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EFFECTS OF FEEDING RATE AND LARVAL DENSITY ON DEVELOPMENT IN *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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ABSTRACT: Larval density and food availability can act as limiting factors in the container habitat of *Aedes albopictus* mosquito vector species. Under laboratory conditions, two experiments were conducted to investigate the effects of feeding rates and larval density on the immature development of *Ae. albopictus* larvae. A cohort of 30 larvae were reared in 300 ml tap water with low and high amounts of food supplied for feeding rates experiment. For density experiment, 20 larvae in low density and 60 larvae in high density treatment was reared with same amount of food in 200 ml tap water. Each treatment was replicated five times. Larvae were reared from hatching to adult emergence with food provisioned per capita and per instar basis. A one-way ANOVA revealed significant effects of the experimental treatments on survivorship, age at pupation and development time. Maximum survivorship of 93.33 ± 4.08% was observed from high food treatment and a reduced survival rate of 75.67 ± 6.08% was found from high density treatment. A prolonged development time of 25.34 ± 0.73 days was resulted from low food treatment and a short development period of 10.29 ± 0.50 days from high food treatment. Male-biased sex ratios (male: female) were recorded from low food (81: 41) and high-density (150:77) treatments respectively. This study supports that survivorship and larval development are influenced by larval density and availability of nutrition levels in container breeding habitats, with potential implications for developing vector control strategies.

Key words: Feeding rates, larval density, *Ae. albopictus*, container habitat, life history traits.

INTRODUCTION

Mosquitoes constitute the most significant category of insects in the field of medical entomology because of their crucial role in spreading numerous infectious diseases that affect humans (Weaver and Reisen, 2010). *Ae. albopictus*, the Asian tiger mosquito, bites throughout the day. It thrives in urban and suburban environments where it may easily feed on people which makes it an important pest species (Faraji *et al.,* 2014).

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Culicid larvae are docile and undemanding, consuming nearly everything in their natural or artificial environments as well as organic particles in the water through a variety of feeding techniques (Walker *et al.,* 1988; Merritt *et al.,* 1992). *Aedes* mosquitos are container-breeding mosquitos. *Ae. albopictus* is the predominant species in the container-breeding habitats. This mosquito species completes its immature development in small-volume artificial containers associated with the human environment, such as flasks, bottles, flower vases, and used tires (Vezzani, 2007).

Among the many variables that influence the traits of mosquito larvae's life cycle, feeding rates and larval density have emerged as key determinants (Agnew *et al.,* 2002). Feeding rates represent the availability and accessibility of food resources. Deficiency of food has been demonstrated to result in a longer period for development. (Aznar *et al.,* 2018), delayed time to pupation (Banerjee *et al.,* 2015). The availability of food resources may also have an impact on the vector competency. When adults from larvae with inadequate nutritional reserves emerge, they are comparatively small (Lehmann *et al.,* 2006) and to produce eggs, they require greater blood feeds, which could enhance their capacity to serve as vectors (Muturi *et al.,* 2011). The relative density of the developing larvae can influence the mature mosquito's fitness in addition to the quantity and quality of the available resources (Agnew *et al.,* 2002; Arrivillaga and Barrera 2004). Per capita resource availability would vary depending on differences in the density of developing larvae under comparable space and resource conditions, which would impact the mosquito's rate of development and rate at which its biomass accumulates (Reiskind and Lounibos 2009). Nutrition and excessive larval density affect both the physical fitness and also mode of blood feeding behavior of the female *Ae. albopictus* mosquitoes (Manorenjitha *et al.,* 2012).

Ecologists have spent years trying to find out how intraspecific competition at different densities during the larval phase affects the performance and fitness of adults (Paaijmans *et al.,* 2009). Because density-dependent intraspecific competition may alter the adult vector capacity, the outcome of intraspecific competitiveness for a vector species is crucial to disease prevalence from the perspective of medical entomology (Takken *et al.,* 1998). Understanding the elements that influence the population dynamics and development of mosquito species is crucial for implementing effective control measures and mitigating the risk of disease transmitted by the mosquito vectors. Therefore, the aim of this research was to look into the interactions among the same-aged larvae of *Ae. albopictus* in a specific food and density treatment. The impacts of larval density and consumption rates on some of the life history traits were measured to understand the population dynamics.

