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Essential elements of biology, mating behavior, and reproduction of *Zelus pedestris* Fabricius, 1803 (Hemiptera: Reduviidae) fed on *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae)

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Abstract

Zelus pedestris is a generalist predator with a wide geographic distribution. This study aimed to examine the biological, behavioral, and reproductive parameters of the species, under laboratory conditions. The embryonic period lasted about 10±0.81 days and the eggs had up to 100% of viability. It was confirmed 5 nymphal instars with duration of 39±0.75 days and 87.5% survival rate. The cycle was completed in 49±1.07 days. The mating sequence consisted in: frontal approach between males and females; mounting; male's spinning movement over the female and mating. The mating lasted 52.25±3.98 minutes. Females mated only once. The sexual maturity of females occurred in 7.5±0.73 days. For males, it occurred in 7.16±1.0 days. Virgin females laid eggs 13.58±1.58 days after emergence and mated females laid eggs 5.75±1.39 days after mating. The average number of egg-laying events per female was 4.33±1.76, with each egg mass containing 30.4±9.55 eggs. Adult females had a lifespan of 63.19±8.22 days. The data gathered enable the laboratory breeding, as the food source used is readily accessible and its reproductive rate is satisfactory, making this species viable for use in biological control programs.

Keywords: Generalist predator, mass rearing, nymphal development, sexual behavior, oviposition.

Introduction

The Reduviidae family is notably rich in species that primarily prey on potential agricultural crop pests [1]. *Zelus pedestris* Fabricius, 1803 is a species of predatory stink bug that belongs to this family. The average length of the males is 10.60 mm, while the females measure approximately 13.09 mm. The color ranges from dark brown to yellowish-brown, with females being lighter than males. Both sexes have bodies covered with short bristles. This species can be found as part of the entomofauna in various agricultural crops [2].

The predator *Z. pedestris* has been reported in Argentina, Bolivia, Brazil, Colombia, Ecuador, Guyana, Paraguay, Peru, Suriname, and Trinidad and Tobago [2]. This species is geographically distributed in an area of great importance for agricultural production, the well-known neotropical region [3, 4, 2].

In Ecuador, this species has been reported preying on *Perkinsiella saccharicida* Kirkaldy, 1903 (Hemiptera: Delphacidae), a sucking pest regarded as the most significant plague for sugarcane in the country's coastal sugar-producing region [5]. In Brazil, they were found preying on crop pests such as: *Monalonion bondari* Costa Lima, 1938 (Hemiptera: Miridae) in *Theobroma cacao* L.; *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) in *Camellia sinensis* (L.) Kuntze, 1887; and were found in the canopies of young *Carya illinoinensis* (Wang.) trees [4,3,6]. *Z. pedestris* records were made in the states of Bahia, Espírito Santo, Goiás, Maranhão, Minas Gerais, Mato Grosso, Paraná, Rio de Janeiro, Rio Grande do Norte, Rondônia, Santa Catarina, São Paulo and Rio Grande do Sul [2, 3]. Beyond its advantageous geographic distribution, the polyphagy of this genus aids in its adaptability in both natural and cultivated areas [7].

Knowledge of natural enemies has an important meaning in biological control programs, within integrated pest management [8]. Biological control is a technique highly significant, especially because it is ecologically based, employing predators, parasitoids, and entomopathogenic microorganisms to manage agricultural pest populations. Natural occurrence or introduction via application of imported agents may happen [9, 10]. Reduviid predators have been widely used as agents in these programs, mainly because they feed on a wide variety of prey [11]. *Zelus renardii* Kolenati, 1856 (Hemiptera: Reduviidae), is a common example of a reduviid known for preying on various pests found in oil palm trees [12]. *Rhynocoris marginatus* Fabricius, 1794 (Hemiptera: Reduviidae), is another example of a generalist predator, noted for effectively controlling around 20 species of agricultural pests [13]. A species within this category is *Rhynocoris longifrons* Stål, 1874 (Hemiptera: Reduviidae), which is also characterized as a generalist predator for various cotton pests [14].

