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RESEARCH ARTICLE

Biological characterization and life cycle of *Necrobia rufipes* (Coleoptera: Cleridae)

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ABSTRACT. The biological parameters of *Necrobia rufipes* (DeGeer, 1775) are estimated under controlled temperature and relative humidity. The adults, collected from industrial canine food at a supermarket in Campina Grande, state of Paraíba, Brazil, were taken to the Laboratory of Insect Systematics and Bioecology at the Universidade Estadual da Paraíba, in order to ascertain the matrix creation. The individuals were placed in boxes with hermetic sealing, each containing 20 adults, and were fed a diet of 1.8 g of fish flour, a slice of bacon, and four dog food pellets. A total of 160 eggs were individualized in the plastic recipient in a base of paper filter, covered with voil tissue, and secured with an elastic cord. The containers were placed in a B.O.D. camera at temperatures of 20, 24, 28, and 32 °C and relative humidity of 70%. The larvae received a diet composed of 0.45 g of fish flour, a slice of bacon, and dog food pellet. The development of phases was monitored daily. The life cycle from egg to adult was completed in four temperatures and encompassed four larval instars. The development speed at all stage phases was affected by temperature. Higher thermal conditions increase development speed and consequently reduce the duration of the immature and adult stages. The development of *N. rufipes* was completed at 20 and 32 °C, two temperatures that are not considered optimal for insect development. The fact that *N. rufipes* is a pest of storage grains, however, may explain why this insect is able to tolerate and develop under suboptimal temperatures.

KEY WORDS. Biological parameters, Necrophagous, Red-legged ham beetle, PMI.

INTRODUCTION

The study of colonization of dead bodies by insects and other arthropods is known as Medical-legal Forensic Entomology (Hu et al. 2020, Key 1985). Forensic entomologists estimate the Post Mortem Interval (PMI) through analysis of data related to the lifecycle and succession of species of insects found in a decaying body (Tomberlin et al. 2011). These patterns of succession are a variable process that depends on the biotic and abiotic conditions of the environment (Hu et al. 2020).

Coleoptera is the second largest order of insects relevant to forensics. A great number of specimens and species are found during the advanced stages of carcass decomposition in open environments. Dipterans, also common in corpses, do not colonize the later stages in great numbers (Goff 1991). Therefore, Coleoptera comprises the main entomological evidence for determining PMI, based especially on their pattern of succession when dry human skeletons are recovered (Kulshrestha and Satpathy 2001).

Coleoptera have cosmopolitan distribution and are the largest and most diverse group of organisms in the animal kingdom, with more than 380.000 described species, representing approximately 25% of all animal species on planet Earth (Ślipiński et al. 2011, Zhang et al. 2018). Cleridae (Cucujiformia: Cleroidea) is one among more than 200 families belonging to this order and is subdivided into 13 subfamilies (Opitz 2010, Gunter et al. 2013): Clerinae, Enopliinae, Epiclininae, Epiphloeinae, Hydnocerinae, Isoclerinae,

ZOOLOGIA An International Journal for Zoology

Korynetinae, Orthopleurinae, Peloniinae, Platynopterinae, Tarsosteninae, Tillinae, Thaneroclerinae. The family has approximately 3,700 species distributed worldwide, and these in turn are allocated in about 350 genera (Okutaner 2020). In the Neotropical region, there are about 886 species in 61 genera; in Brazil, about 506 species in 71 genera (Gerstmeier and Cupello 2024).

Clerids are beetles with a long, shiny, and pubescent body from 3 to 24 mm long– usually from 5 to 12 mm (Triplehorn and Johnson 2011). Most larvae and adult beetles are considered predators and can be found in a broad variety of environments, such as flowers, tree trunks, fungi, and carrion (Opitz 2002). Some species are recognized as pests, since they damage stored food products such as seeds, dried meat, ham, and sausage (Ashman 1963, Osuji 1977).

