quantitative and qualitative predictors, we used a MARSplines analysis. ICM, duration of bloating (DB), average rate and duration of active decay by blow flies, day of carcass exposure (DE), and average temperatures preceding colonization by *Necrodes* beetles were quantitative predictors, season, and habitat of decomposition were qualitative predictors. The analyses were made using Statistica version 13.3 (1984–2017 TIBCO Software Inc.).

Behavioral assays
Insect colonies

Postfeeding larvae of *C. vomitoria* were bought in a fishing shop prior to the experiments and reared until emergence. Adult flies were reared in 30 × 20 × 20 cm plastic containers, on a 2-cm layer of soil, with glucose solution and a water-soaked sponge. Flies were provided with raw chicken liver.

The beetles came from our colony (established in 2017 and supplemented in 2018 using adult beetles sampled in the Biedrusko military range; 52°31'N, 16°54'E, Western Poland). They were reared in 30 × 20 × 20 cm plastic containers, filled to 1/3 with soil, with water-soaked cotton wool, pork meat *ad libitum* and aluminium foil used as a cover.

Rearing boxes were kept under a fume hood at room temperature (20–23°C) and humidity (50–60%).

Feeding and postfeeding third instar blow flies (assays 1 and 2)

To test the preference of the beetles toward feeding and postfeeding L3 of blow flies, we performed 2 separate behavioral assays. In "assay 1" feeding and postfeeding L3 were supplied separately to the beetles, in "assay 2", they were supplied together to the beetles.

In "assay 1", the type of blow fly larvae, sex of adult beetles, and date of the trial were predictor variables, percentage of larvae killed
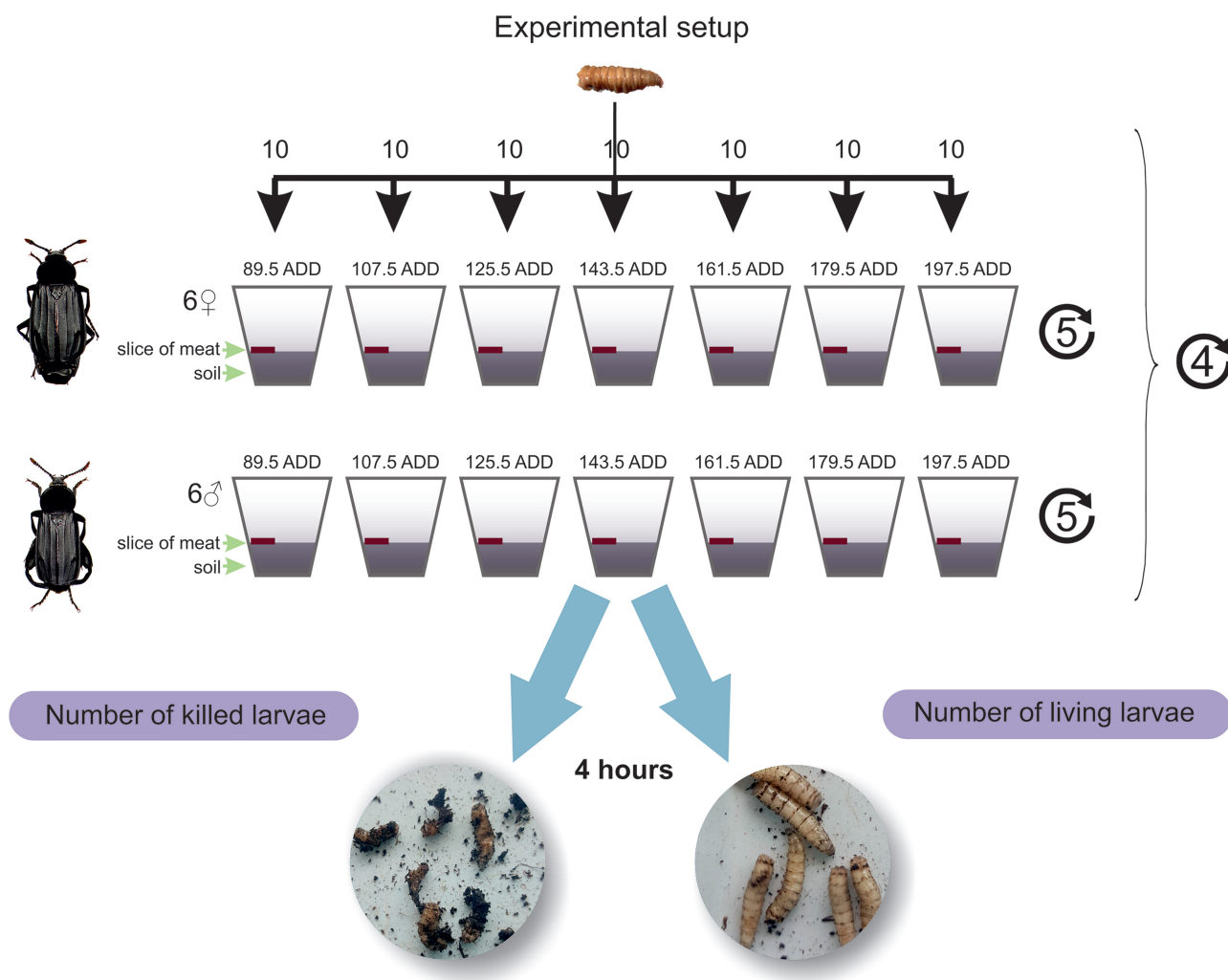
Experimental setup

Figure 1. Experimental design to study a killing frequency of blow fly larvae in various ages and sizes by *Necrodes* beetles.

was a response variable. The type of blow fly larvae was considered on 2 levels: feeding L3 and postfeeding L3 (larvae that ceased feeding and entered into wandering phase). Sex of the beetles was considered on 3 levels: males only (6), females only (6), and mixed (3 males and 3 females). The experiment was replicated 6 times, in 3 trials that were separated in time (2 replicates per trial).