The practical use of predators in biological control methods requires prior studies involving biological, reproductive, and behavioral characteristics [15]. Observations of growth, development, survival and reproduction of predatory insects are essential for the application of successful biological control [16]. Life cycle and fertility parameters have been thoroughly researched for other *Zelus* species, such as *Z. vespiformis* Hart, 1987 (Hemiptera: Reduviidae) [17], a predatory species of pests in Colombian coffee plantations and other pests in Costa Rica, Ecuador, El Salvador, Panama, Trinidad and Tobago, and Venezuela [2]. However, *Z. pedestris* has not yet been investigated for these characteristics.

Given that species within the genus *Zelus* are seen as strategic allies in managing the population of various agricultural pests, it is crucial to understand them and assess their potential as biological control agents. This study aimed to analyze the biology and sexual and reproductive behavior of the species in question.

Materials and Methods

Biological aspects of *Zelus pedestris* in the juvenile stage

Nymphs and eggs of *Z. pedestris*, collected from cocoa plants in Ilhéus, Bahia (14°47'45.5"S 39°10'22.1"W), were transported to the laboratory and kept at 24±3°C, with 70±10% relative humidity and natural light-dark cycle (12h L:12h D) until adult emergence. The rearing unit consisted of a transparent cylindrical plastic cage (17 cm high x 14 cm in diameter) closed at the top with voile fabric. A white paper (A4), folded accordion-style, was placed in the cage to keep the insects further apart from each other.

The insects were provided with an unlimited amount of food every 24 hours, consisting of adult *Drosophila melanogaster* Meigen, 1830 flies (Diptera: Drosophilidae), captured from fruit and vegetable peels (Lahbib *et al.* 2022). The *D. melanogaster* individuals were provided 24 hours after the nymphs hatched. Newly emerged *Z. pedestris* adults were sexed and placed in breeding units at a 1:1 male to female ratio (♂:♀). The collected egg masses (N=12), were carefully moved to new cylindrical plastic cages.

In a laboratory setting, 90 freshly laid eggs were individually placed in separate containers and monitored daily to record the duration of the embryonic period, egg viability, the duration of each instar stage, the number of instars, the nymphal survival rate, and the longevity of female specimens. The insects that hatched from each egg mass were kept together, in accordance with the species' gregarious behavior. For the record of instar changes, the insects' exuviae were counted. The longevity of males was not recorded due to the cannibalistic behavior of females.

Mating behavior of *Zelus pedestris*

To study the mating behavior, newly-emerged adult couples of the same age were used. Each pair (N=9) was isolated in a breeding unit, with one couple per container. The insects were monitored daily during the photophase period until after mating. The pre-copulatory behavior, copulation duration, and timing, were documented using videos and photos taken with an iPhone XR.

Reproductive aspects of *Zelus pedestris*

To understand the reproductive aspects of *Z. pedestris*, the same 15 pairs were used. It was observed sexual maturity of males and females, post-emergence oviposition, post-copulation oviposition, number of egg masses per female, total number of eggs per female, oviposition frequency, and number of eggs per mass. Four virgin females were individually placed in observation cages to assess their egg-laying ability without mating.

Results and Discussion

Biological aspects of *Zelus pedestris* in the juvenile stage

The embryonic period had a duration of 10±0.81 days, as shown in Table 1. The nymphal stage duration was 39±0.748 days, and each instar ranged from 7 to 9 days. The values observed for immature *Z. pedestris* were lower than those described for *Z. vespiformis*, which presented an average duration of 56.95±1.09 days [17]. However, they were close to those observed for *Z. renardii*, which presented an average nymphal phase of 33.18±0.38 days [19]. These differences may have occurred due to the type and amount of food provided, due to abiotic factors such as temperature, or even biotic factors inherent to each species, which together may have acted on the biological aspects of *Z. pedestris* [20]. Studies carried out in the laboratory, revealed that nymphs and adults of the predator *Sycanus falleni* Stal, 1863 (Heteroptera: Reduviidae), showed differences in development when fed on different species. According to the authors, the nutritional composition of prey can influence, in addition to development, in reproductive capacity and survival of insects [21]. However, among all factors, the type of prey is the most important characteristic and is directly related to the biology, physiology, longevity, fecundity and survival of predatory insects [22]. Temperature also interferes with the development and population values of insects [23].

