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Bionomics of Peckia (Euboettcheria) Anguilla and Peckia (Euboettcheria) Collusor (Diptera: Sarcophagidae) in The Laboratory.

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Abstract

Flesh-flies are important mechanical vectors that cause myiasis in man and animals and they also play an important role in forensic entomology. Postmortem interval can be estimated using data available in the literature on the biology of the species. This study aims to elucidate the bionomics of *these two species* in order to provide preliminary data for medical, veterinary and forensic entomology analyses. We analyzed the larval stage durations (L1–L3), weight of the mature larvae (L3), L1-pupae stage duration, L3-pupae stage duration, pupal stage duration, L1–adult duration, adult emergence, atrophies and the viability of larvae and adults. The mean duration of the L1–adult of *Peckia anguilla* was 22.6 days and 21.8 days, in the first and second experiments. Mean lifespan for females and males was 75 and 69.6 days, respectively. The mean duration of the L1–adult of *Peckia collusor* was 25.9 days and 23.8 days, in the first and second experiments. Mean lifespan for females and males was 77.5 and 73.5 days, respectively. *Although the two species presented similar results in relation to their post-embryonic development, P. collusor showed an adult lifespan longer, laying 1983 larvae throughout the experiment, while P. anguilla depositing 2298 larvae.*

Introduction

Forensic entomology is the branch of science responsible for studying insects and other arthropods associated with criminal and civil events. Knowledge about the life cycle and feeding habits of these organisms provides useful information to estimate the postmortem interval (PMI), which can help to solve crimes (Oliveira-Costa et al 2001; Cherix et al 2012).

Named popularly as "flesh flies", the muscoid dipterans that belong to the Sarcophagidae family have a large diverse bionomics which gives them the ability to procreate in almost all regions of the world. The Neotropical Region comprises approximately 750 species, of which 270 are distributed throughout Brazil (Carvalho et al 2012). In Brazil, the genus *Peckia* appears in large numbers on carcasses of vertebrates, including human corpses (Oliveira & Vasconcelos 2010; Vasconcelos et al., 2013). The larvae of the species *Peckia (Euboettcheria) anguilla* (Curran & Walley, 1934) and *Peckia (Euboettcheria) collusor* (Curran & Walley, 1934) have a high affinity for excrement and decomposing organic matter. Therefore, the adults and immature samples of these species can be found colonizing carcasses (Byrd & Castner 2001; Carvalho & Linhares 2001; Barros et al 2008; Barbosa et al 2009). Despite being insects of great ecological importance, they can also act as causes of secondary myiasis and vectors of enteropathogens making them of medical-veterinary and sanitary importance (Greenberg 1973; Dias et al 1984; Leão et al 1996; Oliveira et al 2002).

Knowledge of the biology of these species is extremely important because of their close relationship with humans and this knowledge can be used to control the spread of these pathogen carriers. In addition, this knowledge provides support for forensic entomology and can be used in criminal investigations, such as at a crime scene or in cases of neglect of the elderly, children and the disabled (Benecke et al 2004). The comparison between bionomic data of species of the same genus and subgenus is essential for a good

interpretation of the results, since these insects, even coexisting in the habitat, may have specific ecological, morphological and biological characteristics.

This work aims to analyze the bionomics of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* through post-embryonic development, biotic potential, sex ratio and mean lifespan of adults in the laboratory, in order to provide primordial data for ecological, sanitary and medical-legal studies.

Materials And Methods

Colonies of *Peckia* (*Euboettcheria*) *anguilla* and *Peckia* (*Euboettcheria*) *collusor* were established from the adult samples collected on the campus of the Instituto Oswaldo Cruz (IOC / FIOCRUZ) (S 22° 51' 06" 43° 14' 27" W), a metropolitan area of Rio de Janeiro, Brazil, from August to December 2016. The flies were actively collected with the aid of Falcon tubes inside Shannon-type traps (da-Silva-Xavier et al 2015), containing carcasses of albino rats (*Mus musculus* L.) at the initial stage of putrefaction.

After collection, the insects were transported to the Laboratório de Entomologia Médica e Forense (LEMEF/IOC), where they were identified by a group-specific key (Carvalho & Mello-Patiu 2008) and kept in wooden cages (30 x 30 x 30 cm) coated with nylon mesh and conditioned in a climatic chamber regulated at a temperature of 27 ± 1 °C, relative humidity of 60 ± 10% and a 12h photoperiod. These muscoid dipterans received a saccharose solution of 80% and a putrefactive ground beef diet that also served to stimulate posture.

After larviposition, 400 neolarvas (L1), belonging to the first generation of *P.* (*E.*) *anguilla* and *P.* (*E.*) *collusor* were removed from the meat with the help of fine brushes (number zero) and transferred to plastic containers (5 x 7 cm) containing 2g / larvae of putrefaction ground meat. Two experiments were done with four replicates, containing 50 larvae each. The experiments were performed at intervals of two weeks between them in order to confirm the results obtained.

These containers were placed in larger containers (10 x 10 cm) containing vermiculite (substrate for pupariation) and were kept in a climatic chamber with the same temperature, relative humidity and photoperiod as the colonies. In order to collect information on the bionomics of these species, the mature larvae (L3) were then weighed on a precision scale in order to obtain a biomass mean. After this, the larvae were individualized and packed in test tubes containing 1/3 of vermiculite and capped with cloth and elastic. Thus, the duration and viability of its larval, pre-pupal, pupal and neolarva periods to adult could be recorded.

To study the biotic potential and lifespan of the species, three wooden cages (30 x 30 x 30 cm) containing 15 couples from the first four replicates of the tests were assembled. Ground beef in decomposition stage was offered daily to cages in order to record the number of L1 larvae laid by the females. These were recounted when they reached the L3 stage in order to record the viability of the postures. Mortality of males and females was also checked daily to generate their survival curves.

The survival curves for males and females were represented by the Weibull distribution model. This model shows if arthropods reared in laboratory are comparable with the expected survival curve to the wild ones. One of the advantages of using Weibull distribution for survival analysis is that, by estimating only two parameters, informations on both lifespan and type of survival curve are obtained (Sgrillo1982). Chi-square test was carried out to analyze the survival distribution of the insects in order to confirm if they followed the Weibull distribution model (Sgrillo 1982).

Results

The values of chi-square (0,3869 for males and 0,2093 for females of *P*. (*E*.) *anguilla*; 0,1722 for males and 0,1559 for females of *P*. (*E*.) *collusor*) showed a concordance between observed values and expected values, therefore, the survival distribution followed the Weibull model.

Peckia (*Euboettcheria*) *anguilla*

The larval viability found for larvae of *P*. (*E*.) *anguilla* at 27 °C in the first experiment was 86%, whereas in the second experiment it was 90% (Table 1). The mean duration of the larval period of this species in the first experiment was 11.8 ± 7.4 days (Table 2), ranging from 4 to 29 days. In the second experiment the duration of this period was 10.0 ± 6.4 days (Table 2), ranging from 3 to 24 days.

Table 1 Viability of immature stages of *Peckia* (*Eubottcheria*) *anguilla* (Diptera: Sarcophagidae) from the first experiment (F) and the second experiment (S) maintained under laboratory conditions (27 ± 1° C, 60 ± 10% RH and 12 hours photoperiod).

BIOLOGICAL CHARACTERISTICS	VIABILITY (%)
LARVAL STAGE (F) LARVAL STAGE (S) L1-L3	86 90 90.7
PUPAL STAGE (F) PUPAL STAGE (S)	43.6 53.3
L1 TO ADULT (F) L1 TO ADULT (S)	37.5 48

Table 2 Duration of immature stages of *Peckia* (*Euboettcheria*) *anguilla* (Diptera: Sarcophagidae) from the first experiment (F) and the second experiment (S) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours of photoperiod).

BIOLOGICAL CHARACTERISTICS	DURATION (DAYS)	
	MEAN ± SD	RANGE
LARVAL STAGE (F) LARVAL STAGE (S)	11.8 ±7.4 [a] 10 ± 6.4 [b]	4 – 29 3 – 24
L1-PUPAE (F) L1-PUPAE (S)	13.3 ± 0 [a] 11,1 ± 0 [a]	5 - 30 4 - 25
L3-PUPAE (F) L3-PUPAE (S)	1.4 ± 0.48 [a] 1.1 ± 0.87 [b]	1 – 5 1 – 11
PUPAL STAGE (F) PUPAL STAGE (S)	13.3 ± 2.9 [a] 14 ± 2.2 [a]	3 – 17 12 – 19
NEOLARVAE TO ADULT (F) NEOLARVAE TO ADULT (S)	22.6 ± 5.4 [a] 21.8 ± 4.4 [a]	11 – 39 19 – 34

SD: Standard deviation; Different letters in the columns represent statistical differences when applied to Tukey's Multiple Comparison Test of Means between the different temperatures in the same experiment.

The mean larval mass obtained in the first experiment was 94.4 ± 24.2 mg (Table 3), with a minimum of 17.8 and a maximum of 167.7 mg. In the second experiment the mean larval mass obtained was 88.1 ± 24.9 mg (Table 3), ranging from 21.4 mg to 163.0 mg. The L1 to pupal stage lasted on average 13.31 days in the first experiment and 11.19 days in the second experiment (Table 2).

Table 3 Mass (mg) of larvae L3 mature *Peckia* (*Euboettcheria*) *anguilla* (Diptera: Sarcophagidae) from the first experiment (F) and the second experiment (S) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

BIOLOGICAL FEATURES	MASS (mg)	
	MEAN ± SD	RANGE
L3 (FEMALE) (F)	97.5 ± 22.7 [a]	66 – 156,6
L3 (FEMALE) (S)	92.2 ± 17.5 [a]	44,9 – 133.5
L3 (MALES) (F)	102.9 ± 26.6 [a]	17.8 – 147
L3 (MALES) (S)	96.3 ± 23.5 [a]	35.6 – 148
MATURE LARVAE L3 (TOTAL) (F)	94.4 ± 24.2 [a]	17.8 – 167.7
MATURE LARVAE L3 (TOTAL) (S)		21.4 – 163
LARVAE DSD (F)	88.1 ±24.9 [a]	26.5 – 167.7
LARVAE DSD (S)	89.1 ± 22.5 [a]	21.4 – 163
LARVAE DNFD (F)	81.3 ± 27.7 [a]	85.1 – 139.2
LARVAE DNFD (S)	113.1 ± 24.5	-
	-	

DSD: They did not develop; **DNFD:** They did not finish the development; **SD:** Standard deviation; Different letters in the columns represent statistical differences when applied to Tukey's Multiple Comparison Test between the experiments.