Necrobia rufipes (DeGeer, 1775), also known as red-legged ham beetle, is a cosmopolitan species, a predator, and a primary pest of food products rich in protein (Ashman 1963, Hu et al. 2020). Under ideal climatic conditions, this species may cause infestation, resulting in severe damage, especially in stored products, since this environment is very adequate for its proliferation (Silveira-Neto et al. 1976, Osuji 1977, Gredilha et al. 2005, Gredilha and Lima 2007, Hasan et al. 2020, Santos et al. 2020, Savoldelli et al. 2020). Moreover, *N. rufipes* is associated with human bodies (Iannacone 2003, Oliveira et al. 2012), carcasses of dogs, *Canis domesticus* Linnaeus, 1758 (Zahid et al. 2013), rabbits, *Lepus cuniculus* Linnaeus, 1758 (Al-Shareef and Al Mazyad 2017), and pigs, *Sus scrofa* Linnaeus, 1758 (Carvalho et al. 2000, Bahillo de la Puebla and López-Colón 2006, Rosa et al. 2011). The association with cadaveric remains was reported by Prins (1984) during the casein fermentation stage; by Kulshrestha and Satpathy (2001) during the dry remains stage, and by Zahid et al. (2013) during the active decay stage, the advanced decay stage, and the dry stage of decomposition.

The main goal of this study was to estimate the biological parameters of *N. rufipes* in a laboratory setting, using four different temperatures and an artificial diet. From an applied perspective, this study provides important information for Medical-legal Forensic Entomology investigations, since it allows discrimination of life stages in different parameters, being the first recorded case with complete data on the life cycle of *N. rufipes* recorded for Brazil.

MATERIAL AND METHODS

Adults of *N. rufipes* were found in commercial dog food bags in a supermarket in Campina Grande, state of Paraíba, Brazil. Collected specimens were transferred to the Laboratory of Insect Systematics and Bioecology, Departamento de Biologia, Universidade Estadual da Paraíba (UEPB), where they were kept in plastic containers (20 x 20 x 10 cm) with hermetic sealing plastic lids in groups of 20 adults. One slice of bacon (4.0 x 3.0 x 0.5 cm), 1.8 g of home-made fish flour, and four pellets of meat-flavored dog food (from the same brand in which the insects were obtained) were provided to all insects (diet proposed by the authors).

Eggs were collected daily using a stereoscopic microscope, brushes, and entomological clamps. A total of 160 eggs were placed in plastic containers (50 mL), lined with a piece of paper filter. The top of each container was covered with a piece of voile fabric tightened with rubber bands. The eggs were kept in four environmental chambers set at four different temperatures (20, 24, 28, and 32 °C) and relative humidity of 70%. Beside the containers with the eggs, a cotton ball soaked in the water was placed to maintain the humidity level in the B.O.D. chambers, and a thermo hygrometer was used to register possible alterations in temperature and humidity levels. Four groups of ten eggs were kept in each B.O.D. chamber (40 eggs per chamber).

After emergence, 0.45 g of fish flour, one slice of bacon (1.0 x 1.0 x 0.5 cm), and one pellet of meat-flavored dog food were provided to all larvae. Larval instars were determined through the identification of exuviae. Adults were kept individually in plastic containers (50 mL) covered with a piece of voile fabric tightened with rubber bands. The food provided to all adults was the same as previously provided to larvae. The development of the instars of *N. rufipes* was monitored daily.

The data were registered in spreadsheets and statistical analyses were carried out to assess whether there is a difference between the duration of each development phase according to the temperature of the environment where specimens were reared. Initially, a Shapiro-Wilk test was carried out to assess the assumption of normality of the distribution of the variable of duration of the stages of development. Then, for each stage of development, a Tukey test was applied to compare the duration of these stages according to the temperature conditions. All statistical analyses were carried out using the Table Curve 2D software.

RESULTS

Biological characterization

The eggs are transparent right after oviposition and their coloration changes from milky white to light brown

as they are close to larvae hatching. The number of eggs oviposited varied substantially from three to approximately 15 eggs per group. It is possible that the number of eggs oviposited per group could exceed the maximum of 15, due to problems in visualizing the eggs a few hours after oviposition because they are transparent. Females tended to oviposit more eggs first on the pellets of dog food, and then on the slice of bacon (Fig. 1A).

