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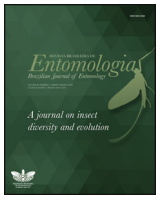

Diet composition affects the rearing of *Mansonia humeralis* (Diptera: Culicidae) immatures under laboratory conditions

Francisco Júnior Oliveira Lima[1], Alexandre de Almeida e Silva[2]*

[1]*Universidade Federal de Rondônia, Programa de Bolsas de Iniciação Científica, Porto Velho, Brasil.*
[2]*Universidade Federal de Rondônia, Laboratório de Bioecologia de Insetos, Departamento de Biologia, Núcleo de Ciências Exatas e da Terra, Porto Velho, Brasil.*

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ABSTRACT

Mansonia Blanchard mosquitoes are aggressive and opportunistic, making it difficult for habitation in certain areas. However, there is no data regarding their rearing in laboratory in Brazil. Therefore, the aim of this study was to evaluate the effect of different diet composition on the development of *Mansonia humeralis* immatures. The plant *Pistia stratiotes* was used as a substrate for larval attachment, and fish food (Tetra Marine® Flakes) and baker's yeast (*Saccharomyces cerevisiae*) in various combinations were used as feed. Eggs and larvae were obtained from mosquitoes collected in the field using protected human catches, and the experiments were conducted in the laboratory (25-27°C and 70-80% relative humidity) with 200 larvae per container (n=15). Mortality rate, larval development time, pupation rate, emergence rate, and the number of eggs and larvae were recorded. Overall, a high mortality rate (0.5 – 0.7) was observed in the first 12 days of the experiment in all diets, up to 50% of adults emerged from the remaining larvae with a male to female ratio of 1.1 to 1.3. The use of yeast in combination with fish food resulted in lower mortality rate (0.52), a shorter larval development time (34 days), a higher pupation rate (0.21) and number of eggs (21 eggs) compared to the other diets. However, fertility was very low (1%) regardless of the diet used. The use of yeast is recommended as part of the diet offered to this mosquito species under the laboratory conditions used in our study.

Introduction

Mansonia Blanchard, 1901, are stenogamous (Laurence, 1960) mosquitoes nearly worldwide distributed and includes 25 species, of which 15 are predominantly found in the neotropical region (Harbach, 2023). They exhibit nocturnal and crepuscular habits and can render certain locations unsuitable for habitation or livestock due to their aggressiveness and opportunistic behavior (Tadei, 1996). Besides, they were reported as potential vectors of Rift Valley Fever in Kenya (Logan et al. 1991) and filariasis in Ghana (Ughasi et al. 2012).

Although there have been no reports of arboviruses transmitted by this mosquito in Brazil, there is a report of a new virus from the Tymoviridae family, named the Mutum virus, in *Mansonia* mosquitoes collected near the Jirau hydroelectric Power Plant on the Mutum Paraná River (Miranda et al., 2022). Additionally, another study conducted by Sousa et al. (2023) documented the natural infection of the Mayaro virus in *Mansonia humeralis* Dyar & Knab 1916, detected through RT-qPCR.

After a blood meal, female *Mansonia* mosquitoes lay their eggs in breeding sites containing aquatic macrophytes in stagnant and shallow, generally isolated backwater, and their immature develop in association with the roots of various plant species (Forattini, 2002), such as *Pistia stratiotes* L. 1753 (Araceae), *Eichhornia crassipes* Solms, 1883 (Pontederiaceae), *Salvinia* sp. Seg. 1754 (Salviniaceae) (Gil et al., 2021), *Limnobium laevigatum* and *Limnobium spongia* (Humb. & Bonpl. Ex Willd) Heine 1968 (Alismatales: Hydrocharitaceae) (Amorim et al., 2022), *Ludwigia helmithorrhiza* Mart. (Myrtales: Onagraceae) (Saraiva et al., 2023).

The colonization of some *Mansonia* species has been reported in Malaysia, including *Mansonia dives* (Schiner, 1868) (Seng et al., 1991), *Mansonia uniformis* (Theobald, 1901), *Mansonia indiana* Edwards, 1930 (Chiang et al., 1985); Thailand, including *Mansonia annulata* Leicester, 1908, and *Mansonia bonneae* Edwards, 1930 (Samung et al., 2006), and in Australia, including *Ma. uniformis* and *Mansonia septempunctata* Theobald, 1905, using wheat as a substrate for immature attachment (Johnson & Russell, 2019a).

