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ORIGINAL ARTICLE

Parasitisation activity of *Spalangia cameroni* and *Muscidifurax zaraptor*, pupal parasitoids of *Musca domestica*

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Abstract

The house fly, *Musca domestica* L. (Diptera: Muscidae), is a significant pest in livestock farms and a major concern for both humans and farmed animals due to its ability to transmit over 200 pathogens. The use of pupal parasitoids is a sustainable strategy for controlling this pest. *Spalangia cameroni* Perkins (Hymenoptera: Spalangiidae) and *Muscidifurax zaraptor* Girault & Sanders (Hymenoptera: Pteromalidae) are commonly used as biocontrol agents for *M. domestica*. The objective of this study was to determine the oviposition peak of female parasitoids in relation to their age and the sex ratio of the adult progeny. For both species, 20 fresh *M. domestica* pupae (24–48 h old) were provided daily to each fertilised female for 14 days, after which the pupae were checked for parasitoid emergence. A control group of 20 pupae without female parasitoids was maintained. The results showed that *S. cameroni* had a higher overall percentage of parasitisation (57.7%) compared with *M. zaraptor* (32.4%). The parasitisation ratio of *S. cameroni* remained almost constant throughout the 14-day period, whereas that of *M. zaraptor* decreased drastically after Day 11. Peak oviposition for *S. cameroni* was on Day 5 with 13 parasitised pupae per female, whereas *M. zaraptor* parasitised eight pupae per day on 4 days during its peak oviposition period (between Days 3 and 8). The newly emerged parasitoids had a skewed sex ratio towards females: 81% for *S. cameroni* and 66% for *M. zaraptor*. The presence of these parasitoid species resulted in fewer new house fly emergences than in the control group, where natural pupal mortality was lower in the absence of parasitoids. These findings may be useful for optimising the mass production and time-use of the two parasitoid species for the management of house flies in livestock farms.

KEYWORDS
biocontrol, Diptera, house flies, Hymenoptera, livestock, mortality, Muscidae, oviposition, pest, Pteromalidae, Spalangiidae, wasp

INTRODUCTION

The house fly, *Musca domestica* L. (Diptera: Muscidae), is a widespread pest closely tied to human environments. It is commonly found in the vicinity of poultry, cattle, horse, pig and dairy farms. The constant presence of this pest in close proximity to humans has allowed it to thrive in different ecosystems and exploit various food sources. Moreover, its adaptability to several environments makes it a formidable adversary, able to evade, adapt to and even develop resistance to the most steadfast control measures (Geden et al., 2021; Gogarten et al., 2019). The widespread use of insecticides (Scott, 2017; Wang et al., 2019) has led to the emergence of resistance in house flies, emphasising the need for safer and more sustainable alternatives, such as biological control (Ardburi & Tangkawanit, 2022). Sustainable management of house flies could include a variety of biological control methods, such as application of microorganisms and augmentation or release of natural and exotic predators and parasitoids (Geden et al., 2021).

For almost 50 years, naturally occurring pupal parasitoids, mainly from the Pteromalidae family, have been used extensively for augmentative biocontrol in animal facilities. However, relying solely on natural parasitoid populations is often not enough to keep house fly populations within acceptable limits. This is mainly because parasitoids take longer to develop than their hosts (Geden et al., 2021). Nevertheless, augmentative releases of parasitoids could be successfully improved if integrated with other pest management methods, as also highlighted by Geden et al. (1995), Skovgård and Nachman (2004), and McKay et al. (2007).

Among the many species capable of parasitising house fly pupae, the most commercialised are the genera *Muscidifurax* as *Muscidifurax raptor* Girault & Sanders, *Muscidifurax zaraptor* Kogan & Legner, and *Muscidifurax raptorellus* Kogan & Legner (all Hymenoptera: Pteromalidae) and the genera *Spalangia* as *Spalangia cameroni* Perkins and *Spalangia endius* Walker (all Hymenoptera: Spalangiidae) (Machtinger & Geden, 2018). Most of these species have similar life histories and reproduction. The female parasitoid locates a suitable fly puparium, drills through it and lays one egg if a solitary species, or several if gregarious (Geden et al., 2021; Gerling & Legner, 1968). The resulting parasitoid larva or larvae subsequently feed on the pupa and emerge as adults within 2–4 weeks. The duration of parasitoid development from egg to adult stage varies from 14 to 30 days at warm temperatures and is influenced by several factors, including temperature, biotype, host, species, sex and environment (Birkemoe et al., 2012; Geden et al., 2021).

One of the most effective parasitoid wasps targeting house and stable flies in livestock is *S. cameroni* (Birkemoe et al., 2012). This species is attracted to the scent of substrates containing house fly larvae and is capable of parasitising pupae at depths of up to 10 cm (Machtinger et al., 2015). Although a solitary species, under laboratory conditions, *S. cameroni* lays more than one egg in a single pupa (Gerling & Legner, 1968). Machtinger et al. (2015) found that *S. cameroni* shows a preference for young and fresh pupae. In addition, *S. cameroni* demonstrates superior puparia location skills, resulting in a higher parasitisation rate compared with *M. raptor*, as indicated by Legner (1967).

Another species of pupal parasitoid wasp, *M. zaraptor*, is used in house fly population management programmes (Weinzierl & Jones, 1998). Similar to *M. raptor*, this species does not discriminate between fresh and frozen hosts (Floate, 2002), a positive feature for biocontrol purposes in terms of mass production for this species. A female *M. zaraptor* can identify parasitised hosts by inserting her ovipositor into the pupa to detect the venom that was injected by previous females. However, *M. zaraptor* females exhibit a preference for ovipositing on non-parasitised pupae, rather than those that have been attacked by other females or species, including *Nasonia vitripennis* (Walker) and *S. cameroni* Perkins in laboratory conditions (McKay & Broce, 2004). In contrast to *S. cameroni*, *M. zaraptor* females appear to respond only to the odours emanating from house fly pupae and may use olfactory cues while seeking hosts (McKay & Broce, 2004).

This study had two primary objectives: (1) to evaluate the effectiveness of *S. cameroni* and *M. zaraptor* wasp parasitisation of the house fly over a defined 2-week period and (2) to investigate the relationship between female wasp age and successful oviposition, with a specific focus on the sex of newly emerged adult wasps. The research also aimed to assess the overall impact of the wasps in reducing house fly emergence.