Necrobia rufipes has four larval instars. Right after emergence, the larva of the first instar is white with a dark brown head and supra-anal processes (Fig. 1B). The coloration of the body of the larva changes from a light pink to darker tones throughout the second (Fig. 1C) and third instars (Fig. 1D), reaching a red-brownish color in the fourth instar (Fig. 1E). The head and the urogomphi also change in coloration throughout the second and third instars. These structures change in the first instar from dark brown to a reddish-brown in the fourth instar.

The larva goes through a pre-pupal stage (Fig. 1F), which is characterized by a short period of rest with no morphological changes in comparison to the fourth larval instar (last larval instar). All individuals that reached the adult stage were observed going through all four larval instars, pre-pupae, and pupae.

Larvae were observed feeding on and hiding in all three food items (fish flour, bacon slice, and dog food pellets) during the four first instars. However, the pre-pupae move to the fish flour or the pellet of dog food to pupate. A white adhering substance was secreted by the pre-pupae, forming a shell around it, where it remained throughout the pupal stage until the adult emerged.

Right after emergence, we observed that some adults presented light green bodies and beige legs, which differ from the characteristic color of the species (metallic blue body and reddish-orange legs) (Fig. 1G–I). It was also observed that adults remained inactive for a maximum of 1–3 days after emergence. After that period, the adults showed considerable agility and copulated frantically. We often observed more than one male trying to copulate with the same female, sometimes two and even three males on top of the same female. In some cases, some males mounted on other male individuals, even protruding the male genitalia, and others just mounted and descended without protruding the genitalia. Another behavior observed was the decapitation of adult individuals. Although there were food resources, the adults, males and females, were confined in containers, which leads us to hypothesize that the stress caused by confinement was responsible for the aggressive behavior.

Life cycle

The cycle from egg to adult of *N. rufipes* was completed at the four temperatures. The shortest mean duration of the embryonic development was observed at 24 °C, and the longest, at 20 °C. A reduction in 42.4% in the duration of this instar of development was verified after the increase in temperature from 20 to 32 °C. The mean duration of development at 20 °C differed significantly when compared to the temperatures of 24, 28, and 32 °C (Table 1).

Table I. Mean duration (in days) and standard deviation (±) of the development stages of *Necrobia rufipes*. Means followed by the same capital letters do not differ statistically by the Turkey test ($p \leq 0,05$).

Stages	Temperature			
	20 °C	24 °C	28 °C	32 °C
Egg	8.5 ± 5.1A	4.4 ± 2.8B	4.8 ± 3.6B	4.9 ± 2.4B
1st instar	26.5 ± 10.2A	19.8 ± 10AB	14.4 ± 7.6B	8.2 ± 3.6B
2nd instar	28.1 ± 11.4A	20.8 ± 21.8AB	15.5 ± 6B	9.2 ± 1.1C
3rd instar	14.4 ± 3B	13.5 ± 0.71B	27.2 ± 6.9A	9.1 ± 2.5C
4th instar	33.6 ± 12.2A	13.5 ± 0.71B	16.5 ± 1.5B	11.3 ± 2.6B
Pre-pupal	20.8 ± 8.6A	11.5 ± 4.9B	7.2 ± 0.8B	6.6 ± 1.1B
Pupal	20.5 ± 8A	8 ± 1.4B	4.7 ± 0.5C	6.1 ± 2.1C
Adult	121 ± 9A	84 ± 5.7B	86.2 ± 7.7B	87.9 ± 3.3B

The larval instar presented a mean duration of 102.6, 67.6, 73.6 and 37.8 days at 20, 24, 28 and 32 °C, respectively. A reduction of 64.8 days can be observed with the increase in temperature from 20 to 32 °C, which can represent a decrease of approximately 63% in the duration of this instar.

The shortest mean duration for each of the four larval instars was observed at 32 °C. For the 1st, 2nd and 4th instars, the longest mean duration was registered at 20 °C, while for the 3rd instar it was at 28 °C. The mean duration of the 1st and the 2nd instars decreased gradually as the temperature increased. A similar pattern was verified between these instars (Table 1).