However, in attempts to establish colonies, there are reports of difficulties regarding the maintenance of macrophytes, rearing conditions, and the feeding of immatures (Chiang et al., 1985). Therefore, studies on improving rearing conditions for the establishment of colonies of these mosquitoes, e.g., Samung et al. (2006), are essential for successful colonization.

*Corresponding author:
E-mail: alealsil@unir.br (A.A. Silva).

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Performing a preliminary investigation in general databases, we have not found reports of established *Mansonia* species colonies in Brazil. Nevertheless, the rearing conditions reported in other countries can guide studies on the biology of local *Mansonia* species. Thus, the aim of this study was to evaluate different diets for the rearing of *Mansonia humeralis* immatures in the laboratory, on biological parameters such as mortality, development time, pupation, adult emergence, as well as the amount eggs and larvae from subsequent mosquito generations.

Material and methods

Ethical aspects

The procedures for mosquito collection carried out with the approval of the SISBIO 58855-3. Human landing catches and human blood collection for blood feeding were authorized by the Research Ethics Committee of the Fundação Universidade Federal de Rondônia, under protocol number 51550621.2.0000.5300.

Study area, mosquito capture and blood feeding in the field

Mansonia captures were conducted near the Ramal do Sical (9°31'60.0"S 64°49'25.3"W), located close to the district of Mutum Paraná in Porto Velho, Rondônia once a month from March to July 2022. Female *Mansonia* mosquitoes were captured using handheld aspirators and protected human catches from 6:00 pm and 7:30 pm and placed in entomological cages (Torres et al., 2022).

After mosquito capture, a blood meal was provided using an artificial feeder adapted from Siria et al. (2018), placed on the top of the cages for 10 minutes. Following the blood meal, a cotton ball soaked in 10% sucrose was placed on the top of the cage. The cages were then enclosed within a polystyrene box and transported to the Laboratory of Insect Bioecology (LaBEIn) at the Federal University of Rondônia (UNIR) and kept at a temperature of 27-29°C and humidity of 70%-80% until the following day when the mosquitoes were separated into new cages containing up to 25 females.

Experimental procedures in the laboratory and diet formulation

Three days after the field blood meal, induced oviposition was performed removing one of the wings from the females anesthetized with ethyl acetate vapors using entomological forceps (Lanzaro et al., 1988). The females, after wing removal, were separated into groups of 15 individuals and placed in 1-liter plastic trays containing 500 mL of dechlorinated filtered water, with polystyrene fine strips as an oviposition substrate. The containers were covered with mesh to prevent escape (adapted from Sucharit et al., 1982).

The eggs of *Mansonia humeralis* were identified using the description provided by Silva Ferreira et al. (2020). The polystyrene strips containing the eggs were placed in plastic containers (8.5 x 36.0 x 43.5 cm) with two liters of dechlorinated filtered water and larval hatching occurred after an average of 4 to 6 days.

For each replicate of each tested diet, 200 first instar (L1) larvae of *Ma. humeralis* were placed in plastic pots (14.0 x 16.5 cm) containing two medium-sized (10cm diameter) *Pistia stratiotes* plants used as attachment substrates and 1400 ml of dechlorinated filtered water. Five replicates were made for each experiment, and the experiment was repeated three times. Rearing media was renewed every 4 days, plants replaced every 8 days, and live and dead larva counts every 12 days to avoid excessive disturbance.

Three diets were used: (i) diluted fish food, DF; (ii) diluted and sprinkled fish food, DSF; and (iii) diluted fish food, sprinkled fish food and yeast, DSYF. The basic component in the diets was Tetra Marine® Flakes fish food containing 46% crude protein, 8.5% of crude oils and fats, 2% of crude fibers, vitamin A 36400IU/Kg, Vitamin D3 2045 IU/Kg, 78 mg/Kg of Maganese, 46 mg/Kg of Zinc and 30mg/Kg of iron.