In "assay 2", the type of blow fly larvae (feeding and postfeeding L3) and sex of adult beetles (males only, females only, and mixed) were predictor variables, percentage of larvae killed was a response variable. This experiment was replicated 10 times.

Blow fly larvae were reared in 2 colonies to have feeding and postfeeding L3 available at the same time. There was about 3 days difference in age between feeding and postfeeding L3 that were used in the trials, i.e. 54 accumulated degree-days (ADD) above 0°C. About 100 mg of eggs was sampled and transferred to fresh chicken liver (~15 g). Larvae were reared in 500 mL plastic containers with a perforated lid, on a thin layer of soil. Colonies were kept in a temperature chamber (ST 1/1 +, POL EKO, Poland) at 18°C and photoperiod 12:12 (L/D). They were inspected each day, to ease ventilation and replenish food (20 g of minced pork meat, added daily starting from the second day).

Adult beetles were taken out of the main colony 24 h before experiments and were transferred to separate containers for males

and females. Beetles were kept under the same conditions as in the main colony but without food.

Experiments were conducted in plastic, transparent, 500 mL containers filled to 1/3 with soil and closed with a perforated lid. A thin slice of pork meat (2 × 3 cm, 2 mm thick) was put on the soil surface and 10 feeding or postfeeding L3 of *C. vomitoria* were transferred on meat (assay 1). In "assay 2", both feeding and postfeeding L3 were put on meat (20 larvae per container). To ease counting of the larvae in "assay 2", we marked postfeeding L3 with a black permanent oil marker (a line usually across dorsal parts of abdominal segments). The marking had no discernible effect on the killing behavior of the beetles, since similar numbers of postfeeding larvae were killed by the beetles in "assay 1" (without marking) and "assay 2" (with marking). Then, beetles were added. Containers were kept in dark at room temperature (~21°C) and humidity (50–60%) for 4 h. Afterwards, beetles were returned to the main colony and living or killed blow fly larvae were counted.

Blow fly larvae of various ages and sizes (assay 3)

Age of blow fly larvae, sex of adult beetles, and date of the trial were predictor variables, percentage of larvae killed was a response variable. Larval age was considered on 7 levels: 89.5, 107.5, 125.5, 143.5, 161.5, 179.5, and 197.5 ADD. ADD were added starting
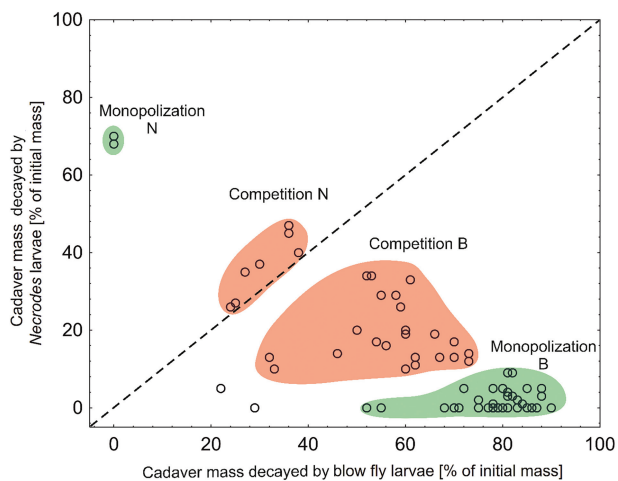
Figure 2. The partitioning of large cadaver pool between blow flies and *Necrodes* beetles. Green areas include cadavers monopolized by 1 group (at least 50% of cadaver mass decayed by the group, with <10% contribution of the other group). Red areas include cadavers with substantial contribution to the decay by both groups (at least 10% of cadaver mass decayed by each group). Monopolization B/N—cadavers monopolized by blow flies or *Necrodes* beetles, Competition B/N—cadavers with larger contribution of blow flies or *Necrodes* beetles.

from the oviposition time (the midpoint between the current and previous inspection of the main colony, colonies were inspected every 24 h). Sex of the beetles was considered on 2 levels: 6 males and 6 females. The experiment was replicated 20 times, in 4 trials that were separated in time (5 replicates per trial; Figure 1).

To get blow fly larvae of various ages (from late L2 until late L3), we reared them in 7 colonies. For this purpose eggs were sampled at daily intervals, resulting in larvae of gradually decreasing age. Eggs left in the main colony were removed, to ensure new oviposition for the next sampling point. Larvae were reared under the same conditions as in assays 1 and 2. Experiments were performed following the protocol of assay 1. Containers with young larvae (89.5 and 107.5 ADD) were left for 2 days after termination of a trial to ensure that all living larvae were counted. In addition, 10 randomly chosen larvae per age were killed (immersion in hot water, preservation in 75% ethanol) to measure their length.

Data analyses
The results of "assay 1" were analyzed using a 3-way analysis of variance (ANOVA) with the type of blow fly larvae, sex of adult beetles, and date of the trial as predictor variables. The results of "assay 2"' were analyzed using a repeated measures ANOVA with the type of blow fly larvae as a repeated measure variable and sex of adult beetles as a predictor variable. To analyze the results of "assay 3", we performed a 3-way ANOVA with the age of blow fly larvae, sex of the beetles, and date of the trial as predictor variables. The percentage of larvae killed was used as a response variable in all analyses. The relationship between average length of the blow fly larvae and the percentage of larvae killed was analyzed using a nonlinear regression with the polynomial model $(y = b_0 + b_1x + b_2x^2)$ and Levenberg–Marquardt estimation of parameters. Calculations were made using Statistica version 13.3 (1984–2017 TIBCO Software Inc.). Datasets used in the analyses are provided as Supplementary Material.