For the 1st instar, its mean duration at 20 °C differed significantly from the ones at 28 and 32 °C. For the 2nd instar, the temperatures of 20, 28 and 32 °C varied significantly among them. For the 3rd instar, there was no significant variation in the mean duration at 20 and 24 °C, but both differed from the results at 28 and 32 °C – these, on the other hand, varied significantly between them. For the 4th instar, the mean duration under the temperatures of 24, 28 and 32 °C did not present statistically significant difference. However, when the temperature was 20 °C, the development differed considerably when compared to the other temperatures (Table 1).

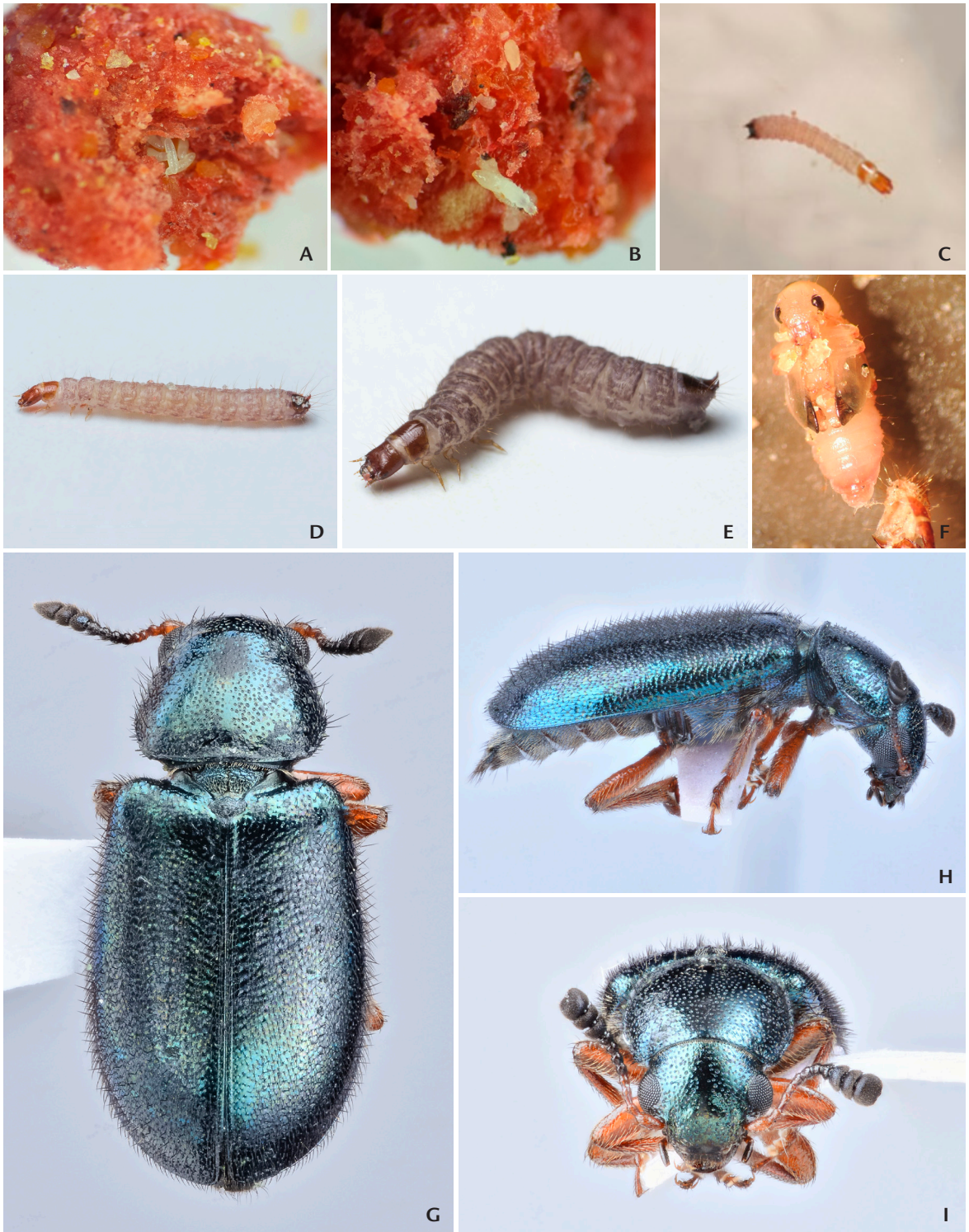

Figure 1. Stages of development of *Necrobia rufipes*: (A) eggs laying on pellet of meat-flavored food for dogs; (B) first larval instar; (C) second larval instar; (D) third larval instar; (E) fourth larval instar; (F) pupal stage; (G) adult in dorsal view; (H) adult in lateral view;(I) adult, frontal view.

The mean time of development of the pre-pupa was slower at 20 °C (with a mean duration of approximately 21 days) and faster at 32 °C (about seven days). Consequently, the mean duration at 20 °C varied significantly when compared to the other temperatures (Table 1). For this stage, we also verified that its mean duration decreased gradually with the increase in temperature.

The pupal stage had its shortest mean duration of development registered at 28 °C, and the longest, at 20 °C. However, there was no significant variation between 28 and 32 °C in this stage, whereas pupal development at 20 and 24 °C varied significantly, both between individual pupae and when compared to the other temperatures (Table 1).

In terms of the mean duration of the adult instar, a variation of 84 to 121 days can be observed, corresponding to the temperatures of 24 and 20 °C, respectively. There was, in this stage, a significant variation between the development at 20 °C when compared to the ones registered at 24, 28 and 32 °C – these presented no significant variation among them. The longest and the shortest mean durations of development from egg to adult (273.4 and 143.3 days) were registered at the temperatures of 20 and 32 °C, in that order. This corresponds to a reduction of 47.6% in the cycle duration. Regarding mortality found throughout the life cycle, it was observed that the highest rate was found at a 24 °C in the egg and 1st instar stages. Even though this temperature is indicated as ideal for rearing some insects, the egg and first instar stages are more susceptible to death.