The nymphal survival rate *Z. pedestris* was 87.5%, significantly higher than the values found for *Z. vespiformis* (11.75%), *Z. renardii* (23.7%) and *Zelus tetracanthus* Stål, 1862 (Hemiptera: Reduviidae) (23.6%) [19, 17, 24]. The high nymphal mortality rate is associated with insect cannibalism [17]. Regarding *Z. pedestris,* feeding was *ad libitum* and daily, ensuring the food source was constantly available to the insects, which helped to reduce cannibalistic behaviour. The short nymphal development time, associated with high survival values, are considered advantages for the development of mass breeding techniques [25]. Likewise, a shorter total development time (egg-adult) suggests a high reproductive capacity for the species. Therefore, *Z. pedestris* has a high potential to be a biological control agent, as given the results observed, and the individuals presented much lower nymphal duration and total cycle values when compared to other individuals of the genus [24, 17, 26].

It was confirmed that *Z. pedestris* did not feed during the first 24 hours after hatching. The nymphs roamed the entire cage all day and at night they exhibited aggregating behavior. This behavior was noted up to the second instar. Furthermore, the egg masses that gave rise to them were the reference point for aggregation. In the first and second instar, collective feeding was often recorded, with up to 7 nymphs per *D. melanogaster*. Most of the nymphs performed ecdysis in the same place, resulting in the grouping of exuviae. It was observed that *Z. pedestris* performs 5 ecdysis throughout its nymphal phase, a very common event for the genus *Zelus* [27, 17, 26, 28]. However, the duration of the stages may differ between species. This factor is related, among other factors, to the type of prey provided for the predator to feed on [29].

The development cycle was completed in 49±1.067 days. The lifecycle results observed in this study differ from those presented by [17] for *Z. vespiformis*, as the authors observed an embryonic period of 23.22±0.21 days and a total lifecycle of 112.65±0.76 days. These values were more than twice as high as those observed for *Z. pedestris* in this study.

The eggs of *Z. pedestris* showed a high viability percentage. Out of the 12 egg masses assessed, 3 achieved 100% nymph emergence (Table 2). Values similar to those found in this study were also observed for two generations of *Zelus longipes* L., 1787 (Hemiptera: Reduviidae) [30]. The high values of viable eggs associated with the short embryonic period reaffirms the potential of this species to establish high population densities [28].

Mating behavior of *Zelus pedestris*

During the pre-copulation phase, males and females remained mostly motionless. Moreover, they were always positioned at the top of the cages. Occasionally, the females remained stationary, and an increase in the male's movement around them was observed. In certain instances, the insects stayed close, with the male moving towards the female. At other times, they were found on opposite sides. When either both or just one was in motion, the "exploratory walk" and the "recognition stop" were observed [31].

The sequence of actions defining mating behavior is categorized into four stages: frontal approach between males and females; mounting; male's rotational movement over the female; and copulation. During the mating phase, the male approached directly, positioning itself in front of the female with frequent antenna touching observed (Fig 1a). Following this acknowledgment, the male positioned himself beside the female and overlaid her with his antennae, keeping the final segments of the flagellum bent downwards, making contact (Fig 1b). Next, the male mounted the female parallelly and began attempting to copulate. At that time, the insect used its mouthparts to support itself on the female (Fig 1c). During successful mating, the male positioned his body to the side of the female's body and remained in this position throughout the period until the decoupling of the aedeagus (Fig 1d). The female's stationary condition and the male's pronounced movement around her suggest the presence of a sex pheromone produced by females. This behavior was similar to that seen in studies with *Z. renardii*, *Z. tetracanthus*, and *Z. leucogrammus*, 1834 (Hemiptera: Reduviidae) [31, 32].