MATERIALS AND METHODS

House flies and parasitoids rearing

The study was conducted at the Applied Entomology Laboratory of the BIOGEST-SITEIA Interdepartmental Center, University of Modena and Reggio Emilia, Reggio Emilia, Emilia Romagna region, Italy. During the summer of 2020, pupae were collected from livestock farms in the Reggio Emilia area to establish colonies of house flies (*M. domestica*), *S. cameroni* and *M. zaraptor*. Adult house flies were kept in polyester mesh cages (32.5 × 32.5 × 32.5 cm), situated in a climatic chamber with a temperature of 27 ± 1°C, 60 ± 1% r.h. and L16:D8 photoperiod. They were given water, honey droplets and a standard diet (see below) to facilitate oviposition. Every 2–3 days, the eggs were transferred to closed containers, and after hatching, the diet was supplemented with 60 g of wheat bran, 40 g of alfalfa pellets and 3-g mixture of milk powder and water. The diet provided was adapted to conform to that of Bell et al. (2010).

The parasitoid colony started 6 months before the beginning of the experiment. The parasitoid species were reared in polyester mesh cages (17.5 × 17.5 × 17.5 cm), located inside a climate-controlled room, with 60 ± 1% r.h. and a temperature of 25 ± 1°C. They were supplied with water, honey droplets, and fresh or frozen *M. domestica* pupae for oviposition, for 24–48 h. Parasitised pupae were substituted every 3 days, and the newly emerged specimens were captured and returned to the cages after 25–27 days for *S. cameroni* and 17–22 days for *M. zaraptor*.

Experimental design

One male and one female of each parasitoid species (no more than 24 h old) were held in a 50 mL conical tube containing honey drops for 48 h to mate. The female was then transferred to a sealed Petri dish with 20 fresh pupae and honey drops, placed in a climatic chamber. Every 24 h for a period of 14 days, the female parasitoid was removed and placed in a new dish with 20 fresh pupae (for *M. zaraptor*, $n = 30$ and for *S. cameroni*, $n = 28$; replicates were performed at different times and from several batches of parasitoids). A daily control group of 20 house fly pupae was established in the absence of the parasitoid. Newly emerged individuals of *M. domestica*, *S. cameroni* or *M. zaraptor* were counted daily and the intact pupae were

dissected after 50 days to detect any non-emerged parasitoids.

Data collection

Parasitisation was calculated by counting the number of parasitoids emerging each day (equal to the number of parasitised pupae) for each species and then by calculating parasitisation ratio using the following equation:

$$\text{Parasitisation ratio} = \text{no. of parasitised pupae} / \text{total no. of pupae offered.}$$

The F1 sex ratio for each parasitoid species was determined by counting the daily number of females and males. The percentage of female/male emergence for each day and species was also calculated by using the following equation:

$$\text{Female emergence percentage} = (\text{no. of emerged females} / \text{total no. of pupae}) \times 100.$$

The number of house flies emerging in the presence of parasitoids was also determined for each day and species by using the following equation

$$\text{House fly emergence percentage} = (\text{no. of emerged flies} / \text{total no. of pupae}) \times 100.$$

Female mortality was recorded daily for each parasitoid species.

Data analysis

Statistical analysis was performed using R STUDIO v.2024.04.1 + 748 (RStudio Team, 2024). A significance level of $\alpha = 0.05$ was used for each test. After descriptive data analysis, models were selected to fit the data with lower Akaike information criterion values. A generalised linear mixed model (GLMM) with a negative binomial distribution (log link function) was fitted for the dependent variable 'parasitisation' (number of emerged parasitoids) using 'days' as a fixed variable and 'tested females' as a random factor, considering the species separately. Comparing the species, a GLMM with binomial distribution (logit link function) was fitted for the dependent variable 'parasitisation ratio' (number of emerged parasitoids over total number of pupae) with 'days' and 'species' as fixed variables and 'tested females' as a random factor. The variable 'sex ratio' of F1 was considered separately for each species in relation to days. The effects of 'parasitoid species' and 'days' on fly emergence were evaluated. The models were fitted using the package glmmTMB and the marginal means were calculated using the package emmeans in the case of comparisons between the species and days.

A Kaplan–Meier survival curve and an associated risk table were used to estimate and visualise the probability of survival over time for each parasitoid species. The survival curves of the two parasitoid species were compared using a log-rank test. The effect of species on survival over time was assessed using the Cox regression model.

RESULTS

Number of emerged parasitoids and parasitisation ratio

The proportion of parasitised house fly pupae varied between the two species (Figure 1). For *M. zaraptor*, significant differences were observed between days, especially between Day 2 (median 22.5% parasitism of the 20 pupae provided) and Days 3, 4, 5, 6 and 7 (35%–50%).

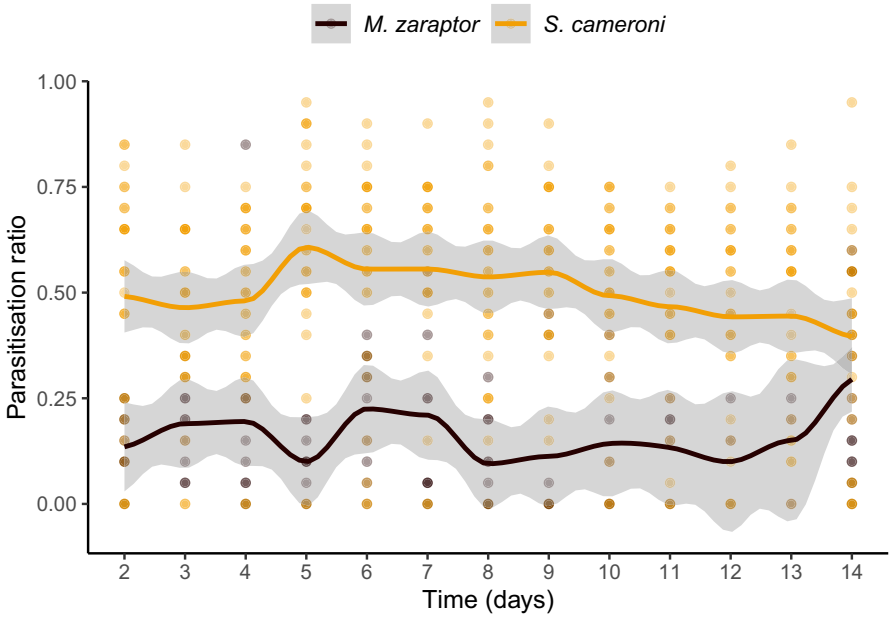

FIGURE 1 House fly parasitisation ratio for the wasps *Muscidifurax zaraptor* (black) and *Spalangia cameroni* (orange) over a 2-week period. The female parasitoid was given 20 fresh pupae each day ($n = 28–30$). The lines indicate the trend of parasitisation, and the dots are observed data at daily intervals. The grey area around the smoothed trend line represents the 95% confidence interval.