Results

Partitioning of large carrion pool between *Necrodes* beetles and blow flies: field observations
To reveal patterns of large cadaver utilization by blow flies and *Necrodes* beetles, we reanalyzed the results of decomposition studies of 90 pig carcasses (various mass, seasons, and habitats). By quantifying carcass mass decayed by larvae of blow flies and larvae of *Necrodes* beetles, we found that majority of carcasses were monopolized by blow flies: 56 cases (62%) compared to 2 cases with the monopolization by *Necrodes* beetles (Figure 2). The contribution of *Necrodes* beetles to the decay was larger compared to that of the flies in 9 cases only.

To identify factors that promote colonization of carrion by *Necrodes* beetles, we first plotted NCS (the combination of completeness and abundance of colonization, see Materials and Methods section) against several predictor variables. Large colonization by *Necrodes* beetles occurred only when carrion reduction by blow flies was absent or minimal (Figure 3A) or when it lasted from 10 to 20 days (Figure 3B). High values of NCS were recorded on larger carcasses only, starting from 15 kg (Figure 3C). NCS increased with an increase in the duration of carcass bloating (Figure 3D). Only spring and summer carcasses were colonized by *Necrodes* beetles, with the largest colonization scores in the early spring (Figure 4A,B). Carcasses exposed in grasslands, in the clumps of birches in grasslands, at the edge of a forest and in alder forest had higher NCS than carcasses in the pine-oak, hornbeam-oak, and birch forests (Figure 4C). In order to investigate these relationships more deeply, we performed MARSplines analysis. Initial carcass mass (ICM), duration of bloating (DB), average rate and duration of active decay by blow flies (ARADB and DADB), day of carcass exposure (DE), and average temperatures preceding colonization by *Necrodes* (T) were used as quantitative predictors, season and habitat of carcass decomposition as qualitative predictors. The model comprised ARADB, DADB, DE, and season. ARADB and DADB were used twice, DE and season once, the other predictors were not used (MARSplines, $r^2 = 0.807$; residual standard deviation = 3,150.7). These results demonstrate that predictors related to the effectiveness of carrion decay by blow flies and seasonality explained most of the variation in the NCS.

By combining simplified colonization scores for *Necrodes* beetles and blow flies (CCS) and plotting it against the average rate of combined active decay, we found that carcasses colonized by both groups (high CCS) decayed at a slower rate, whereas carcasses monopolized by 1 group decayed faster (Figure 5).

Elimination of blow fly larvae by *Necrodes* beetles: field observations and laboratory behavioral assays
To identify pressures of *Necrodes* beetles on communities of blow fly larvae, we first plotted abundance and residency of adult *Necrodes* beetles from our previous decomposition studies against selected measures of carrion colonization by blow fly larvae. This exploration revealed that the rate of active decay by blow flies decreased with an increase in the number of adult *Necrodes* beetles trapped on the carcasses (Figure 6A) and that residency of the beetles was positively correlated with the residency of L3 of *L. caesar*, a dominant blow fly in our studies (Figure 6B). There was also a positive correlation between the number of adult *Necrodes* beetles trapped on the carcasses and the mass of carrion that was still present when blow flies ceased feeding (Figure 6C). These findings suggested that adult *Necrodes* beetles kill competing blow fly larvae to
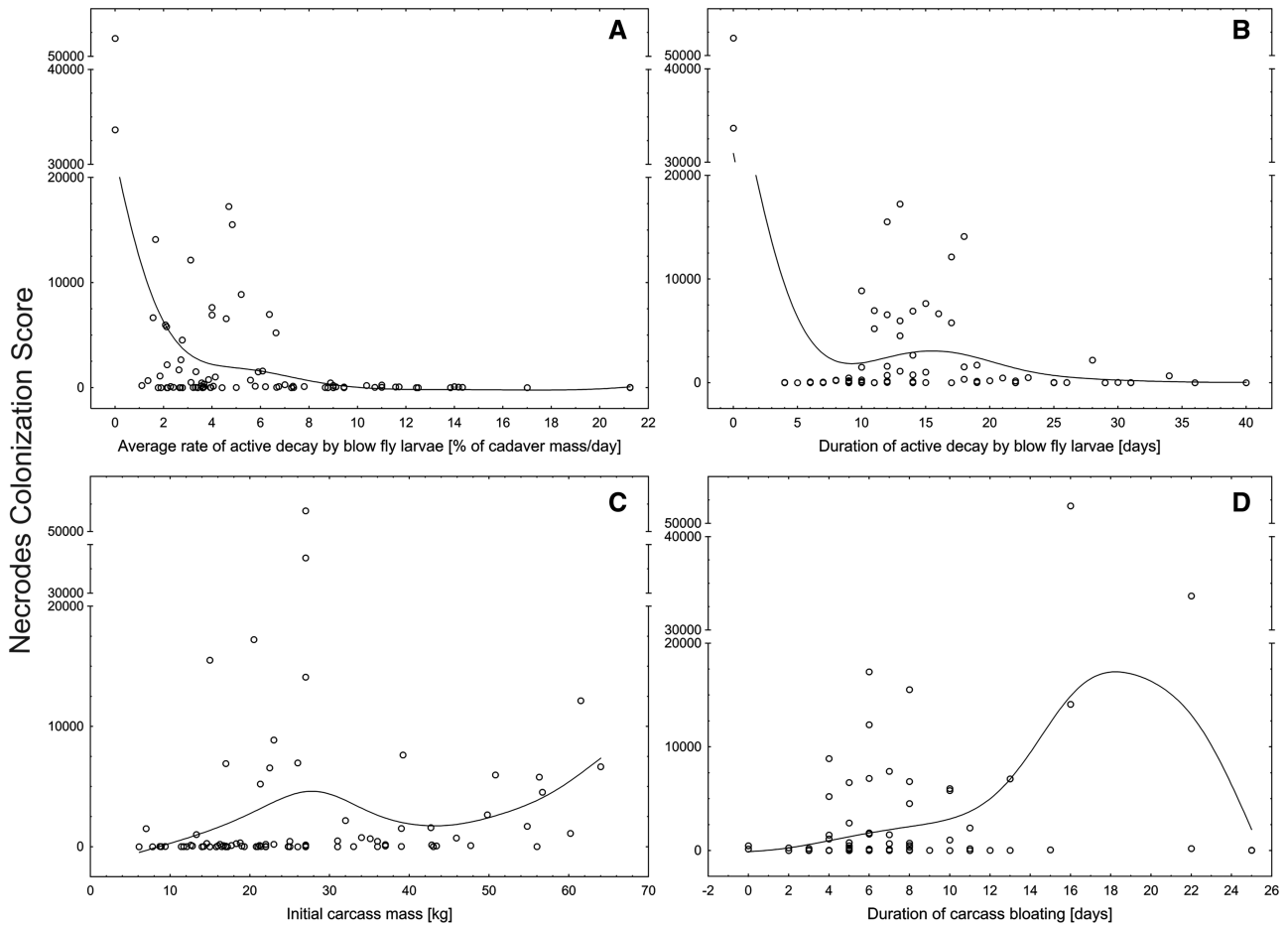
Figure 3. Colonization of pig carcasses by *Necrodes* beetles plotted against the average rate (A) and duration (B) of active decay by blow fly larvae, the initial carcass mass (C) and the duration of bloating (D). Colonization by *Necrodes* beetles was quantified using NCS (see Materials and Methods section). The smoothed lines were fitted with the distance-weighted least-squares smoothing procedure.