In laboratory conditions, it was observed that after mating, most female *Z. pedestris* killed and ate the males. Under severe hunger, generalist predators may resort to cannibalism. This behavior has been observed in *Z. renardii* and is not an uncommon practice [18]. However, [33] observed that females of the same species attempted to prey on males, but were unsuccessful. Likewise, cannibalistic behaviors were not observed among individuals of *Z. longipes* [34]. One explanation for sexual cannibalism is that it occurs when females feed on males to ensure an increase in the quality and quantity of their offspring. Evolutionary reasons underlie this activity. Sexual cannibalism presents a characteristic of resistance for females, who are larger, stronger and more aggressive towards males. Furthermore, providing essential resources for the survival of the offspring, such as parental care [35, 36].

Reproductive aspects of *Zelus pedestris*

It was observed that the sexual maturity was 7.5±0.73 days for females and 7.16±1.0 days for males (Table 3). Furthermore, the virgin females of *Z. pedestris* laid eggs, on average, 13.58±1.58 days after emergence and mated female laid eggs 5.75±1.39 days after mating. It was also found that *Z. pedestris* female fed with adult *D. melanogaster* laid an average of 133±70.31 eggs. The value was similar to that found for *Z. longipes*, which with the same diet yielded 134.10±14.08 eggs per female [37]. The average number of eggs laid per female of *Z. vespiformis* fed with *Galleria mellonella* Linnaeus, 1758 (Lepidoptera: Pyralidae) was 105.55±10.75 [17].

The number of egg masses per *Z. pedestris* female and the number of eggs per mass were 4.33±1.76 and 30.4±9.55, respectively. For these same parameters, a value of 1.55±0.17 and 68.10±2.00 was observed for *Z. vespiformis* [17]. In other words, each female produced fewer egg masses, but the number of eggs per mass was more than twice the average number observed in *Z. pedestris*.

The longevity of adult females of Z. *pedestris* was 63.19±8.22 days. This value was higher than that found for *Z. vespiformis* (25.86±2.94 days). The duration of the adult phase can be influenced by the type of food that predatory insects receive. This is a very common characteristic among reduviids [21]. Therefore, it can be suggested that *D. melanogaster* is an excellent prey option for rearing *Z. pedestris* in the laboratory.

The Fig 2 shows that the minimum and maximum copulation duration of *Z. pedestris* was 42 and 84 minutes, respectively. The average duration of copulation was 52.25±3.98 minutes. This result can be deemed significantly better than the one observed for *Z. renardii* (15.83 minutes) and *Z. tetracanthus* (11.73 minutes) and similar to that observed for *Z. leucogrammus* (55.14±8.35 minutes) [31, 32]. Knowledge about mating is very important, as it provides fundamental information for the massive rearing of insects classified as natural enemies [38].

Long-lasting copulations are widespread in insect species, despite associated potential costs in terms of time, energy, increased predation vulnerability and disease transmission. Sexual conflict resulting from adaptations to sperm competition [39]. Longer copulations can allow sperm transfer and cause increased female fecundity throughout life [40]

In some species of insects, males are able to extend the duration of copulation, with the aim of reducing competition with other individuals and ensuring the transfer of their sperm to females [41, 42].

Regarding the mating period for *Z. pedestris*, it occurs between 08:00 AM and 12:40 PM (Fig 3). This observation suggests that *Z. pedestris* has a specific pattern of mating behavior [43]. *Z. pedestris* females mate only once. This behavior was also verified for *Panstrongylus megistus* (Hemiptera: Reduviidae), which did not accept new males after a single copulation [44]. The low number of copulations observed for the species may be associated with the practice of sexual cannibalism carried out by females after mating.

Table 1: The embryonic period, survival, and life table of immature stages of *Zelus pedestris* Fabricius, 1803 (Hemiptera: Reduviidae) raised in laboratory conditions and fed with *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae).