TABLE 1 Median number of house fly pupae parasitised by *Muscidifurax zaraptor* over a 2-week period.

Time (days)	No. of *M. zaraptor*[a]	Estimate	*z* Value	*p*
2	4.5 (2–11)	-	-	-
3	9 (4.75–12)	0.4	2.36	0.01
4	10.5 (6–12)	0.41	2.42	0.01
5	7 (5–12)	0.36	2.10	0.03
6	9 (5.75–12.25)	0.42	2.45	0.01
7	10 (5.75–12.25)	0.45	2.69	0.006
8	6 (3.75–8.75)	0.1	0.55	0.57
9	8.5 (2–12)	0.22	1.22	0.21
10	6 (4.75–10)	−0.14	1.13	0.25
11	5 (2.75–9)	−0.14	−0.70	0.48
12	2.5 (0–4.25)	−0.59	−2.53	0.01
13	1.5 (0–4)	−0.58	−2.40	0.01
14	0 (0–2)	−1.25	−3.91	<0.001

[a]Interquartile range in brackets. The female parasitoid was given 20 fresh pupae each day ($n = 30$). A generalised linear mixed model (GLMM) was used to compare the parasitisation between Day 2 and the other days. The GLMM's estimate and *z* value of the fitted model are provided.

TABLE 2 Median number of house fly pupae parasitised by *Spalangia cameroni* over a 2-week period.

Time (days)	No. of *S. cameroni*[a]	Estimate	*z* Value	*p*
2	11 (6–13)	-	-	-
3	10 (7–13)	−0.04	−0.39	0.69
4	10 (7.25–13)	0.01	0.15	0.87
5	13.5 (10.25–15)	0.24	2.12	0.03
6	13 (10.25–15)	0.15	1.26	0.2
7	12 (10–14)	0.11	0.94	0.34
8	11.5 (8.25–13.75)	0.12	1.07	0.28
9	12 (9.25–14.5)	0.16	1.40	0.16
10	10.5 (9–13)	0.04	0.37	0.7
11	11 (8–12)	−0.004	−0.04	0.96
12	9.5 (7.25–12)	−0.04	−0.32	0.74
13	11 (6–12)	−0.05	−0.45	0.64
14	8 (4.25–11)	−0.23	−1.74	0.08

[a]Interquartile range in brackets. The female parasitoid was given 20 fresh pupae each day ($n = 28$). A generalised linear mixed model (GLMM) was used to compare the parasitisation between Day 2 and the other days. The GLMM's estimate and *z* value of the fitted model are provided.

Additional differences were observed between Day 2 and Day 12 (12.5%), Day 13 (7.5%), and Day 14 (range of 0%–10%). Parasitisation by *M. zaraptor* generally increased from Day 2 until Day 7, but showed a decrease after Day 11 (Figure 1; Table 1).

Throughout the experimental period, parasitisation by *S. cameroni* showed relative stability, with a peak on Day 5 (median 67.5%), compared with the other days (40%–65%) (Figure 1; Table 2).

Significant differences in parasitisation ratios were observed between the two species, with *S. cameroni* showing a higher ratio on each day compared with *M. zaraptor*. Considering the parasitisation independent of the species, significant differences were observed for Days 2 with Days 13–14. (Figure 1; Table 3).

TABLE 3 Statistical analysis of house fly pupae parasitisation by *Muscidifurax zaraptor* and *Spalangia cameroni* over a 2-week period. A generalised linear mixed model (GLMM) was used to compare the parasitisation between species and among days. The GLMM's estimate and *z* value of the fitted model are provided.

Fixed effects	Estimate	*z* Value	*p*
Day 3	0.11	0.92	0.35
Day 4	0.14	1.19	0.23
Day 5	0.20	1.67	0.09
Day 6	0.19	1.59	0.10
Day 7	0.21	1.74	0.08
Day 8	0.01	0.15	0.87
Day 9	0.14	1.15	0.24
Day 10	0.03	0.31	0.75
Day 11	−0.10	−0.80	0.41
Day 12	−0.24	−1.80	0.06
Day 13	−0.27	−2.04	0.04
Day 14	−0.49	−3.56	0.0003
S. cameroni	0.63	12.26	<0.001

F1 sex ratio

The sex ratio of the F1 generation of the parasitoids was shifted towards females compared with males for both *M. zaraptor* (Figure 2; Table 4) and *S. cameroni* (Figure 3; Table 5). In the case of *M. zaraptor*, more females than males emerged on each day, except for Day 14 where no statistical difference was recorded. Specifically, a higher number of females than males emerged on Day 4. Male emergence remained relatively constant throughout the experiment, with only one to two males emerging per day. In contrast, female emergence varied, increasing from Day 2 until Day 7 and then decreasing until Day 14 (Figure 2; Table 4).

For *S. cameroni*, more females than males emerged on each experimental day. Male emergence remained relatively constant, with only one to two males emerging per day. Female emergence also remained constant, with 6–10 females emerging per day, peaking on Day 5 with a median of 10 females emerging (Figure 3; Table 5).