reduce their population on carrion. Therefore, we performed laboratory behavioral assays to explore this behavior of the beetles.

First, to identify larval stages of blow flies that are preferentially killed by adult *Necrodes* beetles, we made 2 tests with feeding and postfeeding L3 of *C. vomitoria* that were separately or together supplied to the beetles (males only, females only, and mixed). Both assays revealed a clear preference of the beetles toward the feeding L3 (separate *Calliphora* supply: 3-way ANOVA, $F = 97.0$, $P < 0.001$; combined *Calliphora* supply: repeated measures ANOVA, $F = 76.5$, $P < 0.001$; Figure 7A,B). Sex of the beetles was less important in this respect (separate *Calliphora* supply: $F = 3.7$, $P = 0.045$; combined *Calliphora* supply: $F = 2.4$, $P = 0.11$; Figure 7A,B). Then, we tested whether killing the larvae depends on their age and size. By providing the beetles with larvae of different ages (and sizes), we revealed a highly significant effect of larval age on the number of larvae killed by the beetles (3-way ANOVA, $F = 234.8$, $P < 0.001$), with a steep decrease in this number for larvae older than about 90 ADD (Figure 8A). Sex of the beetles had no significant effect ($F = 2.6$, $P = 0.1$; Figure 8A). By analyzing relationship between the number of killed larvae and their size, we found that larvae < 16 mm were regularly killed, whereas the killing frequency decreased for larvae larger than 16 mm ($y = -35.997 + 23.441{*}x + (-1.052){*}x^2$, $P < 0.001$, $r^2 = 0.78$; Figure 8B).

Discussion

Our study provides evidence that the interaction between blow flies and *Necrodes* beetles on carrion is clearly competitive. The flies indirectly affect *Necrodes* beetles by reducing the size of carrion pool that is available for the beetles, whereas the beetles directly eliminate the fly larvae.