Stage	N	Survival (%)	Duration (days) Mean ± SE	Cumulative mean (days) Mean ± SE	Range
Egg	90		10±0,807	10±0,807	9-11
I instar	80	88,88	9±0,759		8-10
II instar	75	93,75	7±0,519		6-8
III instar	71	94,66	8±0,809		7-9
IV instar	71	100	8±1,059		7-10
V instar	70	98,59	7±0,946		6-9
Nymph				39±0,748	
Egg + Nymph				49±1,067	

Table 2: Egg viability by laying of *Zelus pedestris* Fabricius, 1803 (Hemiptera: Reduviidae), observed in the laboratory

Repetition	Total number of eggs	Total number of viable eggs	Viability (%)
1	31	30	96.77
2	35	35	100
3	30	28	93.33
4	42	40	95.23
5	57	50	87.71
6	39	21	53.84
7	41	41	100
8	21	21	100
9	24	7	29.16
10	26	25	96.15
11	32	24	75
12	24	18	75

Table 3: Reproductive parameters of adult *Zelus pedestris* Fabricius, 1803 (Hemiptera: Reduviidae), observed in the laboratory

Reproductive parameters (N=15)	Mean±SE	Range
Sexual maturity of females (days)	7.5±0.73	6-8
Sexual maturity of males (days)	7.16±1.0	6-9
Post-emergence oviposition (days)	13.58±1.58	10-15
Post-mating oviposition (days)	5.75±1.39	3-7
Number of masses per female	4.33±1.76	2-8
Number of eggs per mass	30.4±9.55	14-57
Number of eggs per female	133±70.31	53-305
Female longevity (days)	63.187±8.22	49-72

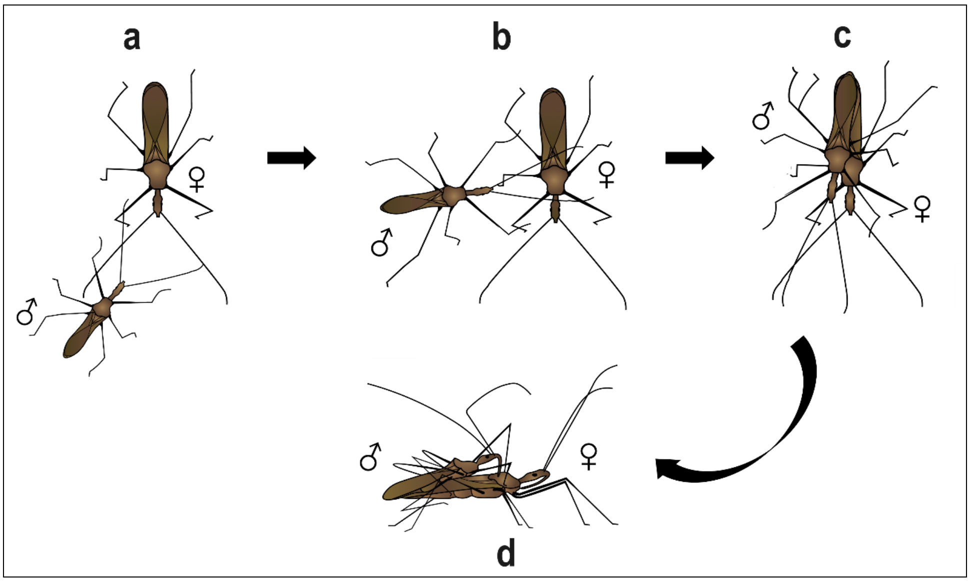

Fig 1: Parameters related to the stages of reproductive behavior of *Zelus pedestris* Fabricius, 1803 (Hemiptera: Reduviidae), observed in the laboratory. (a) Male's frontal approach to the female; (b) Female overlay with the male's last flagellum segments bent downwards, making contact; (c) and (d) Side mounting, use of mouthparts for support on the female and mating