MATERIAL AND METHODS

Experimental protocol: The experiment was carried out from March 2023 to October 2023. The experimental procedures were conducted in the Entomology Research Laboratory and Animal Garden of the Department of Zoology, University of Dhaka.

Collection and rearing of Ae. albopictus larvae: Larvae of different instars were collected from abandoned jars, bottles, earthen pots, clay pots, and cemetery vases from the Animal Garden of Dhaka University. The collected larvae of *Ae. albopictus* were transferred to small plastic bowls using a dropper. Then the plastic bowls were transferred into rearing cages of 1.3ft ×1.3ft×1.3ft size, made of iron frame, covered with small meshed mosquito nets with an opening at one side. The larvae were supplied with fish food and reared to adulthood.

The large number of emerged adults were kept in the rearing cage, and a 10% sugar solution was supplied into the rearing cage for the development and survival of the newly emerged mosquitoes. Female mosquito needs blood meal for ovarian development. A pigeon without feathers and fur on the keel were placed on the rearing cage for 2 hours on every other day as a blood meal source for female mosquitoes.

Small plastic bowls filled with a little amount of water with vertically placed filter paper attached on the side walls were placed inside the adult rearing cage for oviposition. A batch of plastic bowls containing newly laid eggs were transferred to another cage so that a uniform progeny of newly hatched 1st instar larvae (less than 24 hours old) can be obtained for the initiation of the experiment.

Experimental design: The study was conducted in two experimental procedures to investigate the effect of feeding rates and larval density on some of the life history traits of *Ae. albopictus* larvae. For both the two experiments 1st instar larvae of *Ae. albopictus* were reared up to adult in plastic containers with fixed amount of tap water to observe life history traits. Food was provided into the rearing containers on a per capita and per-instar basis (Jannat and Roitberg, 2013). Each experiment consisted of two treatments that was replicated five times.

Feeding rates experiment: In this experiment the food was offered in two distinct categories: low food (LF) and high food (HF) (Table 1). For low food treatment the replications were LF1, LF2, LF3, LF4, LF5 and for high food treatment the replications were HF1, HF2, HF3, HF4, HF5. For each replication the amount of water in the container was 300 ml and there were 30 larvae in each container for both the two treatments.

Table 1. Amount of per capita food supplies (mg per larvae) for *Ae. albopictus* larvae reared in low food and high food treatment

Instars	Low food (LF) treatment (mg per larvae)	High food (HF) treatment (mg per larvae)
1st	0.012	0.1
2nd	0.024	0.2
3rd	0.048	0.4
4th	0.096	0.8

Larval density experiment: In this experiment two different types of larval density were chosen, namely low density (LD) and high density (HD). For low density treatment the replications were LD1, LD2, LD3, LD4, LD5 and for high density treatment the replications were HD1, HD2, HD3, HD4, HD5. In the LD treatment, there were 20 larvae, whereas in the HD treatment, there were 60 larvae in each container. For each replication the amount of water in the container was fixed (200 ml). The amount of per capita food provision was the same for both LD and HD treatment (Table 2).

Table 2. Amount of per capita food supplies (mg per larvae) for *Ae. albopictus* larvae reared in low-density and high-density treatments

Instars	Low density (LD) and High density (HD) treatment (mg per larvae)
1st	0.056
2nd	0.112
3rd	0.224
4th	0.448

There was a control replication for each treatment group comprising equal number of larvae and no food was introduced. The water level in each rearing container was fixed and checked daily, evaporated water was replenished. The predetermined amount of fish food (Quality Gold) was measured and supplied to the larvae every day. Food was supplied on per capita, per-instar basis considering two days for each instar (± two days), there are four larval instars (Jannat and Roitberg, 2013). For instance, in treatment LD1, for 1st instar larvae, 0.056 mg per larva×20 larvae for a day. The open end of each container was covered by a very small meshed net to facilitate aeration and to prevent mosquitoes and other insects to lay eggs or entering the rearing medium. After the initiation of the experiment the containers were monitored two times per day for the presence of pupae and adults. The number of newly transformed pupae was recorded daily and the pupae were placed in the same water medium so that larvae and pupa get the same environment. On the day of emergence, adults were killed and recorded by container name, sex and date. The water in the rearing containers was changed when it appeared sufficiently polluted to affect larval survival. Whenever any dead larvae were found, it was removed quickly and recorded by container name and date. The experiment ended when the last pupa emerged as adults. The life history parameters for any replication are calculated by using the following formula.