Current analyses revealed 3 patterns of large carrion utilization by insect decomposers in Central European nonurban habitats. Most frequently, cadavers were monopolized by blow fly larvae. Carcasses with substantial contribution of both guilds were less frequent, and very rarely *Necrodes* larvae monopolized carrion resources. Blow flies are usually first colonizers and their larvae form aggregations early in the succession, whereas *Necrodes* beetles are typical late-colonizers, with aggregations of their larvae formed late in the succession (Ratcliffe 1972; Hanski 1987b; Matuszewski and Szafałowicz 2013; Anderson et al. 2019). Accordingly, there is a clear separation between these groups along the succession and a resultant partitioning of the resource pool. Blow flies have access to the entire pool of fresh, large carrion, whereas *Necrodes* beetles may utilize a small fraction of the pool, i.e. cadavers with long post-mortem interval (PMI) that were not completely decayed by the flies. Since there are much more intact cadavers with short PMI than with
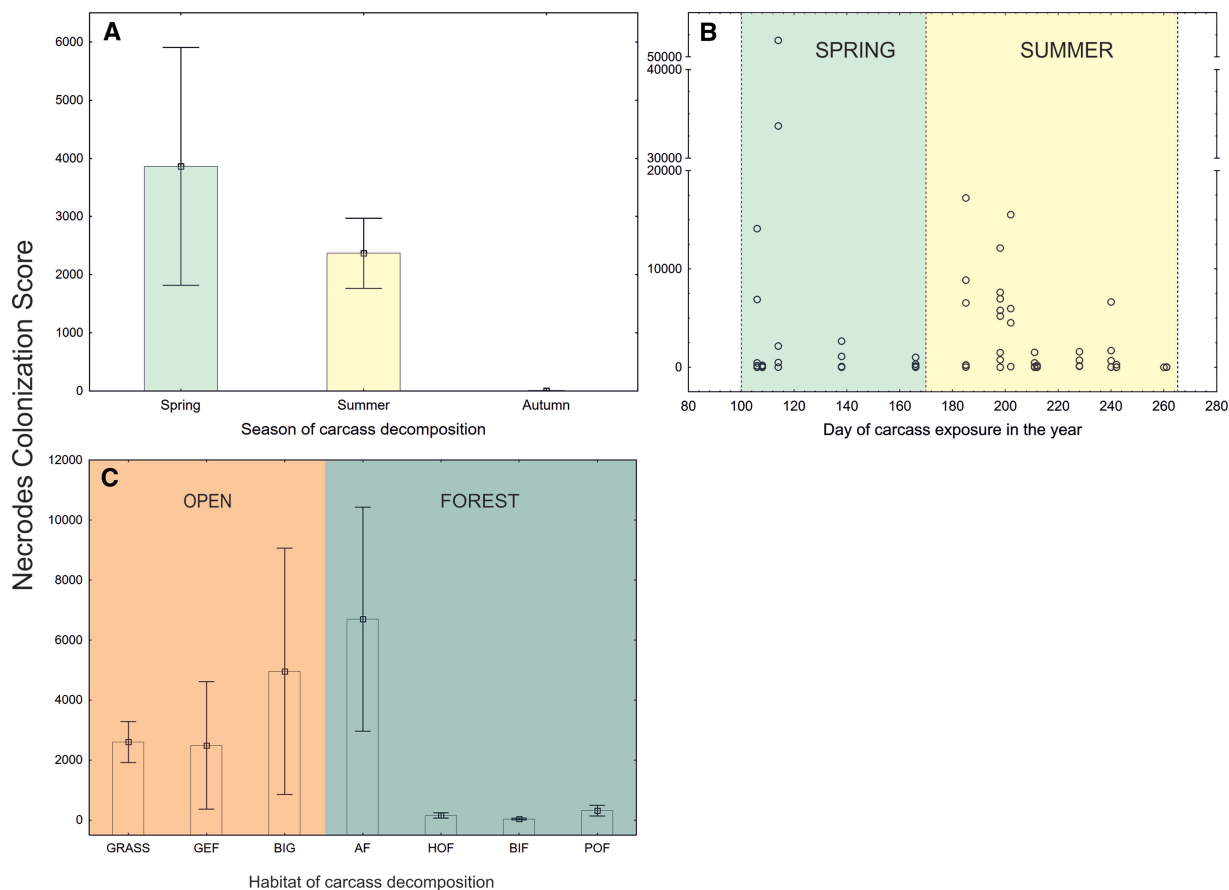
Figure 4. Colonization of pig carcasses by *Necrodes* beetles in different seasons (A) and habitats (C) and on carcasses exposed in different days in the year (B). GRASS: grasslands; GEF: grasslands at the edge of a forest; BIG: clumps of birches in grasslands; AF: alder forest; HOF: hornbeam-oak forest; BIF: birch forest; POF: pine-oak forest. In A and C bars represent means and whiskers are standard errors of the mean. Days of carcass exposure in B are days in which particular carcasses were exposed in the field, numbers on the *x* axis denote the consecutive days during the year.
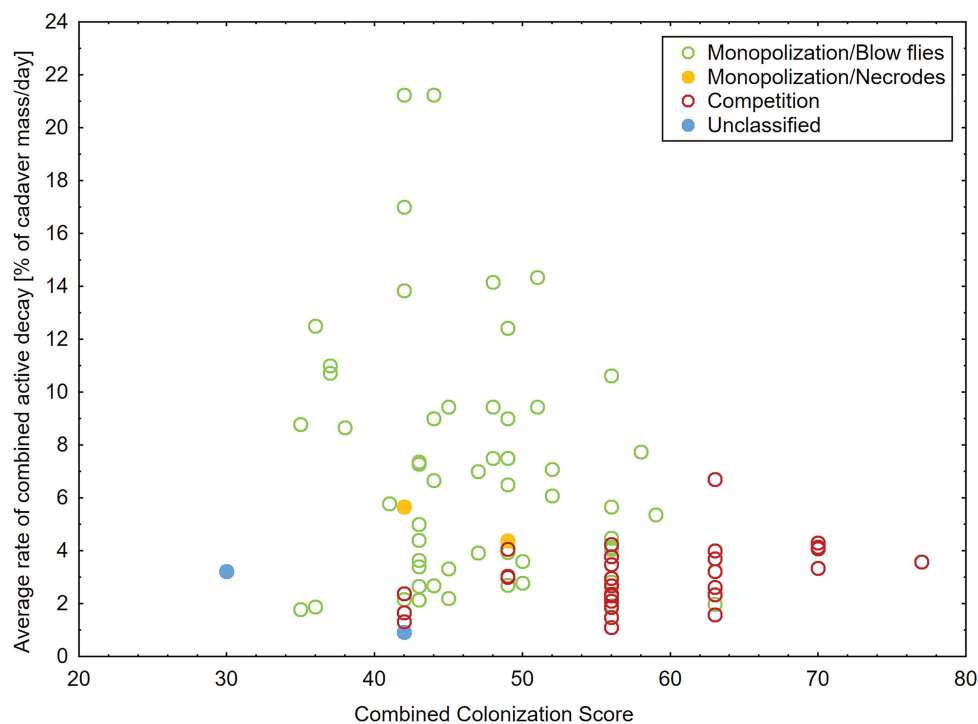