Dissection of pupae

When dissecting pupae, immature stages of the parasitoids were not found for both species. For *M. zaraptor*, a total of 8400 pupae were supplied to female parasitoids during the experiment, and 2990 (36.0%)

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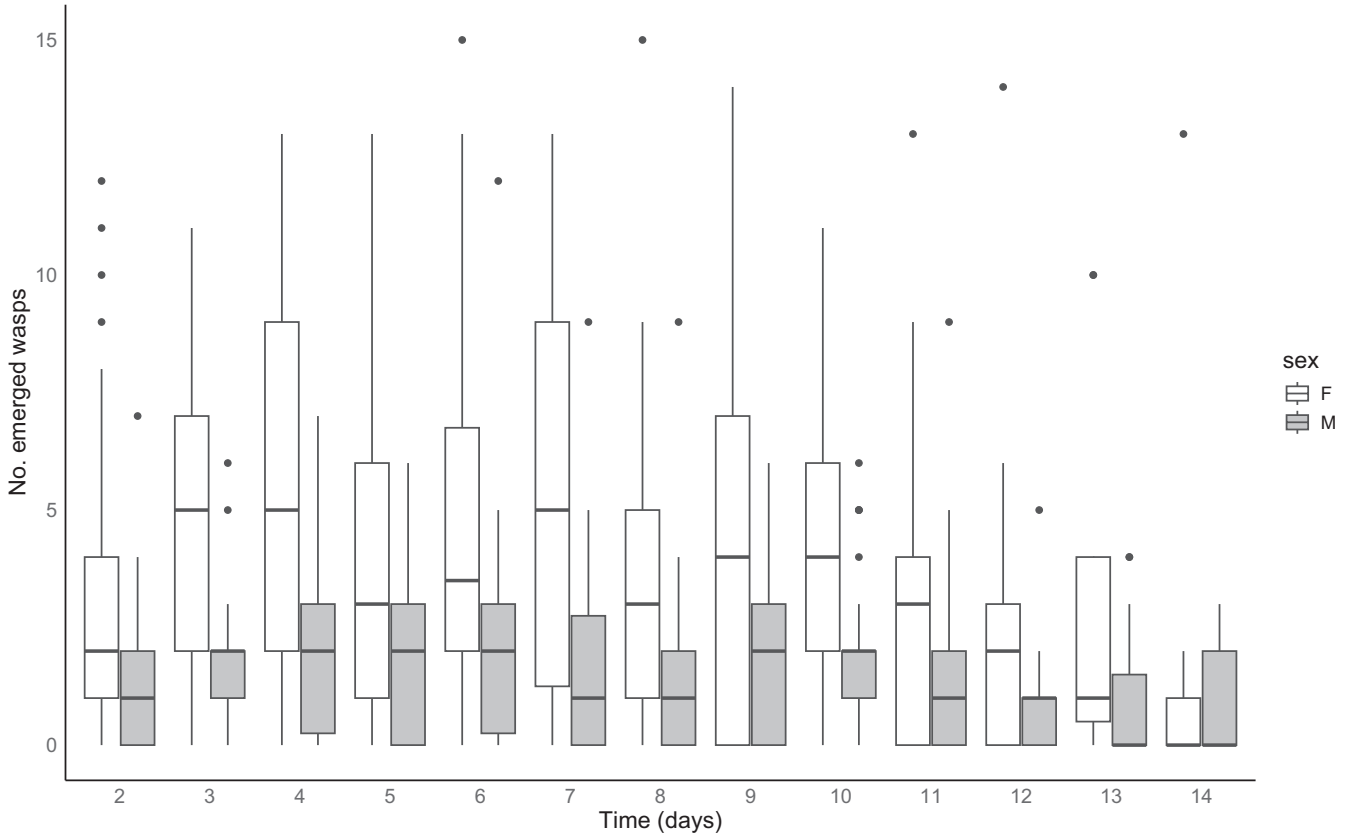

FIGURE 2 Number of emerged F1 females (white) and males (grey) from house flies parasitised by *Muscidifurax zaraptor* over a 2-week period. The P female parasitoid was given 20 fresh pupae each day ($n = 30$). In the boxplots, the boxes indicate the first and third quartiles, the thick line in between shows the median, the whiskers indicate 1.5× the interquartile range, and the dots are outliers. Corresponding statistical analysis is provided in Table 4.

TABLE 4 Median number of female and male parasitoids emerging from house fly pupae parasitised by *Muscidifurax zaraptor* over a 2-week period.

Time (days)	No. of females[a]	No. of males[a]	Estimate	*z* Value	*p*
2	2 (1–3.75)	1 (1–2)	0.77	3.09	0.002
3	5 (2–7)	2 (1–2)	0.98	4.64	<0.001
4	5 (2.25–9)	2 (0.25–3)	0.78	3.78	<0.001
5	3 (1–6)	2 (0–2.75)	0.71	3.29	0.001
6	4.5 (2–7.75)	2 (0.25–3)	0.89	4.19	<0.001
7	5.5 (2–9)	1 (0–2.75)	1.28	5.55	<0.001
8	3 (1–5)	1 (0–2)	0.84	3.53	<0.001
9	4 (0.75–7)	2 (0–3)	0.76	3.25	0.0011
10	4 (2–6)	2 (1–2)	0.65	2.80	0.004
11	3 (0–4)	1 (0–2)	0.67	2.37	0.01
12	2 (0–3)	1 (0–1)	0.7	2.09	0.03
13	1 (0–4)	0 (0–1.25)	1.16	2.17	0.006
14	0 (0–1)	0 (0–1.5)	−0.09	−0.20	0.83

[a]Interquartile range in brackets. The initial female parasitoid was given 20 fresh pupae each day ($n = 30$). A generalised linear mixed model (GLMM) was used to compare sex ratios among days. The GLMM's estimate and *z* value of the fitted model are provided.

did not emerge and were dissected. For *S. cameroni*, a total of 7840 pupae were supplied to female parasitoids during the experiment, and 1472 (18.8%) did not emerge and were dissected.

Emergences of house flies

The presence of *M. zaraptor* and *S. cameroni* caused a reduction in house fly emergence for the whole period