DF: A 16mg/ml solution of crushed Tetra Marine® Flakes fish food was offered daily for all treatments, with 2.5 ml for L1 and L2 in the morning and 5 ml for L3 and L4 during the day, divided into two 2.5 ml portions. DSF: In addition to the DF diet, 20 mg of crushed Tetra Marine® Flakes fish food was sprinkled on the water surface every 4 days during maintenance, regardless of the developmental stage. DSYF: 2 ml of a 10 mg/ml solution of baker's yeast (*Saccharomyces cerevisiae*) from the Lesaffre brand was added every 4 days, along with the DSF diet.

Mortality rate was calculated by dividing the number of dead immatures by the initial number of larvae (Number of dead larvae/Initial number of larvae). Larval development time was estimated by recording the time required for each L1 larva to reach the pupal stage, counted daily. The pupation rate was calculated as the number of pupae obtained divided by the initial number of larvae (Number of pupae/Initial number of larvae), the emergence rate was obtained from the ratio of the number of adults obtained to the number of pupae (Number of adults/Number of pupae) and the male to female ratio was calculated dividing the number of males obtained by the number of females obtained (number of males/number of females).

After emergence, the *M. humeralis* adults were removed using a handheld aspirator and placed according to their respective treatments in plastic cages (14.0 x 16.5 cm), kept daily with cotton balls soaked in 10% sucrose until a blood meal was offered, as previously described. Six days after emergence, a blood meal was provided, and three days later, induced oviposition was performed as described earlier. The females were separated individually and observed daily for the presence of egg-laying. The number of eggs and larvae was counted using a stereomicroscope at 40x magnification.

Statistical analyses

To evaluate the effect of different diets on mortality rate, a two-factor ANOVA (treatment and time) was used. Larval development time was analyzed using the Kruskal-Wallis test (non-parametric ANOVA), with comparisons made using the Dunn method. Pupation, emergence, male/female ratio and fecundity rates were analyzed using analysis of variance (ANOVA), with comparisons made using the Tukey test, using Prism 9 software (Graph Pad Inc).

Results

The average mortality rate of larvae fed with DSF (0.63) was higher than those fed with DSYF (0.52) ($F=5.57$; $P=0.0046$), but both did not differ from DF (0.57). Generally, in the first 12 days, regardless of the diet, approximately 50-70% of the number of first and second instar larvae in all treatments died ($F=122.8$; $P<0.0001$). After this point, mortality increased, reaching values between 0.74 and 0.84 (Figure 1).

The development time was longer for larvae fed with the DF diet compared to the others ($H=46.6$; $P<0.0001$). Generally, except for larvae fed with the DF diet (37 days), larvae from the other treatments, DSF and DSYF, took an average of 34 days to pupate (Figure 2).

The average pupation rate for DF (0.10) and DSF (0.11) larvae was half that observed in the DSYF treatment (0.21) ($F=4.48$; $P=0.02$) (Figure 3).

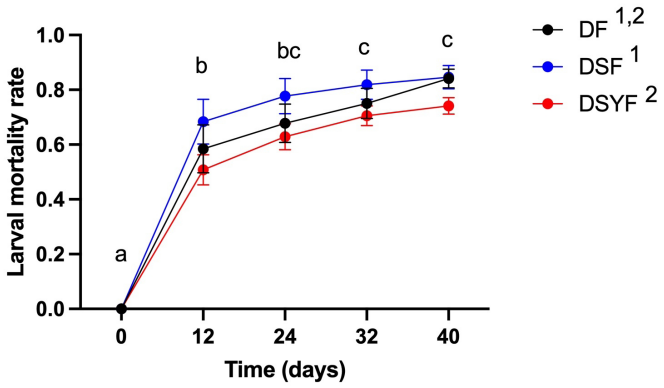

Figure 1 Larval mortality rate of *Mansonia humeralis* reared with different diets. Legend: DF: diluted fish food; DSF: diluted and sprinkled fish food, and DSYF: diluted fish food, sprinkled fish food and yeast. Different letters indicate significant differences (P<0.05) between the observation intervals regardless of the treatment. Different numbers indicate significant differences (P<0.05) between diets.