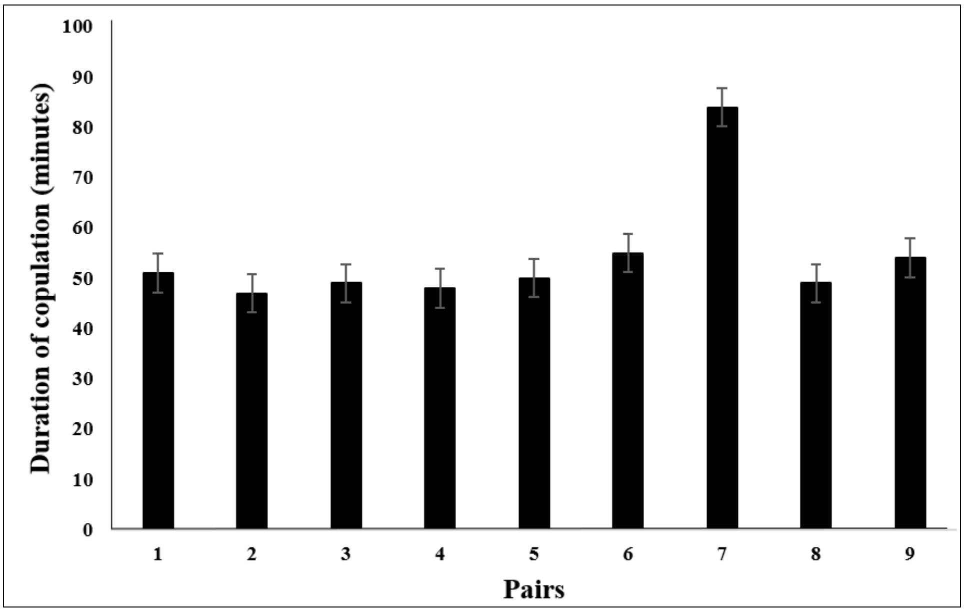

Fig 2: The duration of copulation of *Zelus pedestris* Fabricius, 1803 (Hemiptera: Reduviidae), observed in the laboratory

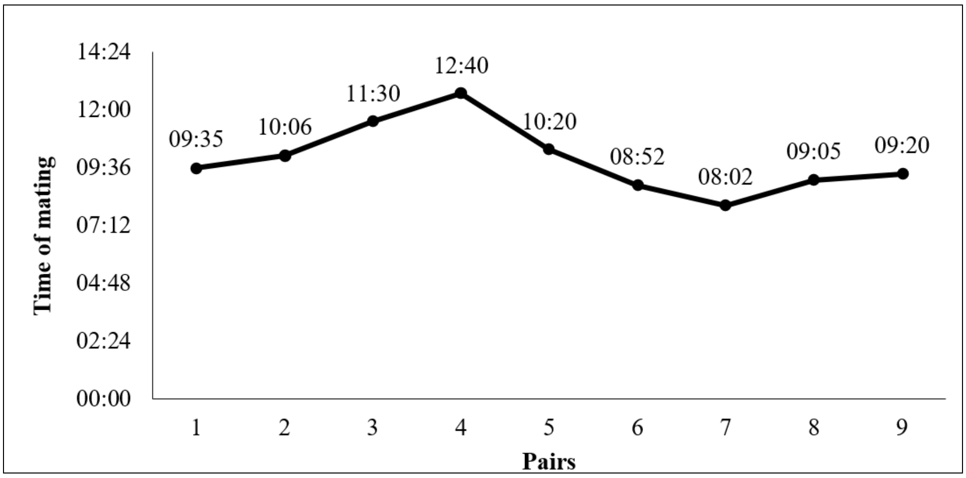

Fig 3: Start time of mating for *Zelus pedestris* Fabricius, 1803 (Hemiptera: Reduviidae), observed in the laboratory

Conclusion

The results obtained in this research demonstrate that *Z. pedestris* can be easily reared under laboratory conditions. Furthermore, they indicate that this generalist predator preys well on individuals of *D. melanogaster*, a species that is easy to obtain. Thus, *Z. pedestris* has the potential to be used in biological pest control, as it presents important characteristics for mass breeding, such as a short life cycle and high nymphal survival rate. However, considering that the experiments presented in this study were carried out in the laboratory, studies under field conditions must be carried out to evaluate the relationship between *Z. pedestris* and any prey that is considered an agricultural pest.

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