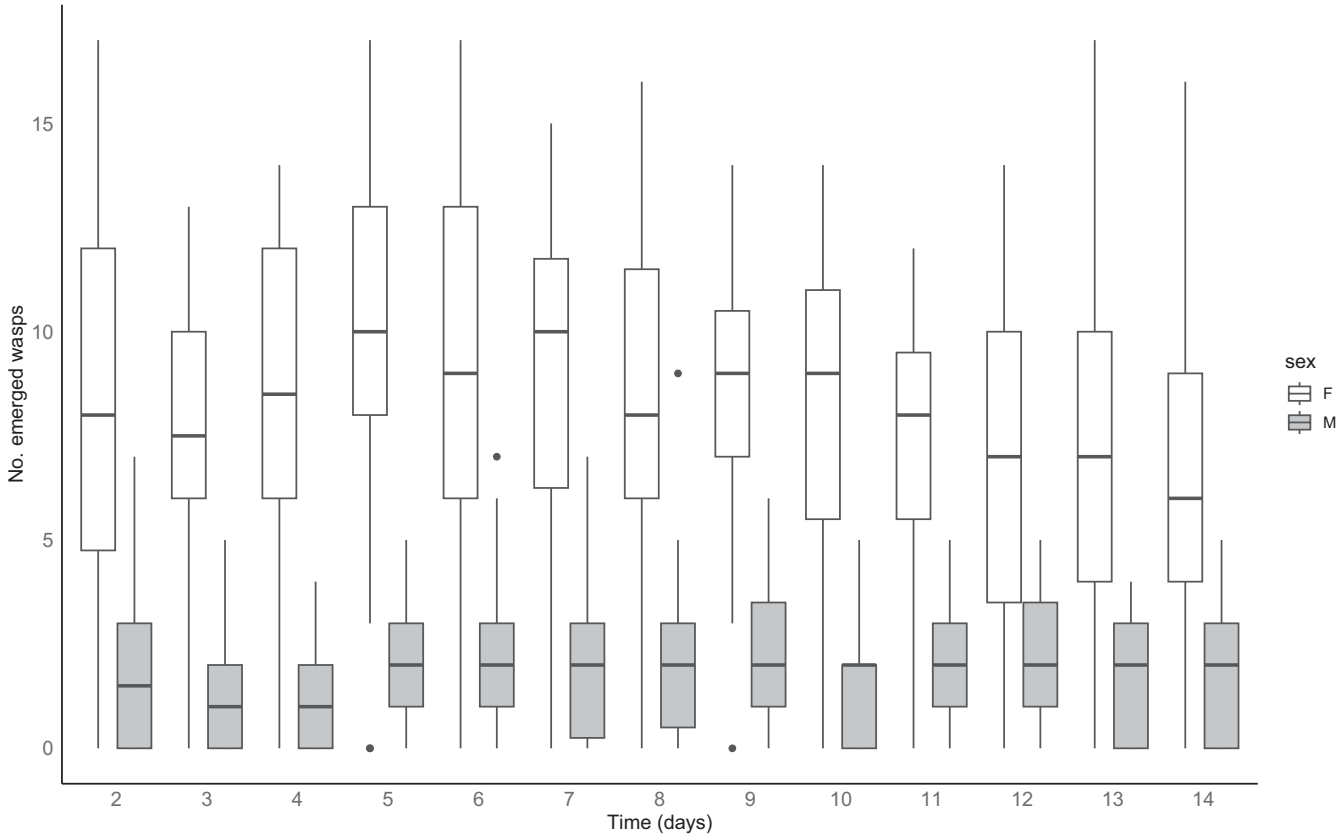

FIGURE 3 Number of emerged F1 females (white) and males (grey) from house flies parasitised by *Spalangia cameroni* over a 2-week period. The P female parasitoid was given 20 fresh pupae each day ($n = 28$). In the boxplots, the boxes indicate the first and third quartiles, the thick line in between shows the median, the whiskers indicate 1.5× the interquartile range, and the dots are outliers. Corresponding statistical analysis is provided in Table 5.

TABLE 5 Median number of female and male parasitoids emerging from house fly pupae parasitised by *Spalangia cameroni* over a 2-week period.

Time (days)	No. of females[a]	No. of males[a]	Estimate	*z* Value	*p*
2	8 (5.5–12)	2 (0–3.5)	1.38	6.94	<0.001
3	8 (6–10)	1 (0.5–2)	1.61	7.54	<0.001
4	9 (6.5–12)	1 (0–2)	1.98	8.57	<0.001
5	10 (8.25–13)	2 (1–3)	1.51	8.25	<0.001
6	9 (6.5–13)	2 (1–3)	1.36	7.26	<0.001
7	10 (6.25–11.75)	2 (1–3.75)	1.47	7.33	<0.001
8	8.5 (6.25–11.75)	2 (1–3)	1.4	7.38	<0.001
9	9 (7–10.75)	2 (1–3)	1.28	7.05	<0.001
10	9 (6.25–11)	2 (1–2)	1.62	7.71	<0.001
11	8.5 (6–9.75)	2 (1–3)	1.24	6.47	<0.001
12	7 (4–9.25)	2 (1–4)	1.13	5.73	<0.001
13	7.5 (5–10)	2 (0.25–3)	1.41	6.83	<0.001
14	6.5 (4–9.25)	1.5 (0–3)	1.41	5.90	<0.001

[a]Interquartile range in brackets. The initial female parasitoid was given 20 fresh pupae each day ($n = 28$). A generalised linear mixed model (GLMM) was used to compare sex ratios among days. The GLMM's estimate of the fitted model is provided.

of the experiment. For *M. zaraptor*, the lowest median value was five emerged flies on Day 5 and Day 9 (25%), whereas an increase in emergence occurred after Day 11, with a median value of 12–13 house flies (60%–65%). For *S. cameroni*, house fly emergence remained relatively constant, with a minimum median of 4.5 emerged flies

(22.5%) on Day 6 and a maximum median value of eight emerged flies (40%) on Day 14 (Figure 4). In addition, a comparative analysis between parasitoid species showed statistically significant differences in the number of emerged flies in the presence of both species. On Day 2 and Days 11–14, more flies emerged in the presence of *M. zaraptor* than *S. cameroni* (Figure 4; Table S1).

A comparison of housefly emergence between the presence of parasitoid species and the control group revealed statistically significant differences throughout the experimental period (Tables S2 and S3). In the control group, the median emergence rate was approximately 90% on each day of the experiment. Although the number of flies emerging during the final stages of experimentation with *M. zaraptor* was relatively high, indicating a reduction in parasitisation, it remained lower than that observed in the control group (Table S2).

Survival rate of parasitoid females

For the parasitoid females, a negative coefficient of the Cox model indicates that *S. cameroni* has a lower hazard (i.e. risk of the event, in this case mortality, occurring) compared with the reference species *M. zaraptor*. The hazard for *S. cameroni* is approximately 19.34% of the hazard for the reference group. This can be expressed as a reduction in hazard of approximately 80.66% compared with *M. zaraptor* (Table 6).