After mature larvae L3 left the diet they took an average of 1.49 ± 0.48 days to begin the pupation process in the first experiment and 1.13 ± 0.87 days in the second experiment (Table 2). In the first experiment, the shortest time a mature larva took to start the pupation process was one day, while the longest was five days. In the second experiment, while some of the larvae also started the pupation process after only one day, others took up to 11 days.

Mature larvae that were unable to continue their development had a mean larval mass of 89.1 ± 22.5 mg (Table 3), with a minimum mass of 26.5 mg and a maximum mass of 167.7 mg in the first experiment, whereas in the second experiment they obtained 81.3 ± 27.7 mg (Table 3), with a minimum of 21.4 mg and a maximum of 163.0 mg. The larvae that initiated the emergency process, but did not finish it, were only present in Experiment 1. They obtained an average larval mass of 113.1 ± 24.5 mg (Table 3) in the first experiment, ranging from 85.1 to 139.2 mg.

The pupal viability was 43.6% in the first experiment and 53.3% in experiment 2 (Table 1). The mean duration of the pupal periods was 13.3 ± 2.9 days and 14.0 ± 2.2 days in the first and second experiments, respectively (Table 2). While in the first experiment, the minimum duration of this stage was 3 days and the maximum period was 23 days, in the second experiment, these values ranged from 6 days to 22 days.

In the neolarva period, the mean number of days obtained for the first experiment was 22.6 ± 5.4 days (Table 2), ranging from 15 to 41 days and with a total viability of 37.5% (Table 1). In the second experiment, this period averaged 21.8 ± 4.4 days (Table 2), with a minimum of 18 and a maximum of 35 days, with a total viability of 48.0% (Table 1). The gender ratio was 0.5 in both tests.

Atrophied specimens were observed only in the first experiment. A total of 18.9% of atrophy was recorded, male adults presented 14.7% of total atrophy and females 14.6% of that same atrophy (Table 4). In the case of males, two specimens presented atrophies on the wings and three specimens were totally atrophied (Table 5). The females only presented six fully atrophied specimens (Table 5).

Table 4 Percentage of atrophied males and females of *Peckia* (*Euboettcheria*) *anguilla* and *Peckia* (*Euboettcheria*) *collusor* from the first experiment (F) and the second experiment (S) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours of photoperiod).

SPECIES	EXPERIMENT	ATROPHY (%)			
		ATROPHIEDMALES	ATROPHIED FEMALES	SUS	TOTAL
Peckia (*Euboettcheria*) *anguilla*	(F)	14.7	14.6	5.0	18.9
Peckia (*Euboettcheria*) *collusor*	(F) (S)	5.3 13.7	10 22.6	0.6 0.7	8.2 19.4

SUS: Specimens with undetermined sex, not being able to identify it.

Table 5 Types of male and female atrophies of *Peckia* (*Euboettcheria*) *anguilla* and *Peckia* (*Euboettcheria*) *collusor* from the first experiment (F) and from the second experiment (S) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours of photoperiod).

SPECIES	EXPERIMENT	TYPES OF ATROPHY		
		ONLY ATROPHIED WINGS	TOTALLY ATROPHIED	TOTAL
Peckia (*Euboettcheria*)a*nguilla* *Peckia* (*Euboettcheria*) *collusor*	(F) (F) (S)	0 ♀ / 2 ♂ 0 ♀ / 0 ♂ 2 ♀ / 2 ♂	6 ♀ / 3 ♂ 7 ♀ / 4 ♂ 15 ♀ / 6 ♂	6 ♀ / 5 ♂ 7 ♀ / 4 ♂ 17 ♀ / 8♂

The maximum lifespan for male adults was 75.0 ± 17.7 days, and the minimum was 14.3 ± 10.7 days. The first death occurred on the 2nd day and the last occurred on the 95th day (Fig. 1). The maximum lifespan for females was 69.6 ± 8.6 days, and the minimum was 5.6 ± 3.5 days. The first recorded death occurred on the 2nd day and the last occurred on the 79th day (Fig 2).

During the experiment, 2298 larvae L1 were deposited, from day 8 to day 79. The number of larvae deposited per female ranged from 0.22 on the 18th day to 10.0 on the 79th day (posture peak) with a

mean of 2.92 larvae per female (Fig 3). Of these larvae, only 2085 developed until the L3 stage, so that the viability of L1-L3 was 90.7% (Table 1).

Peckia (*Euboettcheria*) *collusor*

The larval viability found for larvae of *P.* (*E.*) *collusor* at 27 °C in the first experiment was 75%, whereas in the second experiment it was 89% (Table 6). The mean duration of the larval period of this species in the first experiment was 11.3 ± 0.9 days (Table 7), with a variation of 6 to 13 days. In the second experiment the duration of this period was 6.3 ± 2.2 days (Table 7), ranging from three to 11 days.

Table 6 Viability of immature stages of *Peckia* (*Eubottcheria*) *collusor* (Diptera: Sarcophagidae) from the first experiment (1) and the second experiment (2) maintained under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

BIOLOGICAL FEATURES	VIABILITY (%)
LARVAL STAGE (1)	75
LARVAL STAGE (2)	89
L1-L3 (1)	85.1
PUPAL STAGE (1)	96.7
PUPAL STAGE (2)	74.7
L1 TO ADULT (1)	72.5
L1 TO ADULT (2)	66.5

Table 7 Duration of immature stages of *Peckia* (*Euboettcheria*) *collusor* (Diptera: Sarcophagidae) from the first experiment (1) and the second experiment (2) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours of photoperiod).

BIOLOGICAL FEATURES	DURATION (DAYS)	
	MEAN ± SD	RANGE
LARVAL STAGE (1)	11.3 ± 0.9	6 – 13
LARVAL STAGE (2)	6.3 ± 2.2	3 – 11
L1-PUPAE (1)	13.7 ± 0	1 – 15
L1-PUPAE (2)	8.5 ± 0	4 – 19
L3-PUPAE (1)	2.3 ± 2	1 – 12
L3-PUPAE (2)	3.1 ± 2.5	1 – 15
PUPAL STAGE (1)	12.5 ± 1.3	3 – 17
PUPAL STAGE (2)	14.8 ± 1.3	12 – 19
NEOLARVAE TO ADULT (1)	25.9 ± 3.6	11 – 39
NEOLARVAE TO ADULT (2)	23.8 ± 2.7	19 – 34

SD: Standard deviation; Different letters in the columns represent statistical differences when applied to Tukey's Multiple Comparison Test of Means between the different temperatures in the same experiment.

The mean larval mass obtained in the first experiment was 82.90 ± 6.70 mg (Table 8), with a minimum of 57.7 and a maximum of 94.9 mg. In the second experiment the average larval mass obtained was 84.90 ± 10.40 mg (Table 8), ranging from 25.3 to 150.1 mg. In the first experiment, L1 to pupa lasted on average 13.71 days, while in the second experiment it lasted 8.54 days (Table 7). The L3-pupae stage lasted 2.3 ± 2.0 days in the first experiment and 3.1 ± 2.5 days in the second experiment (Table 7).

Table 8 Mass (mg) of larvae L3 mature *Peckia* (*Euboettcheria*) *collusor* (Diptera: Sarcophagidae) from the first experiment (1) and the second experiment (2) kept under laboratory conditions (27 ± 1 ° C, 60 ± 10% RH and 12 hours photoperiod).

BIOLOGICAL FEATURES	MASS (mg)	
	MEAN ± SD	RANGE
L3 (FEMALE) (1) L3 (FEMALE) (2)	81.6 ± 6.8 [a] 84.7 ± 11 [b]	57.7 – 94.9 37.6 – 107.6
L3 (MALES) (1) L3 (MALES) (2)	84 ± 21 [a] 86.6 ± 9.7 [a]	63.3 – 94.1 60.4 - 119
MATURE LARVAE L3 (TOTAL) (1) MATURE LARVAE L3 (TOTAL) (2) LARVAE DSD (1) LARVAE DSD (2) LARVAE DNFD (1) LARVAE DNFD (2)	82.9 ± 6.7 [a] 84.9 ± 10.4 [a] 83 ± 4 [a] 89.6 ± 27.7 [b] 87.9 ± 0 [a] 88.3 ± 10.3 [a]	57.7 – 94.9 25.3 – 150.1 78.4 – 88.2 25.3 – 150.1 - 81 – 95.7

DSD: They did not develop; **DNFD:** They did not finish the development; **SD:** Standard deviation; Different letters in the columns represent statistical differences when applied to Tukey's Multiple Comparison Test between the experiments.

After mature larvae L3 left the diet they took an average of 2.34 ± 2.06 days to begin the pupation process in the first experiment (Table 7) and 3.19 ± 2.51 days in the second experiment (Table 7). In the first experiment, the shortest time that a mature larva took to start the pupation process was one day, while the longest was 12 days. In the second experiment while some larvae started the pupation process after only one day, others took up to 15 days.

Mature larvae that originated male adults had a larger mass than the L3 that originated females in both experiments. The mean L3 larval mass that originated male adults in the first experiment was 84.0 ± 21.0 mg (Table 8), with a variation of 63.3 to 94.1 mg and the L3 that gave rise to females was 81.6 ± 6.8 mg (Table 8), ranging from 57.7 to 94.9 mg. In the second experiment the mean L3 larval mass that

originated male adults was 86.6 ± 9.7 mg (Table 8), with a variation of 60.4 to 119.0 mg and of the L3 that gave rise to females the mean mass was 84.7 ± 11.0 mg (Table 8), ranging from 37.6 to 107.6 mg.

The mature larvae that were unable to continue their development had a mean larval mass of 83.0 ± 4.0 mg (Table 8), with a minimum of 78.4 mg and a maximum of 88.2 mg, in the first experiment, whereas in the second experiment the mean mass obtained was 89.6 ± 27.7 mg (Table 8), with a minimum of 25.3 mg and a maximum of 150.1 mg. The larvae that started the emergency process, but did not finish it, had a mean mass of 87.9 ± 0 mg in the first experiment (Table 8), without minimum and maximum, since there was only one specimen. In the second experiment, the mean larval mass for this category was 88.3 ± 10.3 mg (Table 8), with a minimum of 81 and a maximum of 95.7 mg.

The pupal viability was 96.7% in the first experiment and 74.7% in the second experiment (Table 6). The mean duration of the pupal period was 12.5 ± 1.3 days and 14.8 ± 1.3 days in the first and second experiments, respectively (Table 7). While in the first experiment, the minimum duration of this stage was three days and the maximum period was 17 days, in the second experiment, these values ranged from 12 days to 19 days.