DISCUSSION

The eggs of *N. rufipes* females were usually deposited on hard-to-reach places. This observation aligns with the findings of Hu et al. (2020) and may be related to the females' preference for oviposition sites that offer protection against solar exposure, mechanical shocks, and potential predators (Triplehorn and Johnson 2011). Oviposition in sheltered sites is possible due to the telescope shape of the ovipositor (posterior abdominal segments of females), which has also been observed in other families of Coleoptera (e.g., in Cerambycidae – Monné and Santos-Silva 2003).

Cannibalism in the larval stages, reported by Hu et al. (2020), was not observed in our study. This may be because the larvae were individualized until the emergence of the adults. However, adults exhibited cannibalistic behavior, especially in breeding boxes where adults had been in storage for longer and did not copulate as often. This same behavior was observed by Hasan et al. (2020).

After the larval stage, the pre-pupa started the process of finding and building shelter for its most critical and susceptible period, the pupal stage. They chose the pellets of dog food and fish flour for this purpose, and in both cases, it was possible to observe when the individuals pupated. After the adults emerged, they remained inactive for a few days (1–3), and some individuals did not have the distinctive coloration of the species in this phase. The initial absence of this distinctive coloration of newly emerged adults can be explained by the absence of the interaction between the light with the liquid adjacent to the cuticle of the insect, which is required for the coloration acquisition process in this species (Liu et al. 2009).

If we compare the complete cycle of development and reproduction of *N. rufipes* with that of other beetles, it is possible to analyze how these species behave under different temperature treatments. In a broader view of the complete cycle of development and reproduction of stored grain pest, studies carried out by Birch (1945) with *Rhizopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrycidae), and by Odeyemi and Hassan (1993) with *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae), established that temperatures ranging from 26 to 35 °C are ideal for development, and reproduction.