The Kaplan–Meier plot and the corresponding risk table show differences between the survival curves of the two species, as confirmed by the log-rank test. Specifically, for *M. zaraptor*, the survival rate drops substantially after Day 8, with the probability of survival decreasing with each passing day. The survival rate in *S. cameroni* is higher than in *M. zaraptor*, with a decrease observed after Day 4 (one dead female), which remains constant until Day 13. After this point, the survival rate starts to decrease in *S. cameroni* (two dead females) (Figure 5).

DISCUSSION

During the experiments, single-mated females were tested to avoid intraspecific competition. *Muscidifurax zaraptor* demonstrated an increased tendency to parasitise as a function of time, whereas *S. cameroni* showed almost constant parasitism. In the research conducted by Legner and Gerling (1967), *S. cameroni* displayed a peak in oviposition on Day 4, which differs from Day 5 peak observed in our study. Furthermore, the trend of

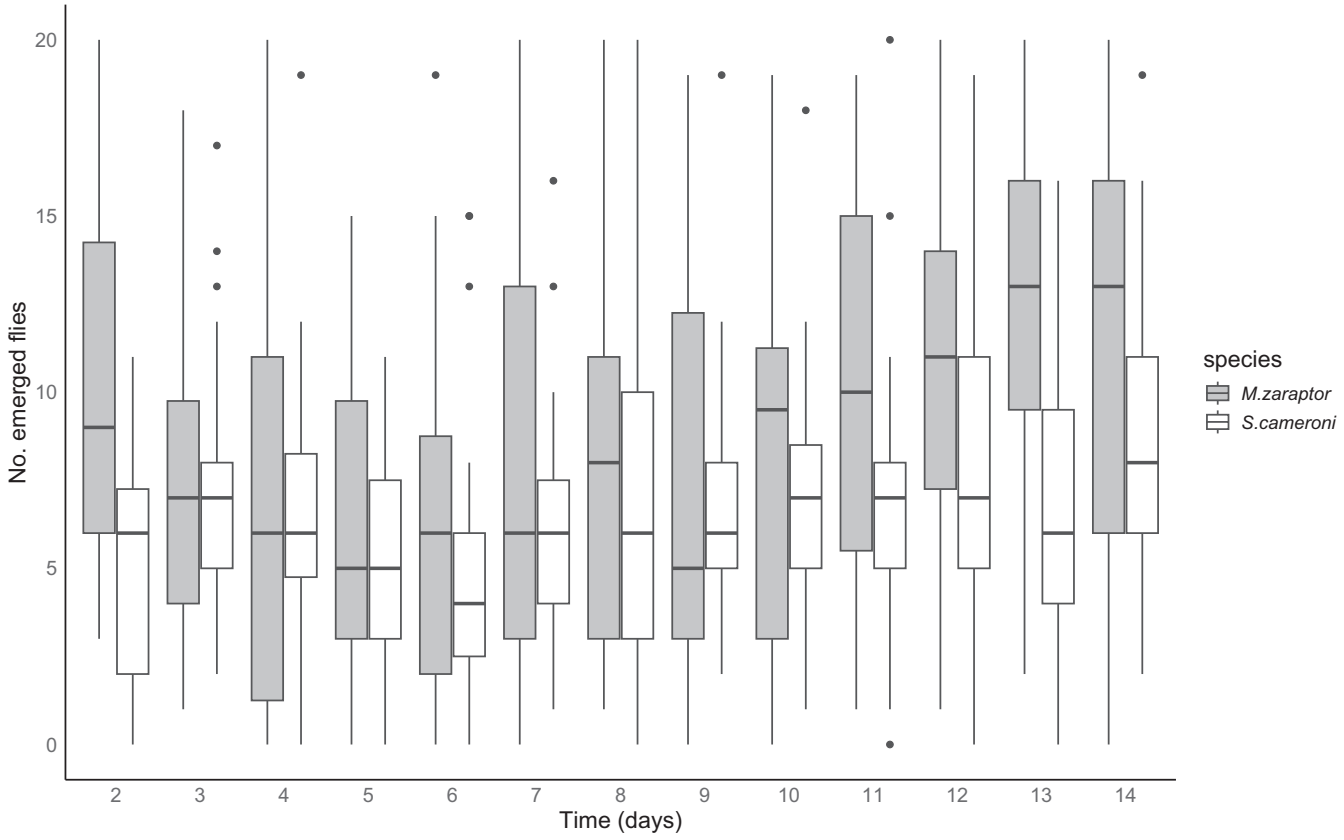

FIGURE 4 Number of emerged (i.e. non-parasitised) house flies after exposure of pupae to the wasps *Muscidifurax zaraptor* (white) and *Spalangia cameroni* (grey) over a 2-week period. The female parasitoids were given 20 fresh pupae each day ($n = 28–30$). In the boxplots, the boxes indicate the first and third quartiles, the thick line in between shows the median, the whiskers indicate 1.5× the interquartile range, and the dots are outliers. Corresponding statistical analysis is provided in Table S1.

TABLE 6 Statistical results of Cox proportional hazards regression analysis assessing the effect of parasitoid species (*Muscidifurax zaraptor* and *Spalangia cameroni*) on survival time.

	Coef	Exp(coef)	SE(coef)	*z*	HR	*p*
Species *S. cameroni*	−1.6431	0.1934	0.6417	−2.56	0.193	0.010

Note: The hazard ratio (HR) for *S. cameroni* indicates the relative risk of experiencing mortality compared with the reference species (*M. zaraptor*). All values are reported for *Spalangia cameroni* compared with the reference species.

Abbreviations: Coef, coefficients; Exp(coef), exponentiated coefficients; SE(coef), standard errors; *p*, *p*-values; *z*, *z*-scores.