In the neolarva period, the mean number of days obtained for the first experiment was 25.9 ± 3.6 days (Table 7), with a variation of 11 to 39 days and a total viability of 72.5% (Table 6). In the second experiment, this period averaged 23.8 ± 2.7 days (Table 7), with a minimum of 19 and a maximum of 34 days, presenting a total of 66.5% of viability (Table 6). The gender ratio was 0.5 and 0.6, in the first and second experiments, respectively.

The percentages of male and female adult atrophies that emerged were recorded, with 8.2% total atrophy in the first experiment and 19.4% in the second experiment (Table 4). In the first experiment, the male adults presented 5.3% of atrophy (Table 4), and four specimens were in a totally atrophied state (Table 5). The females of this experiment presented 10% of total atrophy (Table 4), and seven were totally atrophied (Table 5). In the first experiment, there was one atrophic specimen with undetermined sex, representing 0.6% atrophy in this category (Table 4).

The total percentage of male adults atrophied in the second experiment was 13.7% (Table 4), of which two had atrophied wings and six were totally atrophied (Table 5). The total percentage of female adults atrophied in the second experiment was 22.6% (Table 4), of which two also had atrophied wings and 15 were also totally atrophied (Table 5). There was an atrophied specimen whose sex could not be identified, so that the percentage of atrophy here was 0.7% (Table 4).

The maximum longevity of male adults was 77.5 ± 0.8 days (Fig 4), while for females it was 73.5 ± 7.5 days (Fig 5). The minimum longevity of male adults was 11.5 ± 9.3 days (Fig 4), while for females it was 6.2 ± 4.5 days (Fig 5). The first death of the adult males occurred on the 2nd day and the last occurred on the 79th day (Fig 4). The first death of the female adults was recorded on the 1st day, while the last was on the 79th day (Fig 5).

The results of the survival curves of *P.* (*E.*) *collusor* were concordant with the χ^2 test, as well as the expected results, allowing the survival curves to follow the Weilbull distribution model. In the experiments done with *P.* (*E.*) *collusor* at 27 °C the value of χ^2 for males was 0.1722 and for females it was 0.159. This difference was not considered significant.

Throughout the experiment 1961 L1 larvae were deposited from the 9th to the 71st day. The number of larvae deposited per female ranged from 0.2 on the 40th day to 7 on the 25th day (posture peak), with a mean of 2.36 larvae per female (Fig 6). Of these larvae, only 1668 were able to develop until the L3 stage, so that the viability of L1-L3 was 85.1% (Table 6).

Discussion

Bionomic studies have shown that knowledge about the post-embryonic development of muscoid dipterans is essential to determine the immature ages, especially larvae, as well as the biotic potential and longevity of adults. This is a very important step for forensic entomology, since the PMI can be estimated from this data (Catts & Goff 1992; Oliveira-Costa and Mello-Patiu 2004; Amendt et al 2000). The experiments were carried out in an ideal temperature range for insect development, which makes the data obtained in this work act as the comparative basis of the PMI calculation. Kamal (1958) cataloged the developmental time of the larval stages of 13 necrophagous species of Calliphoridae and Sarcophagidae under laboratory conditions at 27 °C with the same diet. This pioneering work was the basis for all other studies related to the forensic diptera bionomy.

Madubunyl (1986) developed bionomic studies with *Sarcophaga* (*Bercaea*) *africa* at 23-28 °C and obtained a larval viability of 80.69%. The bionomics of *Peckia* (*Squamatodes*) *trivittata* was analyzed by Salviano et al (1996) and these authors obtained a larval viability of 89.82%, at 27 ºC. The results obtained by these authors are similar to those found in the present study for *P.* (*E.*) *anguilla* in the first and second experiments (86 and 90%, respectively) and those found for *P.* (*E.*) *collusor* in the second experiment (89 %). Loureiro et al. (2005) found 99% larval viability in studies with *Peckia* (*Pattonella*) *intermutans* at this same temperature, but differing from all viabilities found for *P.* (*E.*) *anguilla* and *P.* (*E.*) *collusor*. In a study on the biology of Sarcophagidae, da-Silva-Xavier et al (2015) obtained a larval viability of 82% for *Peckia* (*Sarcodexia*) *lambens* and 76% for *Oxysarcodexia amorosa*, both maintained at 27 °C. The value found for *P.* (*E.*) *collusor* in the first experiment (75%) resembles the value found for *P.* (*S.*) *lambens* by da-Silva-Xavier et al (2015).

The results obtained for the duration of the larval period of *P.* (*E.*) *anguilla* and *P.* (*E.*) *collusor* in the two experiments differ from those found in most of the literature on the subject. Oliveira-da-Silva et al (2006) when developing bionomic studies at the Adolpho Ducke forest reserve in Manaus found that the duration of the larval period of *Peckia* (*Pattonella*) *smarti* at 26 °C was 2.4 days in the rainy season and 2.04 days in the dry season at 26.6 °C. At this same location, the authors found 2.25 days of the larval

period of *Peckia* (*Pattonella*) *pallidipilosa* at 26 ºC in the rainy season and 2.1 days in the dry season at 26.6 ºC. Salviano et al (1996) reported that there are significant biological differences, including the time of their development, among the Sarcophagidae species bred in the laboratory to those that develop in their natural environment. Until this date, no experiments have been performed in the field with the species studied in the present study, as well as laboratory experiments with the species studied by Oliveira-da-Silva et al (2006). Because of this, it became impossible to compare these data. However, the results found in both experiments differ from most bionomic studies performed under laboratory conditions.

The values found for the larval period of the species studied in this study can be considered high when compared with other species of the same family analyzed at 27 ºC. Da-Silva-Xavier et al. (2015) obtained 3.51 days of larval period of *P*. (*S*.) *lambens* and *O*. *amorosa,* similar to the 3.7 and 3.9 days found by Ferraz (1995) in her studies of the bionomics of *Peckia* (*Peckia*) *chrysostoma* at 25.9 and 27 °C, respectively. The duration of this period found by Salviano et al (1996) for *P*. (*S*.) *trivittata* was 4.2 days, while Loureiro et al (2005) reported 5.7 days in their studies with *P*. (*P*.) *intermutans.* Nassu et al (2014), analyzing the post-embryonic development of *Microcerella halli*, obtained a mean duration of the larval period of 6.5 days at 25 °C and 6 days at 30 °C, values very similar to the 6.31 days obtained in the second experiment of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* at 27 °C, respectively. It is interesting to note that Nassu et al (2014) also analyzed the development of *Sarcophaga* (*Liopygia*) *ruficornis*, at 25 ºC and 30 °C, and obtained 4.6 and 4.5 days, respectively. The results of Nassu et al. (2014) are close to the other development studies of the genus *Peckia* but differ from the results obtained for *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor*, showing that the post-embryonic development of Sarcophagidae differs even within a genus.

Although the values obtained for the mean larval mass of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* differed from each other, these results were the ones that came closest. da-Silva-Xavier et al (2015) found 33.67 mg for *P*. (*S*.) *lambens* and 28.28 mg for *O*. *amorosa* but they did not observe any significant values to distinguish males from females by larval mass. Loureiro et al (2005) obtained for *P*. (*P*.) *intermutans* 195.63 mg of general mass, while Salviano et al (2006) obtained for *P*. (*S*.) *trivittata* 257 mg for males and 238 mg for females. The larvae that originated male adults of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* had a mean weight higher than the larvae that originated female adults, similar to that observed by Salviano et al (1996). According to Slansky & Scriben (1985), adult insect size and body mass are the two main factors that influence its performance. Size also intervenes directly on mating behavior and dispersion. Body mass reveals the amount of energy and nutrients stored. The two factors together can influence the final fecundity of adult flies, which makes it necessary to include this type of information in bionomic studies. In the work of da-Silva-Xavier et al (2015) the larvae with body mass below 22 mg of *P*. (*S*.) *lambens* completed the pupation but the adult insects did not emerge, which can be considered as a limiting factor for adult development of this species. In the present study, similar results were obtained only in the second experiment of *P*. (*E*.) *collusor*, where the lowest larval body mass found was 25.3 mg, whose larva completed its pupation, but did not emerge. In the second experiments with *P*. (*E*.) *anguilla* at 27 ºC a larva presented 17.8 mg of larval body mass, differing from the mean of larval body mass, but

was able to continue its development, giving rise to a male adult insect. In relation to the species *O. amorosa*, da-Silva-Xavier et al. (2015) did not observe a limiting weight, since adults emerged from pupae with a minimum weight of 14 mg. This finding was also observed in the experiments performed with *P.* (*E.*) *collusor*, the lowest value found for the body mass of an L3 was 60.4 mg, but this was able to carry out the pupation process, develop and generate an adult without anomalies.

Although Fraenkel & Bhaskaran (1973), Cepeda-Palacios & Scholl (2002), Barros-Cordeiro et al (2010), Barros-Cordeiro et al (2014), Nascimento et al (2014) and Flissak & Moura (2018) conducted studies on terms and definitions related to the intra-puparial development of muscoid dipterans, there are many gaps to be filled in this area. To date, the literature of the post-embryonic development of sarcophagids does not take into account the pre-pupal period, making an in-depth interpretation of the data found in the present study impossible. The values found for the pre-pupae period of *P.* (*E.*) *anguilla* and *P.* (*E.*) *collusor* exemplify the need to include this data in bionomics studies because they directly influence the duration time of the complete life cycle of the insect. Oliveira-da-Silva et al (2006) developed bionomic studies in the Adolpho Ducke forest reserve in Manaus found a mean duration of the posture period up to the pre-pupa of 4.58 days for *Peckia* (*Pattonella*) *smarti* during the rainy season at 26.6 °C, and 3.82 days during the dry season at 26 °C. For *Peckia* (*Pattonella*) *pallidipilosa* the average duration of the posture period until the pre-pupae was 3.77 days in the rainy season and 5.27 days in the dry season. Although they recognized this period, it was not considered, thus pointing out the lack of importance attributed to it. In addition to contributing to a better data collection on the pupae of these dipterans, as well as their general biological aspects, the inclusion of the duration of this period in biological studies may be of prime importance for a better estimation of PMI.

The pupal viability of *P.* (*E.*) *collusor* in the first experiment (96.7%) was similar to the pupal viabilities obtained by Salviano et al (1996) for *P.* (*S.*) *trivittata* (92.75%), by Madubunyl (1986) for *S.* (*B.*) *africa* (89.83%) and by Loureiro et al (2005) for *P.* (*P.*) *intermutans* (86.7%). The pupal viability of *P.* (*E.*) *anguilla* in both experiments (43.6% and 53.3% in the first and second experiments, respectively) approached the results obtained by Ferraz (1995) in her studies with *P.* (*P.*) *chrysostoma* (69.9%) and those obtained by da-Silva-Xavier et al (2015) for *P.* (*S.*) *lambens* (65.24%).