Figure 5. Combined colonization of pig carcasses by blow flies and *Necrodes* beetles plotted against the average rate of combined active decay. CCS represents completeness and abundance of colonization by both groups (see Materials and Methods section).
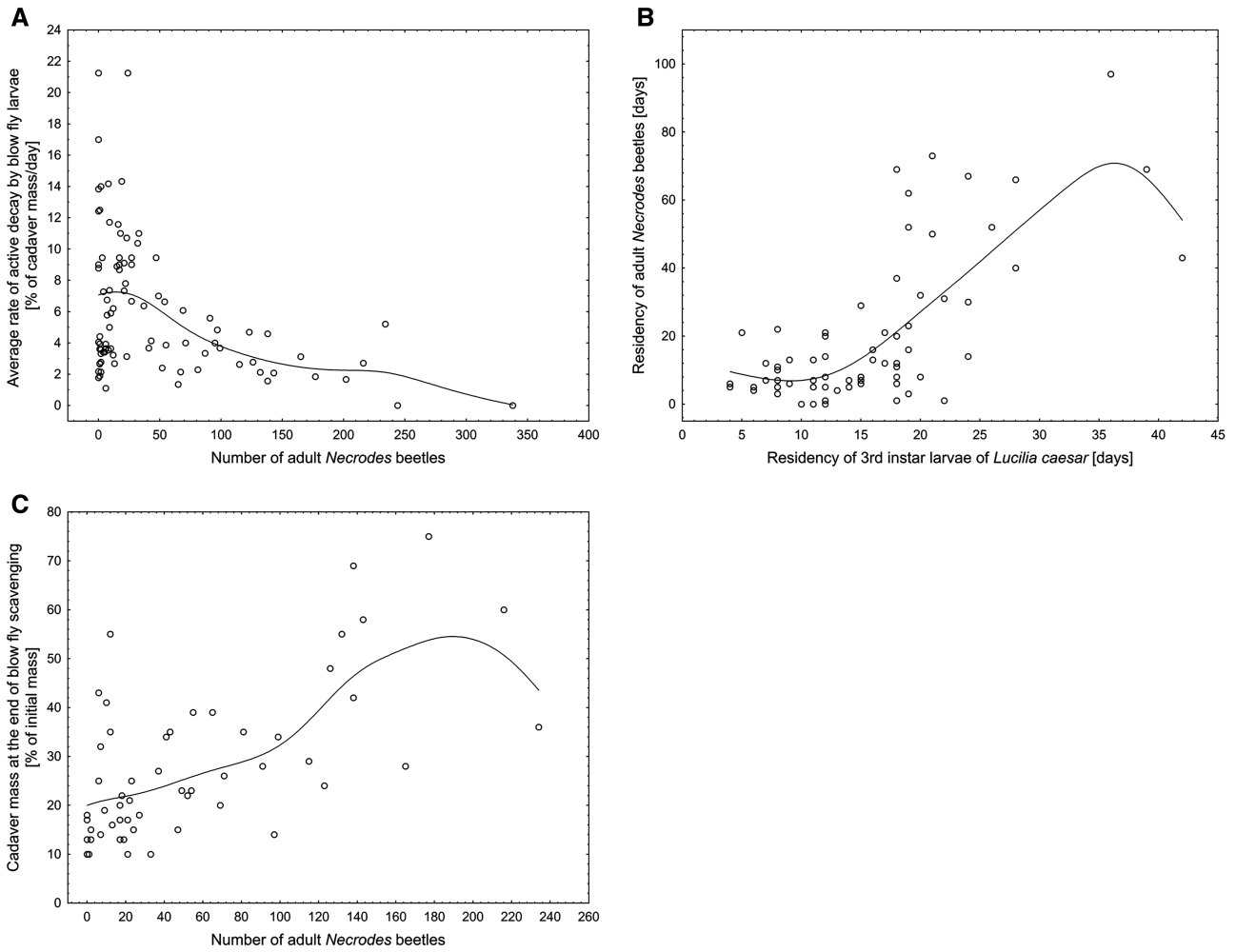
Figure 6. Abundance and residency of adult *Necrodes* beetles trapped on pig carcasses plotted against the average rate of active decay by blow fly larvae (A), residency of L3 of *L. caesar* (B) and cadaver mass that was left when blow flies ceased feeding (C). The smoothed lines were fitted with the distance-weighted least-squares smoothing procedure.
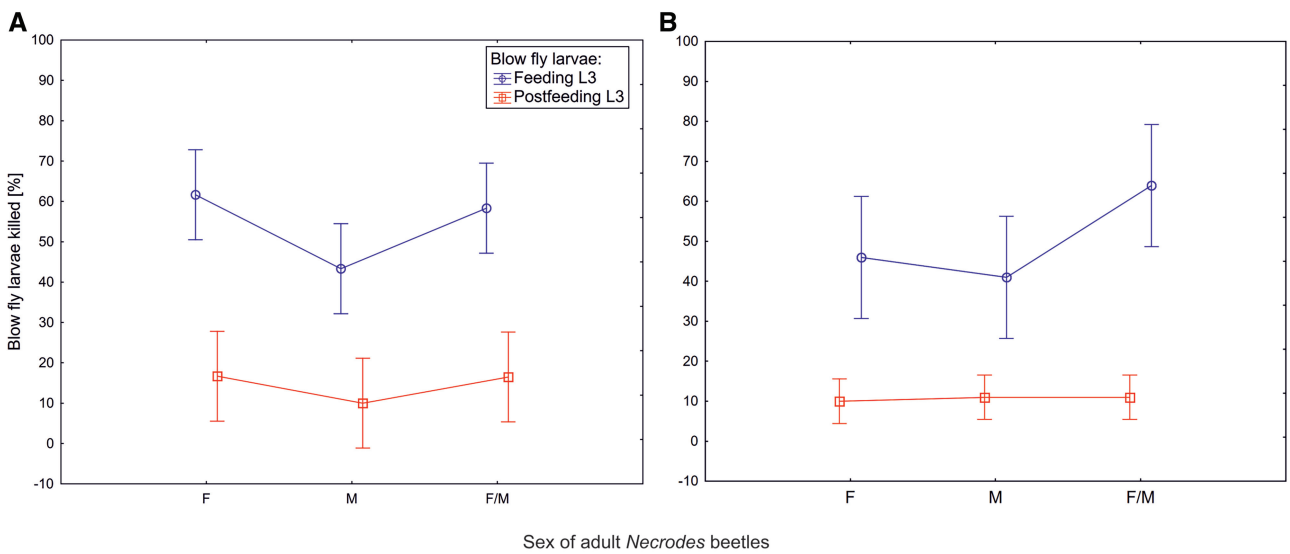