Mean age at pupation = $\frac{\sum(\text{No. of days to pupation} \times \text{No. of larvae pupated})}{\text{Total larvae pupated}}$

Mean development time = $\frac{\sum(\text{No.of days to emerge as adult} \times \text{No.of adult emerged})}{\text{Total adult emerged}}$

Survivorship (%) = $\frac{\text{No.of adult emerged}}{\text{Initial no.of larvae}} \times 100\%$

All the statistical analysis and graphs were done by using MS Excel 2019, Minitab Statistical Software version 2017 and R version 4.5.1. The results are presented as mean ± standard error. One-way ANOVA was used to indicate if there exists any significant effect of feeding rates and larval density on survivorship, mean age at pupation and mean development time. A post hoc analysis of Tukey's HSD (honestly significant difference) test was used to determine the treatment-specific mean difference of survivorship, mean age at pupation and mean development time.

RESULTS AND DISCUSSION

Effects of two feeding rates on survivorship, mean age at pupation and mean development time of Ae. albopictus larvae: The life history characteristics of *Ae. Albopictus* larvae were significantly influenced by both low food (LF) and high food (HF) treatments according to the one-way ANOVA test. The Tukey's HSD test showed that mean survivorship of *Ae. Albopictus* larvae significantly differed between the two treatments (p value 0.000925*) and it was 80.14 ± 4.09 % for LF treatment and 93.33 ± 4.08 % for HF treatment (Table 3,4). The most significant result (p value <0.0001*) was found for mean age at pupation that was 23.35 ± 0.78 days in LF treatment and 8.48 ± 0.46 days in HF treatment (Table 3,4). Statistical analysis showed that larva in LF treatment had significantly longer mean development time of 25.34 ± 0.73 days and larvae in HF treatment had comparatively shorter development time of 10.29 ± 0.50 days (p value <0.0001*) (Table 3,4).

Effects of two larval densities on survivorship, mean age at pupation and mean development time of Ae. albopictus larvae: The one-way ANOVA test also indicated that both the low density (LD) and high density (HD) treatments had significant impact on the life history parameters of *Ae. albopictus* larvae. The Tukey's HSD test revealed that the mean survival rate differed significantly (p value 0.000925*) between the two treatments and the greater survival rate was 90 ± 5 % for LD and comparatively lower survival rate was 75.67 ± 6.08 % for HD treatment (Table 5,6). Statistical analysis showed that larvae in LD treatment had comparatively shorter mean age at pupation of 10.08 ± 0.53 days than the HD treatment which took 12.39 ± 0.49 days for pupation and this difference is significant (p value <0.0001*) (Table 5,6). The treatment specific mean development time also differed significantly (p value <0.0001*) and that was 11.76 ± 0.53 days for LD treatment and a comparatively longer time of 14.12 ± 0.49 days for HD treatment (Table 5,6).

Table 3. Effect of two feeding rates, low food (LF) and high food (HF), on survivorship, mean age at pupation and mean development time of *Ae. albopictus* larvae

Source	DF	Sum of squares	F ratio	P value
Survivorship	1	435.336	26.05052	0.000925*
Mean age at pupation	1	552.941	1347.24	<0.0001*
Mean development time	1	566.1058	1434.632	<0.0001*

[* Indicates a statistically significant effect (ANOVA α = 0.05)].