The mean pupal period of *P.* (*E.*) *anguilla* and *P.* (*E.*) *collusor* corroborates with the observations of several other authors, who affirm that the duration of the pupal period of dipterans of the family Sarcophagidae, in temperatures close to 27 ° C, ranges from 10 to 20 days (Nishida 1984; Ferraz 1995; da-Silva-Xavier et al 2015). The mean duration was 13.3 ± 2.9 days and 14 ± 2.2 days for *P.* (*E.*) *anguilla* and 12.5 ± 1.3 days and 14.8 ± 1.3 days for *P.* (*E.*) *collusor*, in the first and second experiments, respectively; these results are close to the values observed by Salviano et al (1996) for *P.* (*S.*) *trivittata* (15.7 days), by Loureiro et al (2005) for *P.* (*P.*) *intermutans* (13.87 days), by Oliveira-da-Silva et al. (2006) for *P.* (*P.*) *smarti* (17.93 days) at 26.6 °C and for *P.* (*P.*) *pallidipilosa* (15.87 days) at 25.9 °C, and by Nassu et al (2014) for *S.* (*L.*) *ruficornis* (12 days) and *M. halli* (14 days). The duration of the pupal stage found for *P.* (*E.*) *anguilla* and *P.* (*E*) *collusor* differ from that obtained by da-Silva-Xavier et al. (2015) for *P.* (*S.*) *lambens* (8.26 days). Although the values found differ from those seen by Ferraz (1995) for *P.* (*P.*)

chrysostoma at 18 °C (23.5 days), the values are still predicted for the variation of days of the average pupal period of the Sarcophagidae family (da- Silva-Xavier et al 2015).

The results of da-Silva-Xavier et al. (2015) for *P*. (*S*.) *lambens* viability from the neolarva period to adult was 54.5%, similar to the results obtained for *P*. (*E*.) *anguilla* in both our experiments (37.5 and 48%, in the first and second experiments, respectively). The mean duration of the adult neolarva period found for *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* in the present study was higher than that observed by Loureiro et al (2005) for the neolarva time to adult for *P*. (*P*.) *intermutans*, which ranged from 17-20 days, under the same conditions of temperature and humidity. Still within the genus *Peckia*, Gomes et al. (2003) for *P*. (*P*.) *chrysostoma*, also under the same conditions of temperature and humidity, reported a mean period of neolarva to adult of 19.33 ± 1.59 days. The mean age of adult neolarva obtained by da-Silva-Xavier et al (2015) for *P*. (*S*.) *lambens* was 11.53 days, with a minimum of nine and a maximum of 15 days. This suggests that the post-embryonic developmental period of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* is larger than that of other species of the genus *Peckia.* This is also observed when the results obtained for these species are compared with other species of Sarcophagidae, such as *S*. (*L*.) *ruficornis* and *O*. *amorosa.* Nassu et al (2014) found 17.5 days of total post-embryonic development time for *S*. (*L*.) *ruficornis*, while da-Silva-Xavier et al (2015) obtained 13.6 days for *O*. *amorosa*. The species that presented a period of neolarva to the adult similar to that of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* was *M*. *halli.* Nassu et al. (2014) obtained a total post-embryonic development time of 21.8 days for this species.

The results found for the gender ratio of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* (0.5-0.6, respectively) resemble those found by Salviano et al (1996) for imagos from larvae of 100-199 mg for *P*. (*S*.) *trivittata.* The average lifespan of female adults of *P*. (*E*.) *anguilla* presented greater longevity than males. This result differs from that observed by Salviano et al (1996) for *P*. (*S*.) *trivittata*, where male longevity at 27 °C was higher than that of females (14.7 days). The longevity between species of Sarcophagidae differ greatly between them. For *P*. (*E*.) *collusor*, for example, there was no statistically significant difference between the mean longevity of male and female adults. Under the same conditions of temperature and humidity as the present experiment, Salviano et al (1996) obtained a mean longevity for *P*. (*S*.) *trivittata* of 11.9 ± 1.1 days for females and 14.7 ± 1.3 days for males. While, da-Silva-Xavier et al. (2015) observed a duration of 39.33 days for females and 57.33 days for males of *P*. (*S*.) *lambens* and 83.66 days for females and 84 days for males of *O*. *amorosa.* The longevity results of male and female adults of *P*. (*E*.) *collusor* suggest that this species adapts to laboratory conditions better than *P*. (*E*.) *collusor*. Unlike *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* all the cited species obtained a longer longevity in males than in females. Ferraz (1995) obtained a longer longevity of females of *P*. (*P*.) *chrysostoma*, similar to that obtained in the present study. The greater longevity of females observed in this study and in the study of Ferraz (1995) do not corroborate the affirmation of Salviano et al (1996), who justified a lower longevity of females in relation to males, due to the ovarian development. On the other hand we believe that males expend a lot of energy at the time of copulation and consequently have a lesser longevity.

Throughout the experiment, females of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* deposited a total of 1326 and 1983 larvae in ground meat, respectively. Values similar to those found by da-Silva-Xavier et al (2015) for *P*. (*S*.) *lambens* (1433). These same authors also reported 4781 larvae were deposited for *O*. *amorosa.* The females of *O*. *amorosa* had a much higher longevity than *P*. (*E*.) *anguilla*, which would explain the greater number of deposited larvae. The longevity of *O. amorosa* females was similar to that found for females of *P*. (*E*.) *collusor*, but the latter deposited a smaller number of larvae. These results corroborate the high biotic potential of *O*. *amorosa* under these laboratory conditions. The posture peak of *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor*, was 3.7 and 6.4 days, respectively, suggesting a greater preference of *P*. (*E*.) *collusor* in depositing its larvae in ground beef. On the other hand da-Silva-Xavier et al (2015) found a posture peak of 10.17 larvae per female of *P*. (*S*.) *lambens*, showing that this species has a higher biotic potential, since more larvae were deposited in less time (39.33 days). It is interesting to note that *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor* in our study and *P*. (*S*.) *lambens* and *O*. *amorosa* (da-Silva-Xavier et al 2015) had a range of 7 to 11 days before the first larviposition occurred, and ground beef in putrefaction was offered to the four species from the first day of the experiment. This interval may be a period in which females utilize the protein present in the meat for ovarian maturation or sexual maturation.

The results of this study have added to the knowledge of the species *P*. (*E*.) *anguilla* and *P*. (*E*.) *collusor*. These species have potential sanitary and forensic importance, since they have already been found on vertebrate carcasses. Moreover, these species reproduce easily on decomposing animal substrates (Barros et al 2008; Yepes-Gaurisas et al 2013). Besides *P*. (*E*.) *collusor* has already been found colonizing corpses, there are no reports in the literature that prove the larviposition of larvae of *P*. (*E*.) *anguilla* directly on human cadavers. On the other hand, this does not mean that the species does not colonize corpses or animal carcasses; moreover, their breeding and feeding habits have not yet been fully studied (Vanin et al 2011). As this species has a preference for rural regions and closed forests, *P*. (*E*.) *anguilla* could be an important indicator of the location of the crime and any movements of a corpse, if its forensic importance is proven (Dias et al 1984).

Declarations

Authors' contribution

Raquel Fernandes Silva Chagas do Nascimento - execution of all work.

Alexandre da Silva Xavier - revision and writing of the work.

Lorrane de Andrade Pereira - Bionomy bioassay.

Carlos Manuel Dutok Sánchez – statistic and revision of the work.

Margareth Maria de Carvalho Queiroz - planning, supervision and writing of the work.

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Figures

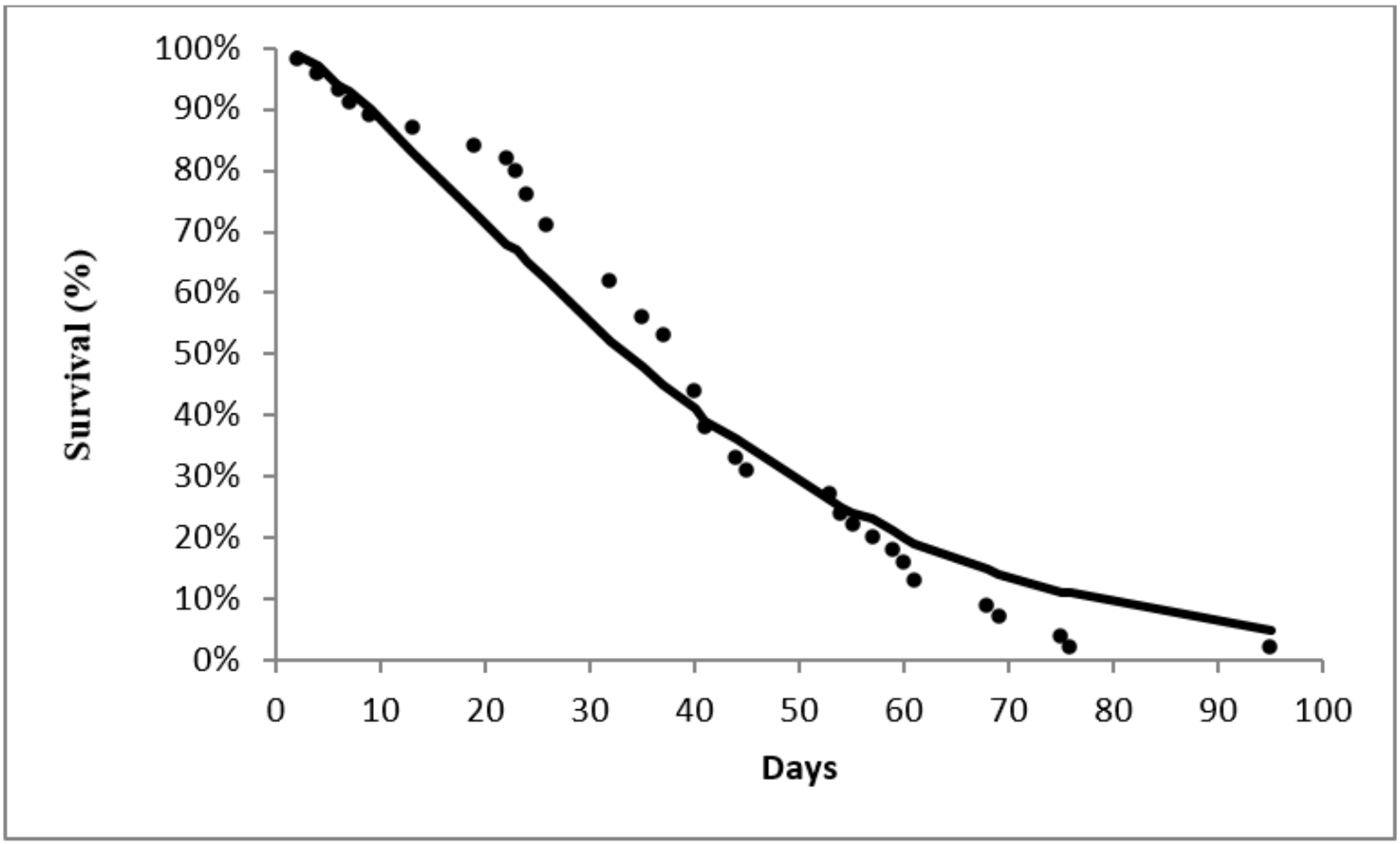


Figure 1

Survival curve following the Weilbull distribution model with the observed results (points) and the expected results (line) for male adults of Peckia (Euboettcheria) anguilla (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