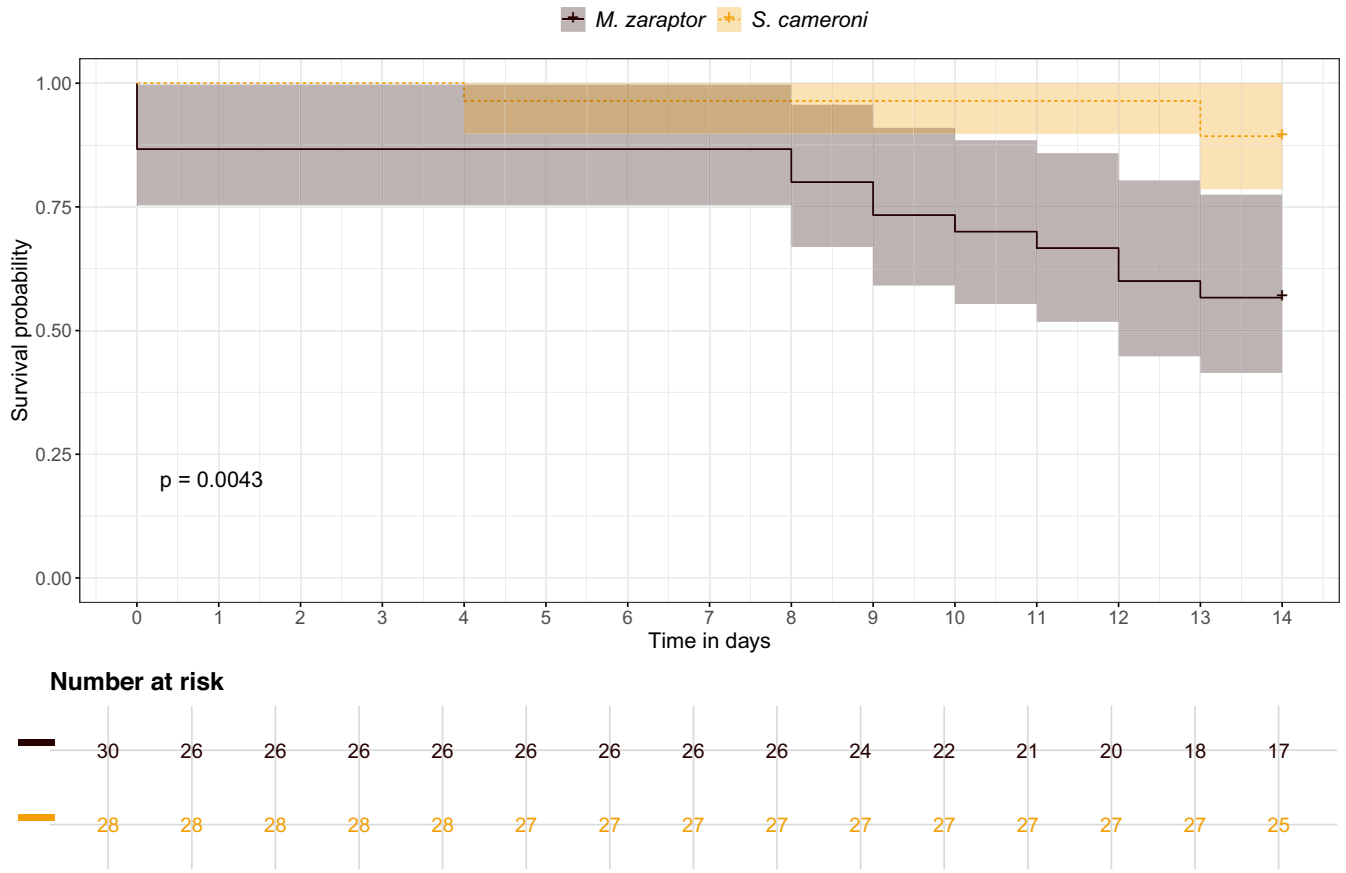

FIGURE 5 Kaplan–Meier survival curves depicting the survival probabilities over time (in days) for *Muscidifurax zaraptor* (grey, full line) and *Spalangia cameroni* (orange, dotted line) when parasitising house fly pupae over a 2-week period. The curves are accompanied by 95% confidence intervals (shaded areas), with different line types distinguishing between species. A risk table below the plot summarises the number of individuals at risk for each species at specified time points.

parasitisation showed a decrease after Day 10, in contrast to our study where the decrease was observed on the final days of the experiment. This variation may be attributed to differences in the population tested with various origins. As shown for scelionid parasitoids of *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), individual populations can exhibit different parasitisation behaviour (Costi et al., 2020). In Legner & Gerling's study, the parasitisation trend of *M. raptor* was similar to that of *M. zaraptor* in our study. Both species showed a decrease in parasitisation after Day 11 of the test. The level of parasitism recorded for *S. cameroni* was higher than for *M. zaraptor* throughout the experiment, in contrast to the results of Mann et al. (1990), where *M. zaraptor* had a higher number of parasitised pupae than *S. cameroni*. Indeed, at the peak of parasitism, the median number of parasitised pupae in our study was 8 for *M. zaraptor* and 11 for *S. cameroni*, compared with 9.3 and 5, respectively, in the study by Mann et al. (1990). The divergence in results may be related to competition between females. Although the ratio of parasitoids to pupae was the same as in their study (1:20), only one female was tested at a time in our study in contrast to the five females tested simultaneously in the study by Mann et al. (1990). In another study, Morgan et al. (1989) found that the trend of parasitisation in *S. cameroni* was influenced by time. In contrast to our results, in their study, the trend of parasitisation in this species decreased after Day 6, with a peak on Day 4 (Morgan et al., 1989).

For the parameter sex ratio, in both species, the ratio shifted towards females compared with males, but with different proportions depending on the number of parasitised pupae, as in the study by Floate (2002). Analysing their results, in *M. zaraptor*, the number of F1 females was about 20.3, whereas the number of males was 5.3 on fresh pupae, whereas in *S. cameroni*, it was 4.3 females and 2.0 males (Floate, 2002). In contrast to Floate's study, we observed a higher number of newly emerged F1 parasitoids in pupae parasitised by *S. cameroni* compared with *M. zaraptor*. Specifically, during the first 2 days of our experiment (48 h, Day 3), the median number of F1 females for *S. cameroni* was double that Floate's study, whereas for *M. zaraptor*, there were 5× fewer females and 4× fewer males than in Floate's study. This could also be related to competition between females. In Floate's, 2002 study, the authors tested five females rather than a single individual, with a parasitoid–host ratio of 1:19. Species *M. zaraptor* produced more offspring in conspecific competition than *M. raptor* (King & Seidl, 1993) and *S. cameroni* (King, 1996). This suggests a discrepancy between our results and those of other studies. The proportion of females for *S. cameroni* was similar to *M. raptor* in fresh pupae (Geden & Kaufman, 2007); 63.8% in 24 h at a parasitoid–host ratio of 1:10, lower than in our study for *S. cameroni* but similar to *M. zaraptor*.