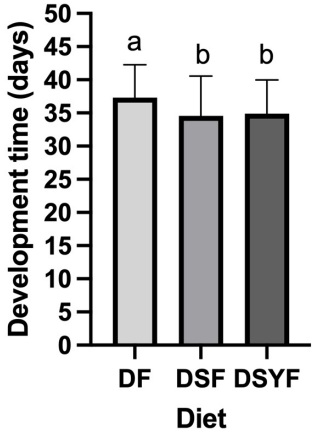

Figure 2 Larval development of *Mansonia humeralis* reared with different diets. Legend: DF: diluted fish food; DSF: diluted and sprinkled fish food, and DSYF: diluted fish food, sprinkled fish food and yeast. Different letters indicate significant differences (P<0.05) between diets.

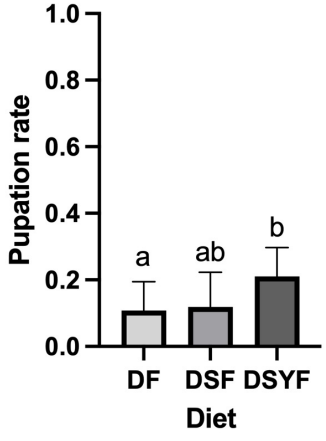

Figure 3 Pupation rate of *Mansonia humeralis* reared with different diets. Legend: DF: diluted fish food; DSF: diluted and sprinkled fish food, and DSYF: diluted fish food, sprinkled fish food and yeast. Different letters indicate significant differences (P<0.05) between diets.

In general, about 50% of adults emerged from the pupae produced by larvae fed with different diets, and the male to female ratio varied from 1.11 (DF) to 1.31 (DSF), but no differences were observed between the treatments for both variables (F=0.28; P=0.76; F=0.61; P=0.56, respectively) (Figure 4A and 4B).

The number of females that oviposited was higher for females that were fed with DSYF during the larval phase (60%) (X^2=10.86; P=0.0044) compared to those fed with DF (21%) and DSF (17%) (Figure 5A). The mean number of eggs recorded varied from 52 (DF) to 59 (DSYF) without significant differences (F=0.54; P=0.58) (Figure 5B). The larval eclosion of the second generation was less than 1% regardless of the diet provided to the F1 larvae.

Discussion

Rearing immatures is a challenge reported for other *Mansonia* species (e.g., Sucharit et al., 1982 and Samung et al., 2006). The high mortality of early-stage larvae, as observed for *M. humeralis* under laboratory conditions (0.5), was also reported by Samung et al. (2006) for some *Mansonia* species, e.g., *Mansonia bonneae* (0.63), *Mansonia dives* (0.69), and *Mansonia annulata* (0.89). However, the authors show that larval mortality depends on the species studied since the mortality of *Mansonia uniformis* (0.19), *Mansonia indiana* (0.18), and *Mansonia annulifera* (0.35) was much lower. Nevertheless, Samung et al. (2006) used diets very different from those used in the present study, limiting the comparison with their results.

The use of yeast as part of the diet for *M. humeralis* in this study resulted in the lowest mortality rate and is a component that was successfully employed in the rearing of *Mansonia uniformis* by Johnson & Russell (2019a), who used a combination of fish food and yeast and cleaning interval of 10 days. However, an important factor for the larval survival of another Mansoninii species studied by these authors, *Coquillettidia linealis* Dyar, 1905, was the amount of food offered, i.e., 0.6mg/ml of a mixture of ground fish food and yeast (1:1) offered to 40 larvae by Johnson & Russell (2019b). In this study, we offered a much smaller amount, i.e., 0.14mg/ml for approximately 200 larvae, based on empirical data because larger quantities of food resulted in a film on the surface and strong putrefaction odors.

Johnson & Russell (2019a) reported that, preliminarily, the use of infusions rich in microorganisms, e.g., flagellated, ciliates, improved survival rates, particularly for 1st instars of *Cq. linealis*. Thus, finding a way to increase the amount of food offered to *Ma. humeralis* or associating it with the use of infusions rich in microorganisms may be an alternative to reduce mortality in the early larval stages.

Development time of *M. humeralis* under our rearing conditions (27-29°C) was usually longer (34-37 days) than other *Mansonia* species, e.g., *M. uniformis* (16-24 days), *M. indiana* (16-26 days), *M. annulifera* (16-25 days) under temperatures varying from 28-41°C under insectary conditions and partially shaded verandah (Samung et al., 2006).