Table 4. Effect of two feeding rates, low food (LF) and high food (HF), on the treatment specific mean difference of survivorship, mean age at pupation and mean development time of *Ae. albopictus* larvae

Source	Treatment	Total no. of larvae	Mean ± SE
Survivorship	LF	150	80.14 ± 4.09 (a)
	HF	150	93.33 ± 4.08 (b)
Mean age at pupation	LF	150	23.35 ± 0.78 (a)
	HF	150	8.48 ± 0.46 (b)
Mean development time	LF	150	25.34 ± 0.73 (a)
	HF	150	10.29 ± 0.50 (b)

[Means with different letters are significantly different (Tukey's HSD α = 0.05)].

Table 5. Effect of two larval densities, low density (LD) and high density (HD), on survivorship, mean age at pupation and mean development time of *Ae. albopictus* larvae

Source	DF	Sum of squares	F ratio	P value
Survivorship	1	513.6589	16.58643	0.00357*
Mean age at pupation	1	13.34025	49.97191	0.000105*
Mean development time	1	13.924	53.58579	<0.0001*

[* Indicates a statistically significant effect (ANOVA α = 0.05)]

Effects of feeding rates and larval density treatments on the male and female sex ratio of Ae. Albopictus: The proportion of males to females among all mosquitoes that emerged were significantly influenced by both feeding rates and larval density treatments. A male biased sex ratio of 81: 41 resulted from low food treatment whereas a female biased sex ratio of 60: 80 was found in high food treatment (Table 7). In low-density treatment male and female sex ratio was 39: 51, which indicates a female biased sex ratio. On the other hand, a male biased sex ratio of 150: 77 was observed from high density treatment (Table 7).

For species that show complex developmental life cycles, like mosquitoes, the larval environment is always a crucial determinant of their adult fitness (Takken *et al.*, 1998). The current study indicates that the density of larvae and food availability in the container habitat affect individual fitness and adjustment to space and food during the life cycle of *Ae. albopictus* larvae.

Table 6. Effect of two larval densities, low density (LD) and high density (HD), on the treatment specific mean difference of survivorship, mean age at pupation and mean development time of *Ae. albopictus* larvae

Source	Treatment	Total no. of larvae	Mean ± SE
Survivorship	LD	100	90 ± 5 (a)
	HD	300	75.67 ± 6.08 (b)
Mean age at pupation	LD	100	10.08 ± 0.53 (a)
	HD	300	12.39 ± 0.49 (b)
Mean development time	LD	100	11.76 ± 0.53 (a)
	HD	300	14.12 ± 0.49 (b)

[Means with different letters are significantly different (Tukey's HSD α = 0.05)].

Table 7. Male and female ratio of total emerged mosquitoes from feeding rates and larval density treatments of *Ae. albopictus* larvae

Treatment	Initial no. of larvae	No. of emerged adults	Male: Female
LF	150	122	81: 41
HF	150	140	60: 80
LD	100	90	39: 51
HD	300	227	150: 77

The survival rate of *Ae. albopictus* larvae was impacted by both the feeding rates and larval density when reared in the laboratory. In high food (HF) treatment a greater survival rate was observed 93.33 ± 4.08% which is about 13% more than low food (LF) treatment 80.14 ± 4.09% (Figure 1). Yan *et al.* (2021) and Gunathilaka *et al.* (2019) also reported higher survivorship of *Ae. aegypti* larvae in high larval nutrition medium. Larval crowding reduced survival rates by about 25% in this study where survival rate was 75.67 ± 6.08% in high density (HD) treatment compared to the survival rate of 90 ± 5% in low-density (LD) treatment (Figure 1). For the control group, mortality was 100% at the first instar in both treatments. Density contributed to greater death rates even when food was supplied at optimal rates on a per capita basis and the entire range of resources was available to the larvae in the rearing medium. This result of higher mortality due to crowding supported the evidence of Muriu *et al.* (2012), Jannat and Roitberg (2013). Mean age at pupation of *Ae. albopictus* larvae was 8.48 ± 0.46 days in the high food treatment, whereas it was 23.35 ± 0.78 days for low food treatment (Figure1). The high-density treatment took an average of

2.5 days longer than the low-density treatment to reach the pupal stage. In the LF treatment, while some larvae pupated early but the majority stayed in the 3rd or 4th instar for a prolonged time. Several other studies also observed the prolonged time to metamorphosis due to scarcity of food (Araújo *et al.,* 2012; Lord, 1998).