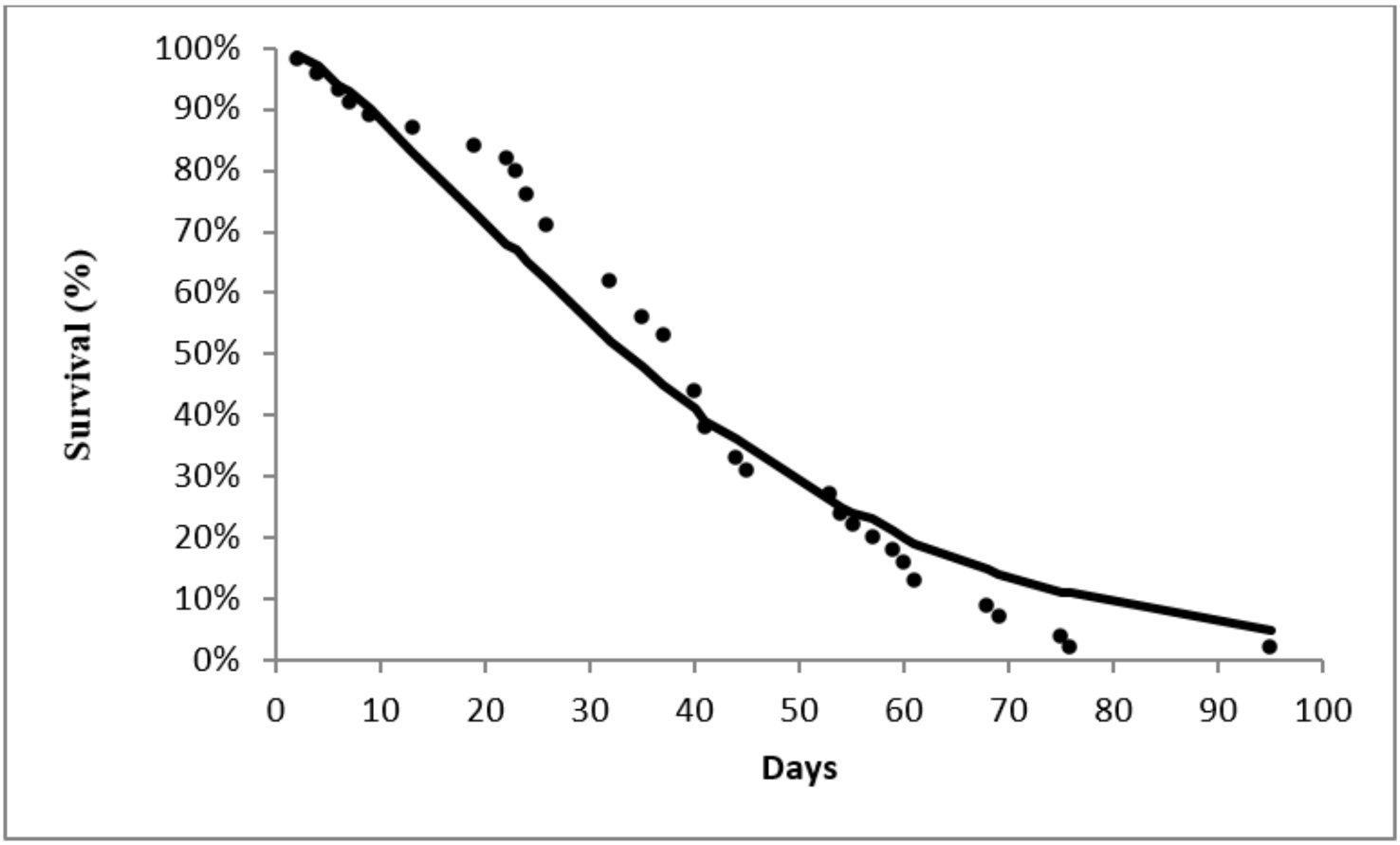


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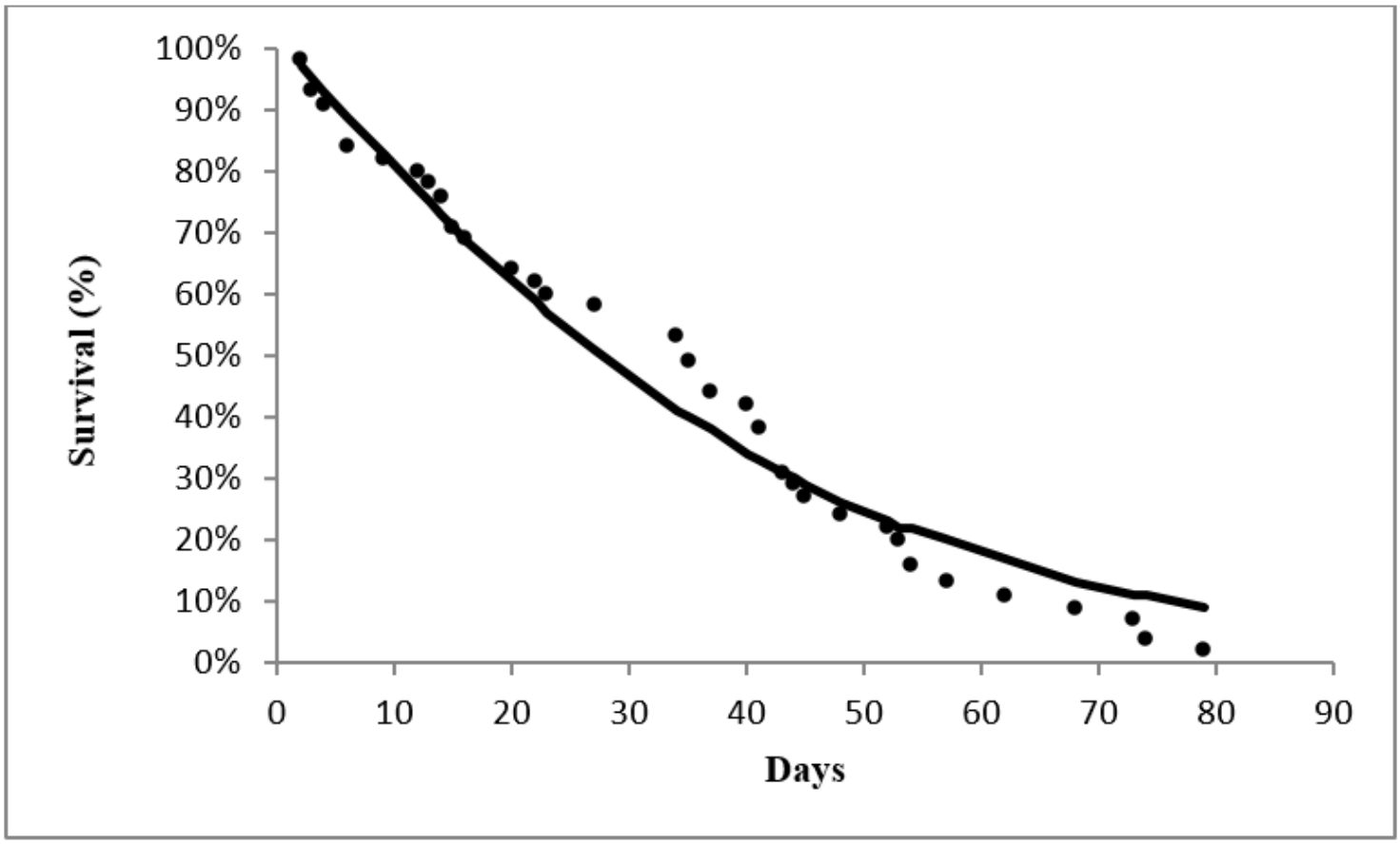


Figure 2

Survival curve following the Weilbull distribution model with the observed results (points) and the expected results (line) for female adults of Peckia (Euboettcheria) anguilla (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