However, the result obtained by Chen et al. (2015), who analyzed the influence of temperature on the development of *Holotrichia oblita* (Faldermann, 1835) (Coleoptera: Scarabeidae), demonstrated that the optimal temperature ranges from 20 to 25 °C. Taghizadeh et al. (2008), when examining the impacts of temperature on the life cycle of *Stethorus gilvifrons* (Mulsant, 1850) (Coleoptera: Coccinellidae), demonstrated that the highest fecundity occurred at 35 °C and that the insects successfully developed at temperatures from 15 to 35 °C, which corroborates the findings of Howe (1965). The latter mentioned that for a species to be successful it must be able to not only survive and multiply when the conditions are favorable (temperatures from about 25 to 33 °C), but also to withstand when the conditions are unfavorable (temperatures ranging from 15 to 22 °C). In opposition to that, Fields (1992) argued that insects from stored products need a high minimum temperature to complete their development, and that an optimal temperature for that ranges from 25 to 33 °C.

If we consider that development efficiency is correlated with a shorter life cycle, temperature will always be the key factor for insects. This is clear both in the study of Kutcherov (2016) and in the present study. When analyzing the cycle of *Gastrolina depressa* (Baly, 1859) (Coleoptera: Chrysomelidae),

Kutcherov concluded that development becomes faster as the temperature increases. This is consistent with the present study, where an increase in temperature from 20 to 32 °C shortened the development in 64.8 days. This result represents a decrease of approximately 63% in the duration of the larval instars.

Analyzing the studies that specifically used *N. rufipes* as a model, we observe a variation in temperatures ranging from 22 to 36 °C (Simmons and Ellington 1925, Ashman 1963, Osuji 1975, 1977, Hasan and Phillips 2010, Hu et al. 2020). Hu et al. (2020) established six temperatures for the study of the development of *N. rufipes*: 22, 25, 28, 31, 34 and 36 °C, with 70% humidity for all temperatures. In their results, the shortest life cycle was found when individuals were exposed to 36 °C and the longest one was found at 22 °C, resulting in 31.2 and 121.96 days of life, respectively.

When comparing the results of Hu et al. (2020) with the data of the present study, we noticed that it had a great variation in days even when the temperatures were equal. For instance, at 28 °C, we found an average variation of 86.2 days, whereas Hu et al. (2020) observed 50.61 in their study.

The temperature of 20 °C in the present study, when compared to 22 °C in the study of Hu et al. (2020), even with a difference of 2 °C, showed an approximate value of 121 (average) and 121.96 days of development, respectively. For the other established temperatures here, 24 and 32 °C, we had an average of 84 and 87.9 days. With similar temperatures (25, 31 and 34 °C), these authors found 66.16, 38.26 and 37.97 days, respectively.

The mortality rates in the present study were higher in the egg and 1st larval instar, at 24 °C. This stands in contradiction with the findings of other studies (Hu et al. 2020, Milanez and Parra 2000, Panizzi and Parra 2009), which suggest that 25 °C is ideal for the development of insects. As portrayed in Fig. 2 (24 °C), high mortality rates were observed at 24 °C: 30% of the subjects were dead in the egg stage, and 78.6% in the 1st larval instar. This corresponds to more than 75% of the total population.

The highest rates of mortality, considering the four temperatures to which the subjects were exposed, were observed in the egg and 1st larval instar (Fig. 2). This correlates with nutrient deficiency during maternal nutrition,