Figure 7. The killing frequency of *Calliphora vomitoria* feeding and postfeeding L3 by adult *Necrodes* beetles. A – feeding and postfeeding L3 supplied separately to the beetles, B – feeding and postfeeding L3 supplied together to the beetles, F – females only, M – males only, F/M – males and females, symbols – means, whiskers – 95% confidence intervals.
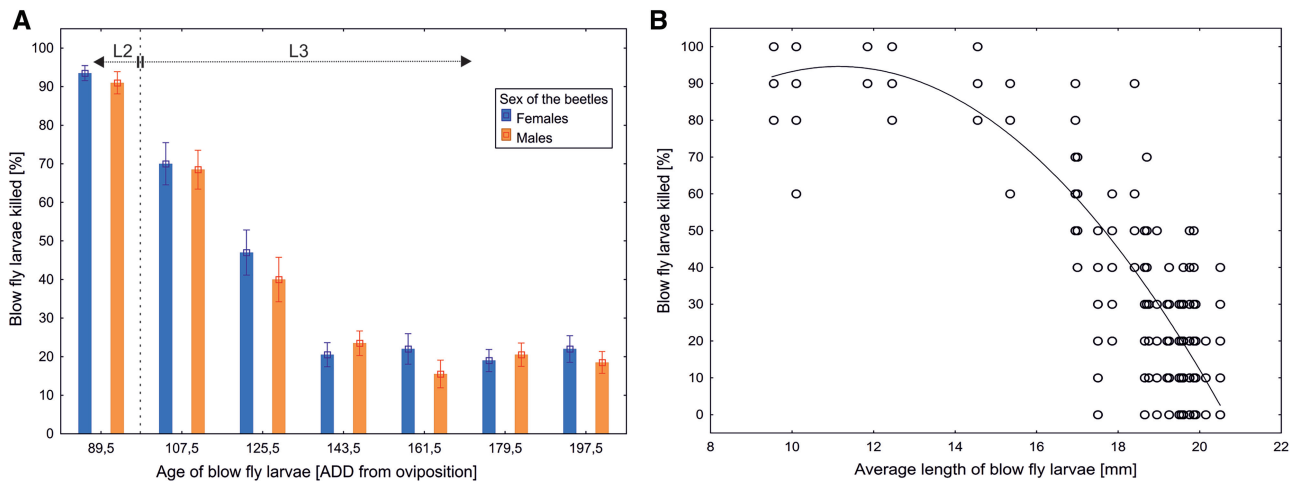
Figure 8. The killing frequency of *Calliphora vomitoria* larvae by adult *Necrodes* beetles in relation to larval age (A) and size (B). ADD – accumulated degree-days (over 0 °C, starting from oviposition), L2 – second instar larvae, L3 – third instar larvae, symbols – means, whiskers – standard errors of the mean. The model line in B is the polynomial model.

Table 2. Competitive and facilitative interactions between insect decomposers on carrion [following the presentation scheme used in Figure 9 by Kaplan and Denno (2007)]

	Interference competition	Exploitative interaction	Mixed competition
Interactions			
Examples	*Nicrophorus* beetles (inter and intraspecific) Dermestid beetles (inter and intraspecific)	Calliphorid flies (inter and intraspecific) Silphid beetles (intraspecific)	Calliphorid flies vs *Necrodes* beetles *Lucilia/Calliphora* flies vs *Chrysomya* flies Piophilid flies (e.g., *Stearibia*) vs. *Necrobia* beetles
Mechanisms	Direct interaction (e.g., fights for resources in *Nicrophorus* beetles, cannibalism in dermestid beetles) with possible effects on carrion	Indirect interaction related to the elimination of carrion (–) or changes in the feeding environment (+)	Indirect effects related to the elimination of carrion by one species combined with direct effects related to the elimination of competitors by the other species
Succession	Interaction between concurrent colonizers	Interaction between concurrent colonizers	Interaction between concurrent or successive colonizers

N: necrophagous species; C: carrion; (–) competition; (+) facilitation; solid lines: direct effects; dotted lines: indirect effects; the same colors represent concurrent colonizers, different colors represent successive colonizers.

long PMI, the monopolization of carrion by the flies was naturally much more frequent than the monopolization by the beetles.

By analyzing conditions that favor colonization of carrion by *Necrodes* beetles, we found that it is determined by the effectiveness of carrion decay by blow flies (in terms of its rate and duration). Blow fly larvae feed in aggregations and the size of an aggregation, as a product of the number of feeding larvae, determines rate and completeness of carrion decay by the flies (Rivers et al. 2011; Gruner et al. 2017; Scanvion et al. 2018). The size of larval aggregation depends primarily on the number of ovipositing females, and, therefore, is mainly a product of the size of the blow fly population

and species preferences along environmental gradients. A small number of blow flies results in small aggregations of their larvae and in effect low rate of active decay driven by the flies and larger part of carrion resources left for *Necrodes* beetles. In addition, small size of larval aggregations prolongs active decay by blow flies, with positive effects on colonization by *Necrodes* beetles. Similarly, in experiments with removal of early decomposers, abundances of silphid beetles increased as more blow fly larvae were removed from carcasses (Michaud and Moreau 2017). Previous experiments on decomposition of large cadavers in temperate climates revealed a delay in carrion colonization by blow flies during early spring (Bourel

et al. 1999; Matuszewski et al. 2010; Cogswell and Cross 2021). The highest NCS were recorded in this study also during early spring, with the only 2 cases of carcass monopolization by *Necrodes* larvae. Several blow fly species (e.g., *Lucilia* spp.) overwinter as the postfeeding L3 and during early spring their adult population is small (Macleod and Donnelly 1957; Cruickshank and Wall 2002; Draber-Mońko 2004). Therefore, the colonization of carrion by blow flies may be minimal or delayed in this season and more cadavers than in summer or autumn may reach (in the intact condition) the point that favors colonization by *Necrodes* beetles.