Dietary compositions and food quantities are well-established factors that affect mosquito fecundity, such as *Ae. aegypti* (Linnaeus, 1762) (Yan et al., 2021) and *Anopheles* mosquitoes, *Anopheles gambiae* Giles, 1926, and *Anopheles stephensi* Liston, 1901 (Takken et al., 2013). Yan et al. (2021) offering about half the amount of food to *Ae. aegypti* larvae resulted in a 50% reduction in the fecundity of females obtained from those larvae. This suggests that the composition and quantity of the diet offered to F1 larvae of *Ma. humeralis* need to be adjusted to obtain more egg laying females, increase the number of eggs as observed for wild *M. humeralis* females (Torres et al., 2022) and larvae (F2) from females obtained in the laboratory, thus enabling the establishment of a viable colony.

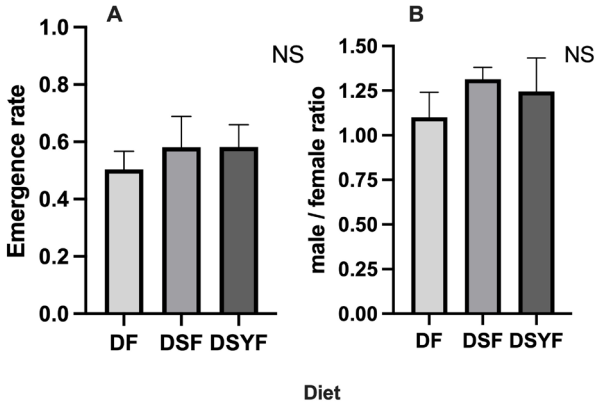

Figure 4 Emergence rate (A) and male to female ratio (B) of *Mansonia humeralis* reared with different diets. Legend: DF: diluted fish food; DSF: diluted and sprinkled fish food, and DSYF: diluted fish food, sprinkled fish food and yeast. NS= No significant statistical differences (P>0.05).

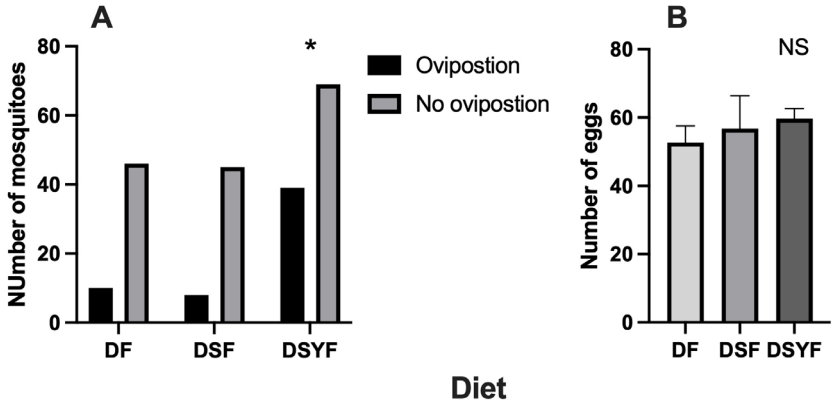

Figure 5 Number of females that laid eggs (A) and number of eggs laid (B) by *Mansonia humeralis* emerged from larvae reared with different diets. Legend: DF: diluted fish food; DSF: diluted and sprinkled fish food, and DSYF: diluted fish food, sprinkled fish food and yeast. * Indicate significant differences between diets. NS= no significant statistical differences (P<0.05) between diets

Conclusion

The use of a larval diet for *Ma. humeralis* containing fine grinded fish food and yeast (*Saccharomyces cerevisiae*) reduced the development time in 3 days and doubled the pupation rate compared to the other diets. In addition, the number of egg laying females obtained from larvae raised on this diet was higher, but fertility was very low (1%) regardless of the diet used. The use of yeast in the diet for rearing *M. humeralis* immatures is recommended under the experimental conditions used in this study.

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Conflict of interest

The authors declare that there is no conflict of interest to disclose.

Author contributions statement

FJOL Investigation and Writing – Review and Editing. AAS Conceptualization, data Analysis and Original Draft Preparation. All authors have read and approved the final manuscript.

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