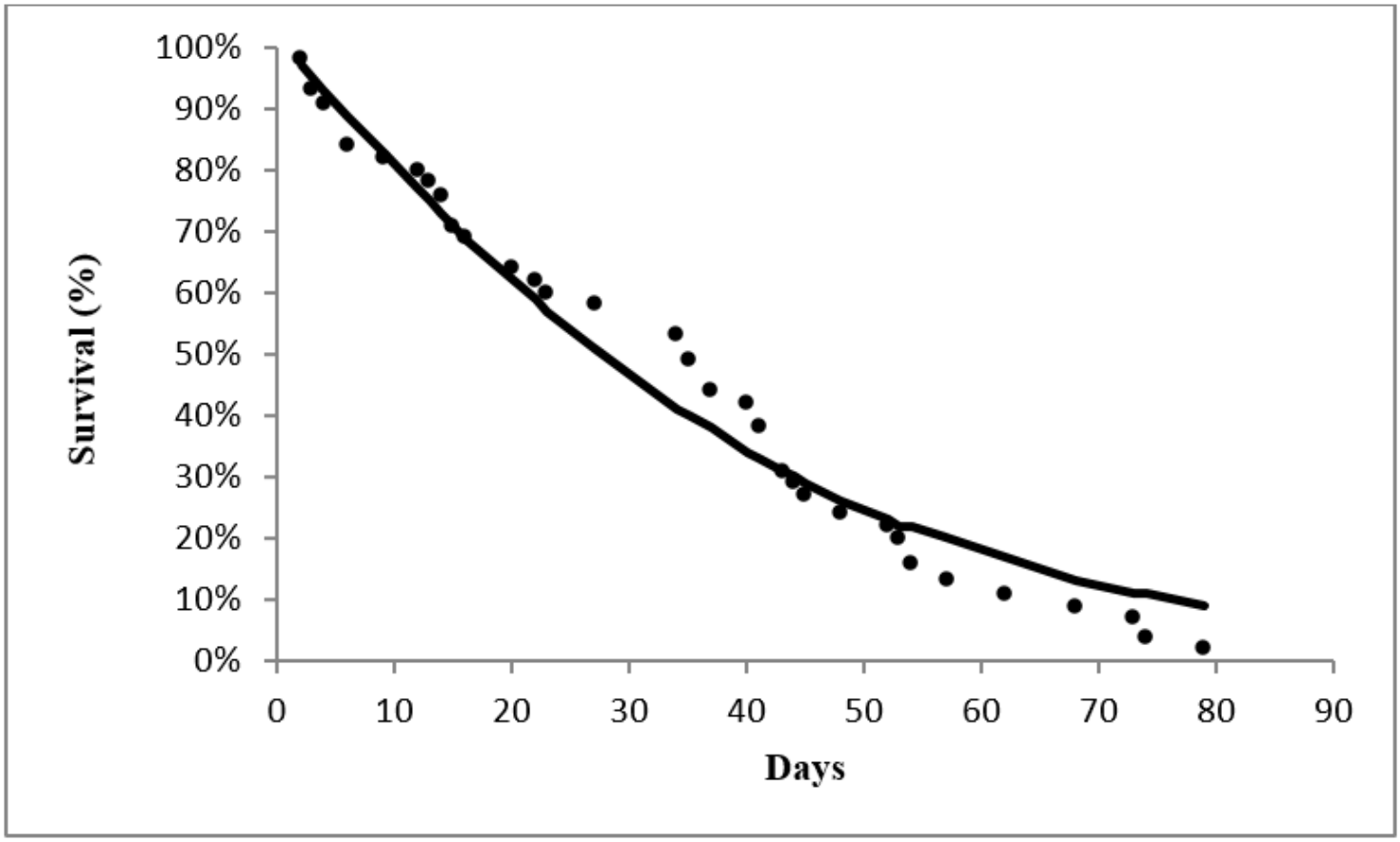


Figure 2

Survival curve following the Weilbull distribution model with the observed results (points) and the expected results (line) for female adults of Peckia (Euboettcheria) anguilla (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

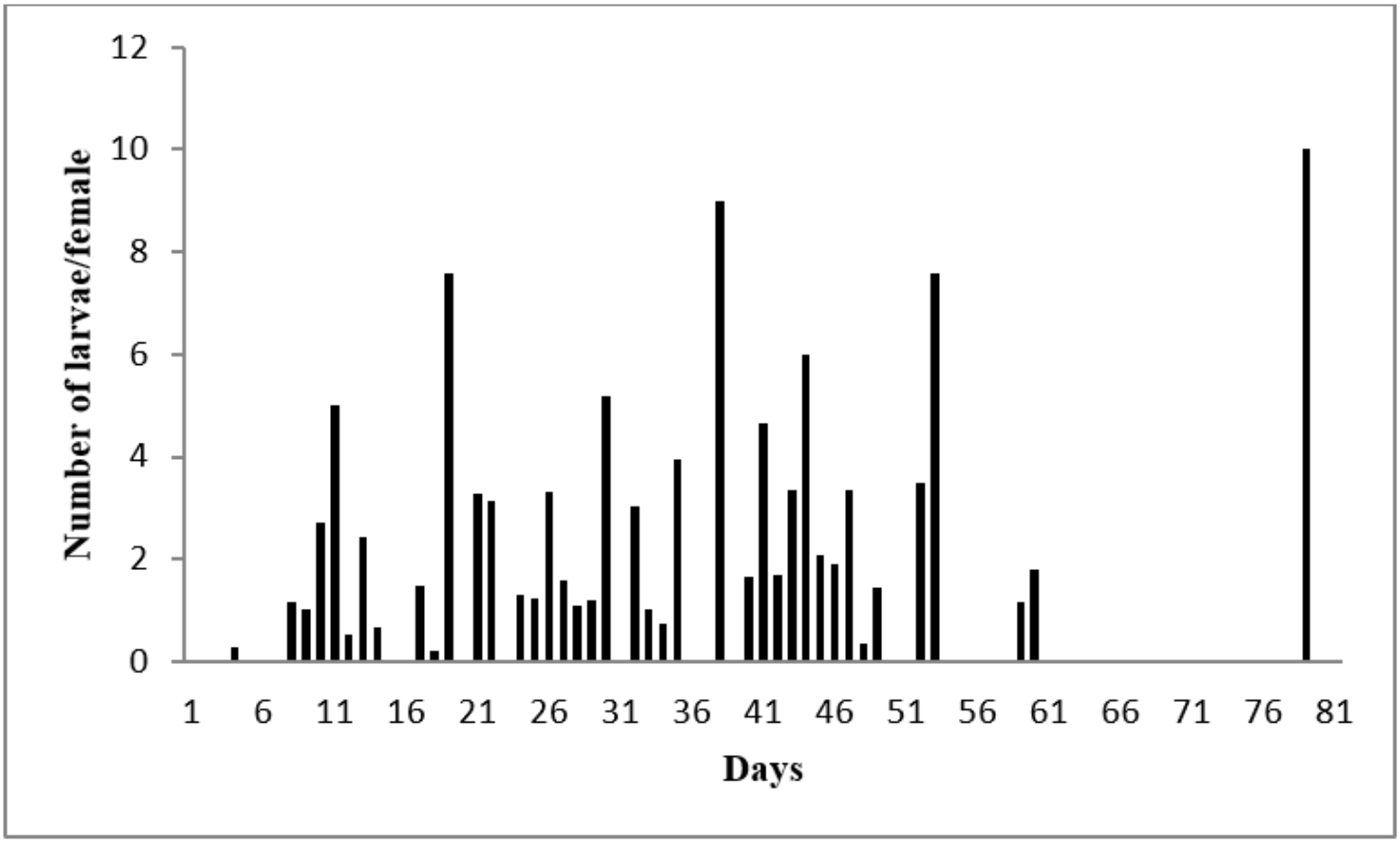


Figure 3

Number of larvae deposited in ground beef at initial stage of decomposition divided by the number of females of Peckia (Euboettcheria) anguilla (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

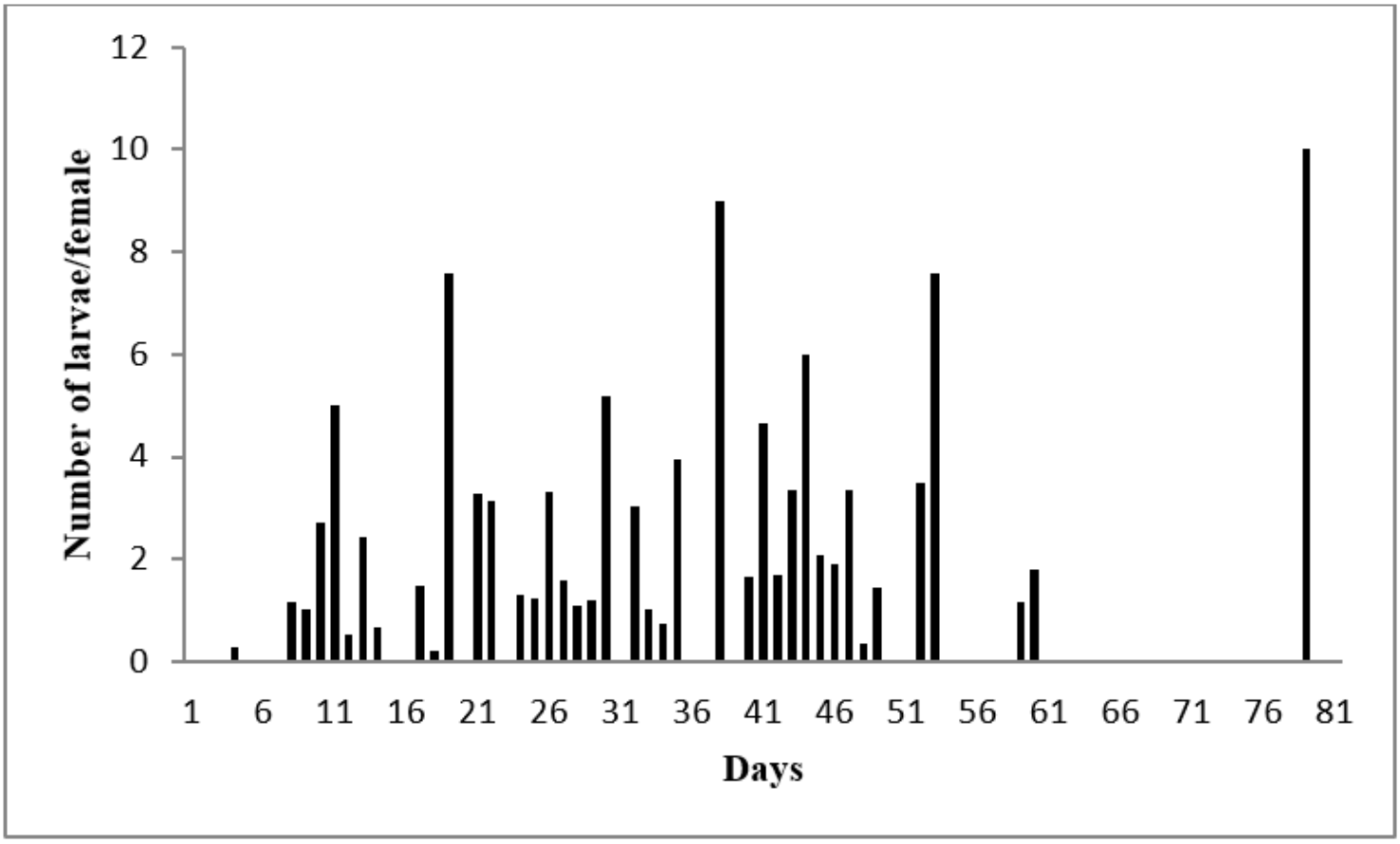


Figure 3

Number of larvae deposited in ground beef at initial stage of decomposition divided by the number of females of Peckia (Euboettcheria) anguilla (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