Adult Silphinae beetles that frequent carrion are usually regarded as predators, and *Necrodes* beetles are well-recognized for their voracious feed on maggots (Ratcliffe 1996; Charabidze et al. 2016). Conventional view treats this behavior as an acquisition of food. Results of this study demonstrate that adult *N. littoralis* prefer younger and smaller larvae, and very rarely kill late feeding or postfeeding L3 that are the largest maggots. Since nearly all late L2 were killed and the frequency of maggot killing decreased after they reached initial phase of L3, our study demonstrated that killing maggots by the beetles is not just a foraging behavior. Blow fly larvae pass through 3 larval stages and L3 is further divided into feeding and postfeeding larva (Rognes 1991). Their growth is the fastest during early L3, when larvae may double their length and increase in mass even 10-fold (Wells and Kurahashi 1994; Grassberger and Reiter 2001). For instance, mean dry weight of *Chrysomya megacephala* increases during this short period (10 h at 27°C) from ∼1.5 mg into ∼15 mg (Wells and Kurahashi 1994). Accordingly, blow fly larvae are most destructive for carrion during this short period and killing them shortly before may limit the decay of carrion. Therefore, killing blow fly larvae by *Necrodes* beetles is related more to the competition over carrion and reduction of the population of maggots than to the foraging of the beetles. Current results provide no indication of the mechanism by which adult *Necrodes* beetles choose late L2 and early L3, further studies are, therefore, necessary to elucidate this mechanism. Size of the larvae, their chemical properties or movement (most postfeeding larvae move vigorously to leave carrion) may be important in this respect.

Similar killing behavior of Nicrophorinae beetles is usually interpreted in terms of the competition and resource guarding. For instance, *Ptomascopus* beetles guard carrion for their offspring by clearing it of the maggots and this behavior was categorized as a simple form of parental care (Trumbo et al. 2001; Suzuki and Nagano 2006). Another example are *Nicrophorus* beetles that eliminate blow fly eggs and larvae by removing hair or feathers from carrion, by transferring phoretic mites on a resource or by simply killing the larvae (Pukowski 1933; Wilson and Knollenberg 1987; Scott 1998). Since *Necrodes* beetles also secure carrion for their offspring (similarly to *Ptomascopus* beetles), this study highlights similarities between Nicrophorinae and Silphinae beetles.

The interaction exposed in this study is a combination of indirect, exploitative effects of the flies and direct, interference effects of the beetles. Such a mixed competition is frequent among carrion insects, which indicates that a distinction between exploitative and interference competition does not fully represent competitive interactions on carrion (Table 2). In addition, mixed competition slows down decomposition, as it has been evidenced by the low rate of combined active decay on carcasses with high colonization scores of both groups (Figure 5). The way insect taxa interact is related to the sequence in which they colonize carrion. Exploitative and interference competition occur usually between concurrent colonizers. Fights for carrion between *Nicrophorus* beetles, an example of interference competition, require synchronized location of the resource by competitors (Trumbo 1990; Scott 1998). In larval aggregations, mutual and indirect effects that comprise exploitative interactions occur usually between conspecifics or congeners that concurrently colonize carrion (Rivers et al. 2011; Charabidze et al. 2021). On the other side, mixed competition involves successive colonizers. As a rule, an earlier colonizer affects its competitor indirectly, by reducing available resources, whereas a later colonizer has direct, interference effects to reduce the exploitative effects of its competitor. Moreover, the mixed competition occurs frequently between different life stages of competitors, for instance larvae of blow flies and adult *Necrodes* beetles. The asymmetry of the competition is also related with the succession. Owing to the preference of blow flies toward fresh carcasses and *Necrodes* beetles toward decomposed carcasses, the carrion pool available for the flies is much larger than the pool accessible by the beetles. These differences result in generally superior performance of blow flies over *Necrodes* beetles and are probably related to the larger species diversity of carrion blow flies. On the other hand, blow flies and *Necrodes* beetles are clearly separated in the succession (Matuszewski et al. 2011). Therefore, competition might contribute to this separation.

A recent study provided evidence that *Necrodes* beetles reveal indirect parental care by preparing carrion resources for larvae (Matuszewski and Mądra-Bielewicz 2021). Current findings indicate that the beetles clear carrion of the fly competitors to secure the resource for their offspring. These findings further support the hypothesis that *Necrodes* beetles care for their offspring. Simple forms of indirect parental care that comprise guarding of the resource are prevalent among Nicrophorinae beetles (Scott 1998; Suzuki and Nagano 2006). In Silphinae beetles parental care has been identified only in *Necrodes* beetles, although there are other taxa that visit large carrion and may exhibit similar forms of parental care (e.g., *Diamesus* or *Ptomaphila*). To understand the evolution of parental care in carrion beetles, more studies of Silphinae beetles are necessary.

Ethical Statement

This manuscript describes laboratory experiments using carrion beetle species *Necrodes littoralis* (Linnaeus, 1758) (Silphidae). The species is not under protection. Moreover, we analyzed data from previous experimental decomposition studies using pig carcasses. Since the original experiments utilized only carcasses (bought from local pig farms), no permission or approval from the Ethics Commission was necessary.

Data Accessibility

Datasets supporting this article are provided as supplementary material.

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Supplementary Material

Supplementary material can be found at https:// academic. oup. com/ cz.

Authors' Contributions

S.M. developed the concept for the study and the article, analyzed the data and wrote the draft of the manuscript. A.M.B. performed behavioral assays. Both authors prepared raw data for the analyses, discussed the results, prepared figures and reviewed the manuscript.

Conflict of interest

We have no competing interests to declare.

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