Another parameter that influences the sex ratio is parasitoid–host ratio. In *Spalangia endius*, higher host densities increase the number of parasitised pupae, whereas in *M. raptor*, parasitisation decreases with higher host densities (Ables & Shepard, 1974). This agrees with our experiments and explains that in *M. zaraptor*, parasitoid emergence was lower in the 1:20 parasitoid–host ratio (our study) than in the 1:19 parasitoid–host ratio in Floate's study (2002). This suggests that the number of parasitised pupae is lower at higher pupal densities, which is consistent with the study by Mann et al. (1990) on the variation of parasitisation as a function of pupal density. It is noteworthy that *M. zaraptor* did not have a single peak, but a range of oviposition peaks different from *S. cameroni*. The production of two or more female peaks by *M. zaraptor* instead of one peak increases the likelihood that some offspring will find hosts of optimal age, while reducing the number of females searching for hosts at any given time (Coats, 1976). An alternative explanation for this production, which may depend on the cyclic maturation of ovarioles, does not fully explain why males were not produced in peaks. It is possible that the cyclic production of females, but not males, occurs to limit the competition between females competing for hosts (Coats, 1976). Competitive abilities are more important for this species than a high reproductive rate (Coats, 1976).

During the last days of the experiment, the survival rate of female *S. cameroni* was higher than that of *M. zaraptor*. The time of mortality *of S. cameroni* in a study by Morgan et al. (1989) was 3.0, 8.77 and 11.4 days for 50%, 90% and 95% of the parasitoids, respectively. This is in contrast to our study, in which the mortality of the *S. cameroni* females tested was only observed on Day 4 and on the last day of experimentation. For *M. zaraptor*, mortality was influenced by the age of the females from Day 8 to Day 14 of the experiment. This is partially consistent with Coats' study where adult survival decreased after Day 11 (Coats, 1976).

The emergence of house flies was higher in the presence of *M. zaraptor* than *S. cameroni*, according to the parasitisation ratio. The highest percentage of house fly emergence for *M. zaraptor* was recorded in the last days of the experiment, corresponding to the lower parasitisation. In *S. cameroni*, the emergence of house flies was constant over the whole period as far as the parasitisation trend is concerned. In both species, the presence of female parasitoids had reduced the emergence of house flies compared with the control. Pupal parasitoids may be effective in suppressing the fly population in biological control programmes because of their ability to reduce the population of house flies. The true potential of parasitoids as biological control agents can be assessed after field trials (Malik et al., 2007). The effectiveness of these parasitoids in the field differs from their performance under laboratory conditions because of environmental factors that influence parasitoid abundance and distribution (Skovgård & Nachman, 2004). Parameters such as sensitivity to insecticides, use of low-quality commercial colonies, microhabitat preferences, host availability, and lack of optimal timing and methods of release (Machtinger et al., 2015; Petersen & Meyer, 1985) must be taken into consideration. Furthermore, fly immigration from neighbouring livestock areas can rapidly increase fly populations (Machtinger et al., 2015). A monitoring programme should be established to assess fluctuations in fly populations, to aid decisions on when to implement additional pest management strategies and to evaluate the effectiveness of the pest management programme. Monitoring records can be maintained and used to anticipate increases in fly populations in subsequent years (Machtinger et al., 2015).

CONCLUSION

The main finding of this study is that *S. cameroni* showed higher parasitisation activity and survival compared with *M. zaraptor*. This has important implications for the selection of parasitoid species for the biological control of house flies. The results of our investigation indicate that female parasitoids exhibit a decline in their parasitisation capacity with each passing day. This implies that aged parasitoids produce fewer offspring over time. However, this decline did not result in a proportional increase in fly emergence compared with the control group. A reduction in flies was observed, but with significant variations between days. These results emphasise the need to consider not only the age of female parasitoids, but also the period of observation, as such dynamics may influence the overall effectiveness of fly population management. In addition to the age-related dynamics of female parasitoids, it was

found that the sex ratio favours females relative to males. Despite a decline in female parasitisation ability over time, the predominance of females may still be significant for managing the emerging fly population. Further research should investigate the complex relationship between sex ratio, age of female parasitoids and their effectiveness, taking into account other individuals. Investigating intra- and interspecific competition dynamics, as well as the potential influence of males on these factors, would be beneficial. Investigating how parasitoid interactions with other individuals, of the same or different species, influence their efficacy in regulating the house fly population may provide a more comprehensive picture.

Currently, there is a lack of scientific evidence comparing the oviposition activities of *S. cameroni* and *M. zaraptor*, two key species that are extensively mass-reared for biocontrol purposes (Machtinger & Geden, 2018). Understanding the oviposition patterns of these species over time would allow strategic timing of parasitoid releases, minimising the risks of intra- and interspecific competition while optimising cost-effectiveness for farmers. In addition, many Italian farmers face problems such as receiving commercial bags of already hatched pupae with deceased parasitoids, as it emerged from a preliminary analysis on a consistent sample of commercially available bags on the Italian market (D'Arco, unpublished data). Knowledge of species-specific hatching rates over time could significantly mitigate such challenges and increase the effectiveness of biocontrol programs. This research could provide insights into optimising biological control strategies by considering not only the intrinsic characteristics of parasitoids, but also their interactions within a more global ecological context.

AUTHOR CONTRIBUTIONS

Sara D'Arco: Conceptualization (equal); data curation (lead); formal analysis (equal); software (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Elena Costi:** Conceptualization (lead); formal analysis (lead); methodology (equal); software (equal); visualization (equal); writing – review and editing (equal). **Letizia Prodi:** Data curation (equal); writing – review and editing (supporting). **Tutku Yatman:** Data curation (equal); writing – review and editing (supporting). **Lara Maistrello:** Funding acquisition (lead); supervision (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare not to have any competing interests regarding the publication of this work.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Median number of house flies emerging from pupae that were exposed to the pupal parasitoids *Muscidifurax zaraptor* and *Spalangia cameroni* (i.e. non-parasitised flies) over a 2-week period.

Table S2. Median number of house flies emerging from pupae that were exposed to the pupal parasitoids Muscidifurax zaraptor and in absence of parasitoid (control) (i.e. non-parasitised flies) over a 2-week period.

Table S3. Median number of house flies emerging from pupae that were exposed to the pupal parasitoids Spalangia cameroni and in absence of parasitoid (control) (i.e. non-parasitised flies) over a 2-week period.

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