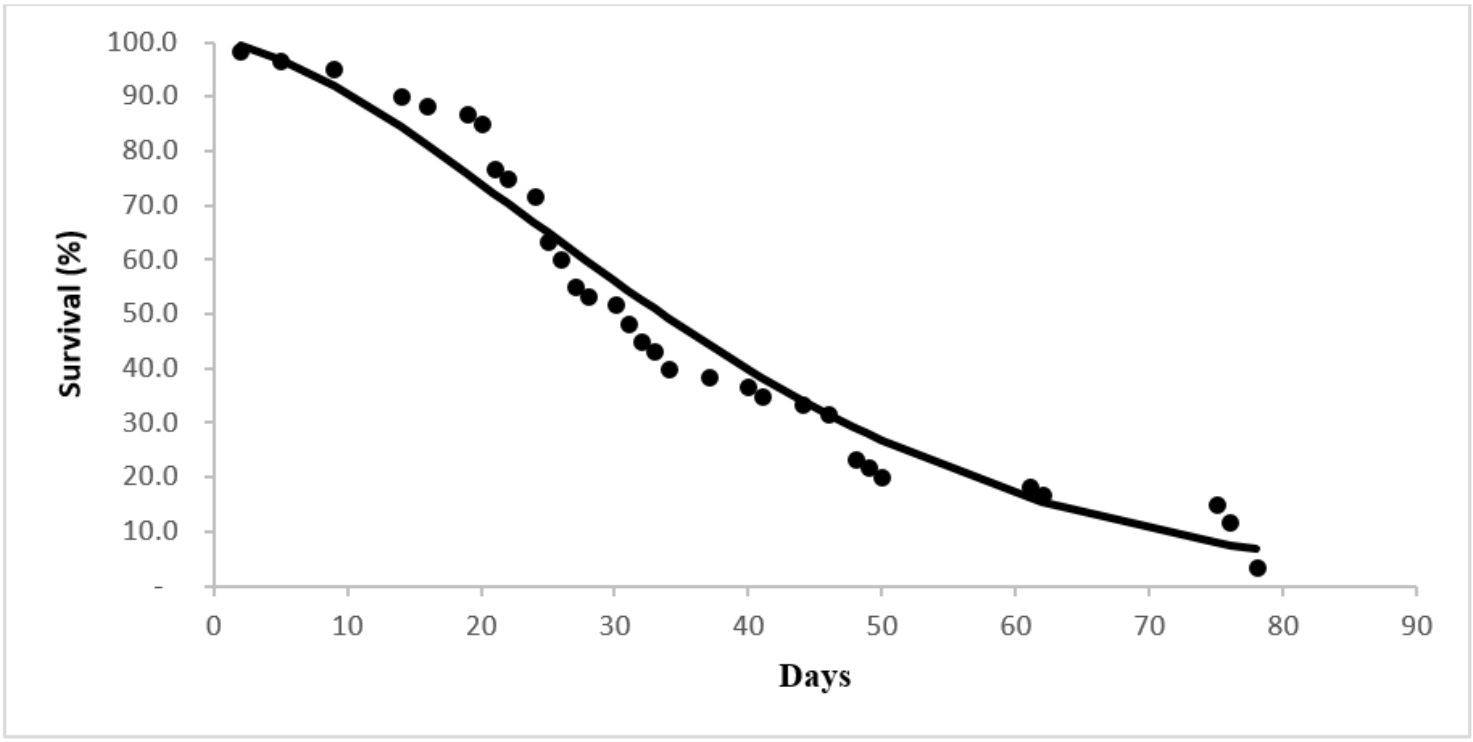


Figure 4

Survival curve following the Weilbull distribution model with the observed results (points) and the expected results (line) for male adults of Peckia (Euboettcheria) collusor (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

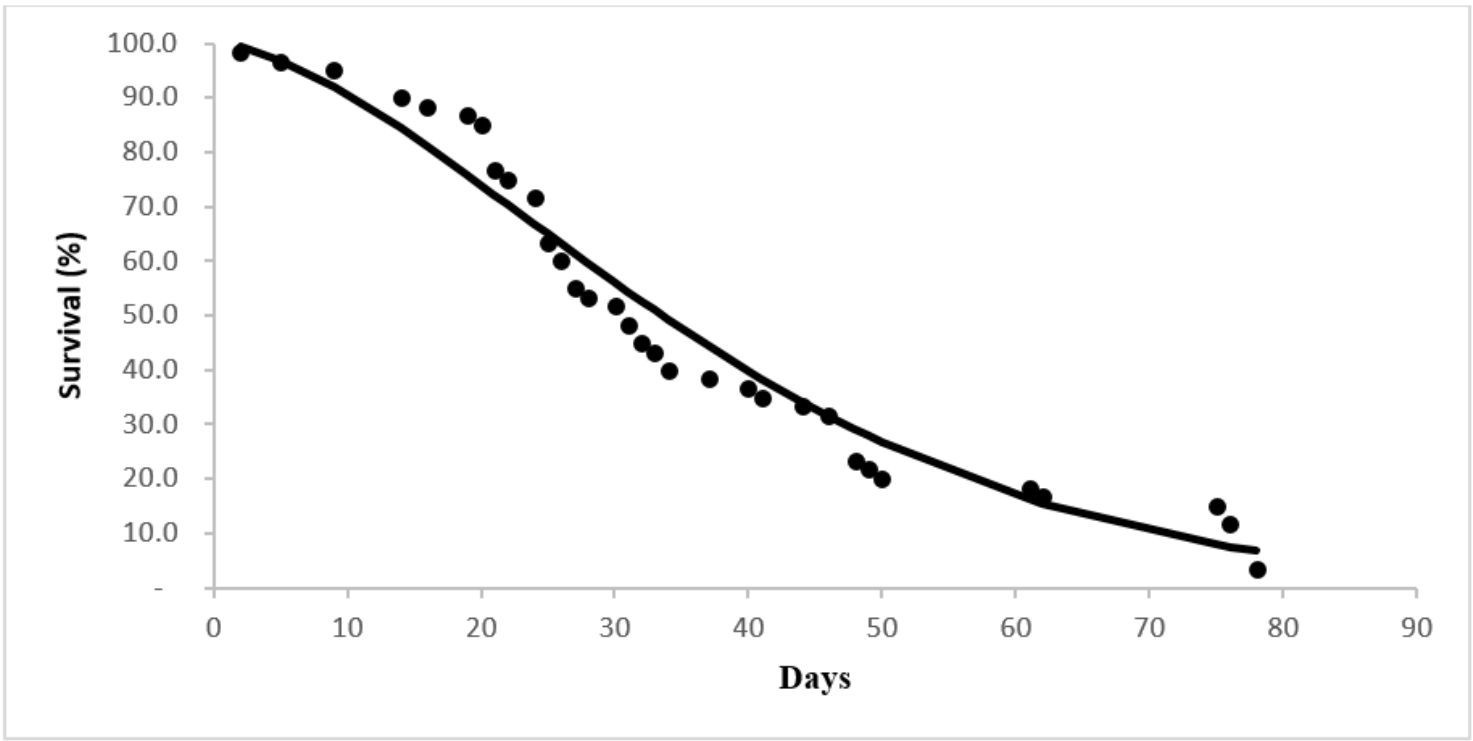


Figure 4

Survival curve following the Weilbull distribution model with the observed results (points) and the expected results (line) for male adults of Peckia (Euboettcheria) collusor (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

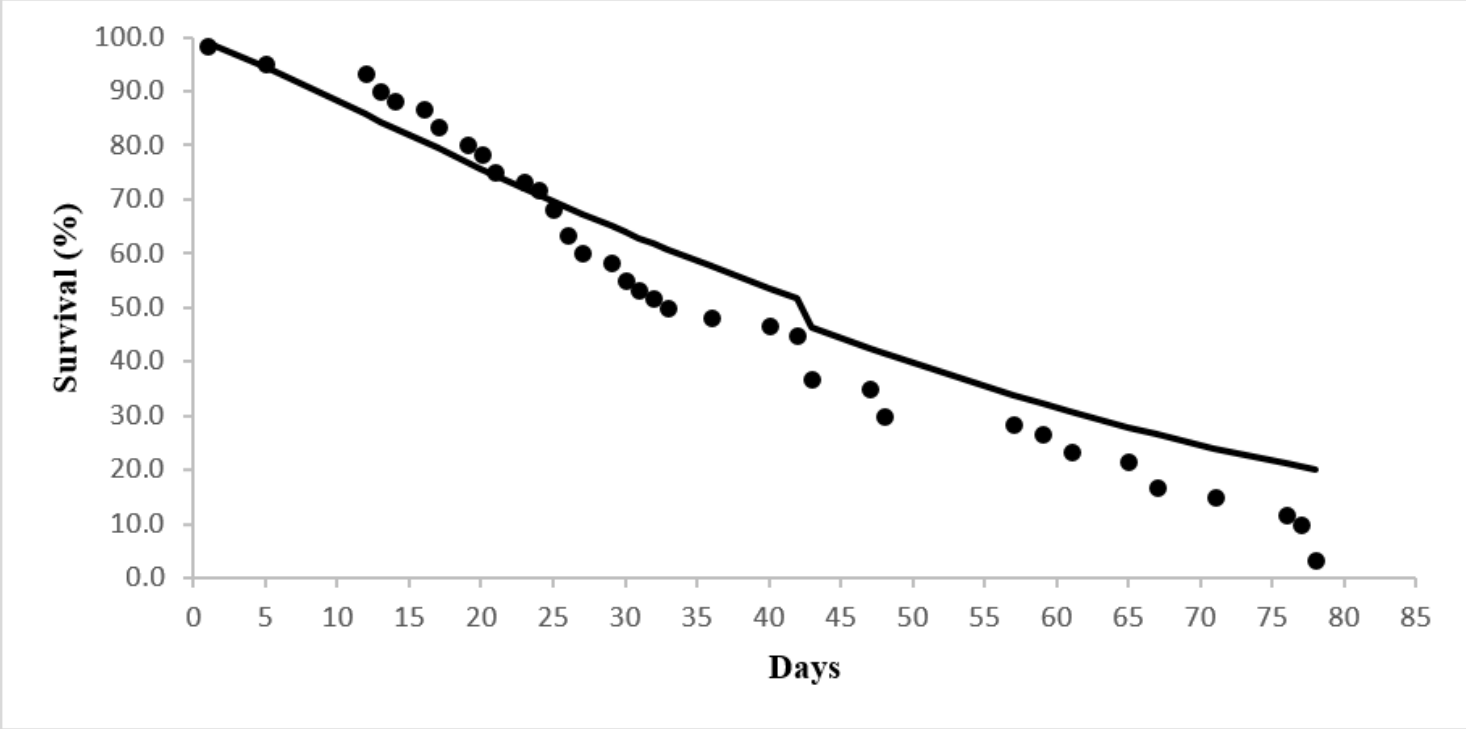


Figure 5

Survival curve following the Weilbull distribution model with the observed results (points) and the expected results (line) for female adults of Peckia (Euboettcheria) collusor (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 ° C, 60 ± 10% RH and 12 hours photoperiod).