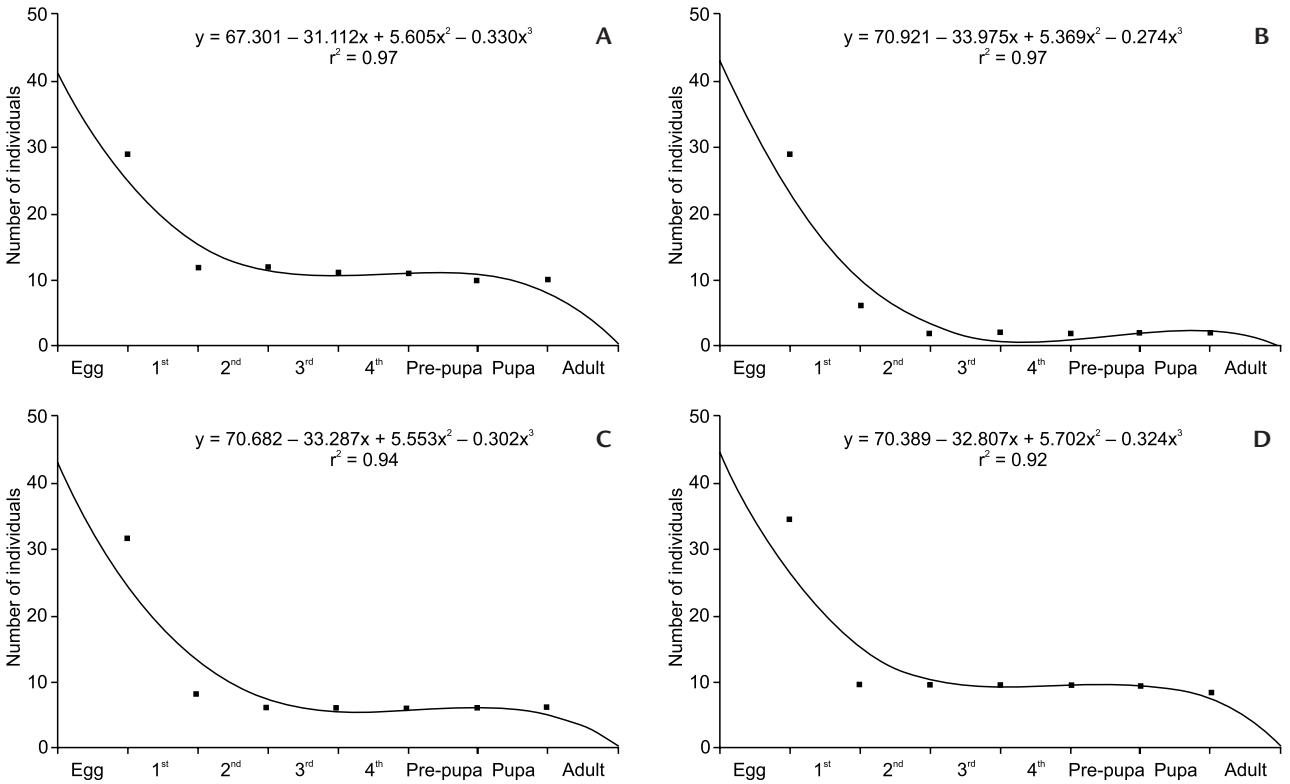

Figure 2. Survival of the development instars of *Necrobia rufipes* at the different temperatures: (A) 20 °C, (B) 24 °C, (C) 28 °C, and (D) 32 °C.

such as the absence of sterols (responsible for reproduction, oogenesis, lipoprotein transport, sclerotization of the cuticle, precursor of steroid hormones, and growth of immature forms), and a possible low level of phagostimulants (physical and chemical), present in the diet offered to the 1st instar larvae (Panizzi and Parra 2009).

The diet chosen for the maintenance of adult and immature individuals followed the criteria of previous studies (Ashman 1963, Hasan and Phillips 2010, Osuji 1975, Simmons and Ellington 1925), which showed satisfactory results with bacon, ham, dog food pellets and even dried fish meal. Although, observations made by Hasan et al (2022) indicated that copra is inferior as a food source for *N. rufipes* growth and development. The intention was to provide satisfactory quantitative and qualitative food, in order to eliminate the mortality effects caused by a nutrient-poor diet. Therefore, we hypothesize that the high mortality rate is due to the previous environment, in which the adults were confined to a bag of feed, where they did not have access to a balanced diet. The effects of those conditions were noticed in the early life stages of the first generation in the laboratory.

Ultimately, it was shown that temperature had an impact on the rate of development for every stage examined. We confirmed that higher temperatures cause the rate of development to rise and the duration of the immature and adult stages to decrease. The pest insects from stored grains' great adaptability to temperature variation can be used to explain why *N. rufipes* developed to completion at 20 and 32 °C. Therefore, we draw the conclusion that all the temperatures examined permit *N. rufipes* immature instars to fully grow, giving rise to morpho physiologically viable adults.

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