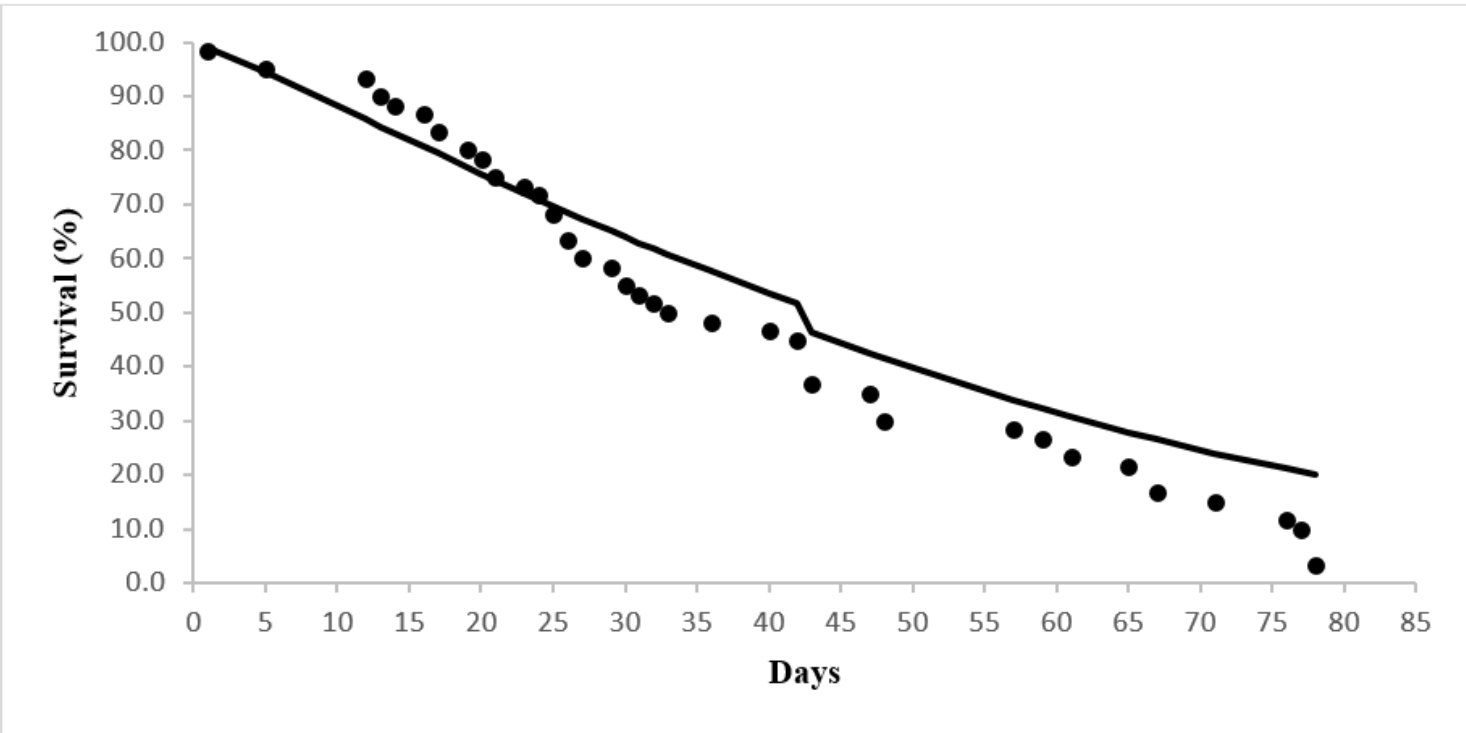


Figure 5

Survival curve following the Weilbull distribution model with the observed results (points) and the expected results (line) for female adults of Peckia (Euboettcheria) collusor (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 ° C, 60 ± 10% RH and 12 hours photoperiod).

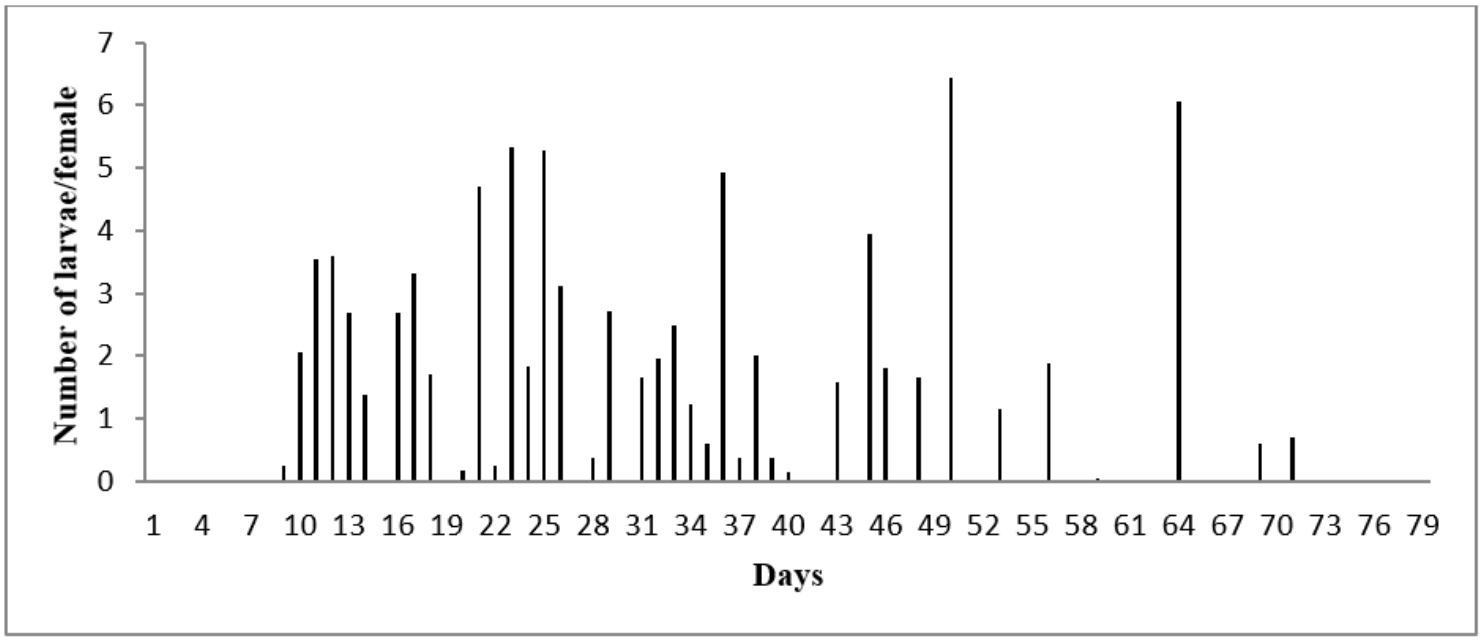


Figure 6

Number of larvae deposited on ground meat at initial stage of decomposition divided by the number of females of Peckia (Euboettcheria) collusor (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).

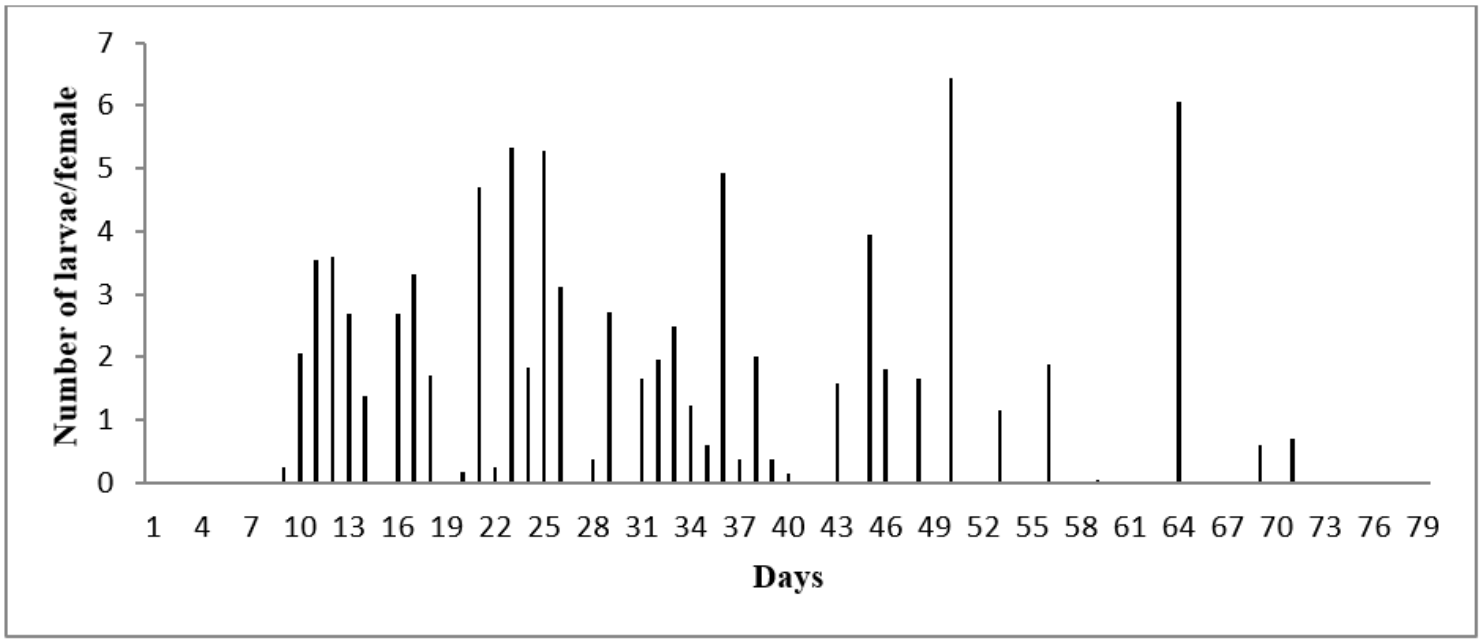


Figure 6

Number of larvae deposited on ground meat at initial stage of decomposition divided by the number of females of Peckia (Euboettcheria) collusor (Diptera: Sarcophagidae) kept under laboratory conditions (27 ± 1 °C, 60 ± 10% RH and 12 hours photoperiod).