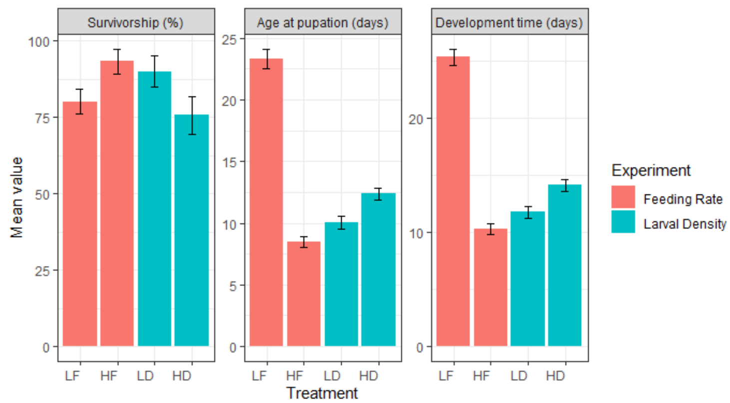

Fig. 1. Comparison of survivorship, age at pupation and development time (Mean ± SE) of *Ae. albopictus* larvae from feeding rates and larval density treatments.

Since, in larval density treatments the larvae were not experiencing poor per capita food supplies, it is possible that they were attempting to escape an adverse condition and had enough food supplies to undergo pupal metamorphosis.

This study showed that scarcity of food has a detrimental impact on the development time of *Ae. albopictus* larvae. Larvae in high food treatment (HF) completed immature development within 10.29 ± 0.50 days on the contrary larvae in low food treatment (LF) took a prolonged time of 25.34 ± 0.73 days (Figure 1). Larvae from low density treatment (LD) took around 3 days more for immature development than HD treatment. Similar result of decreased development time due to large amount of food provision was observed by Araújo *et al.* (2012), Zirbel *et al.* (2018) and Aznar *et al.* (2018). Manorenjitha *et al.* (2012) observed that due to food scarcity and overcrowding, the larval growth of *Ae. albopictus* has been noticeably delayed, taking up to 36 days. According to Clement AN (1992), mosquito species that breed in containers are exposed to prolonged periods of food scarcity may be able to significantly prolong their life cycle.

In this study, both the feeding rates and larval density affect the sex ratio of *Ae. albopictus* larvae. A male biased sex ratio of 81: 41(male: female) was obtained from low food (LF) treatment and 150:77 was observed from high density (HD) treatment. The emergence of greater number of males and a smaller number of females *Ae. albopictus* adult mosquitoes from higher larval density treatment and low food treatment are consistent with the earlier outcomes of the study experimented by Yadav *et al.* (2017) and Puggioli *et al.* (2016). Puggioli *et al.* (2013) commented that a sex ratio skewed to male might suggest an effect of underfeeding. This study found a female biased sex ratio of 60:80 from high food (HF) treatment and 39:51 in low density (LD) treatment. Field populations of *Anopheles gambiae* have also been found to exhibit a female biased sex ratio (Mutuku *et al.,* 2006). Cipollini (1991) indicated that male-biased death rates at high density larval populations resulted a sex ratio skewed to female in some insects like weevil, *Acanthos celidesobtectus*. Female mosquitoes need more food than male to grow reproductive organ and for longer duration of their life cycle. This could be the reason why the male survived on such little food in an overcrowded environment while the female perished from inadequate nutrition and resulted such distorted sex ratio (Yadav *et al.,* 2017).

In laboratory settings, this study demonstrated that density-dependent competition and changes in nutrition availability affect physiology and performance of *Ae. albopictus* mosquito in ways that affect life expectancy. These components of the population dynamics model are crucial for organizing any vector control strategy in the case of a container habitat that supports starvation and density-dependent competition.

CONCLUSION

This study revealed that the life history parameters including survival rate, age at pupation, development time, and sex ratio of *Ae. albopictus* are significantly affected by food provision and larval density which will help to understand the pattern of population growth of *Ae. albopictus* mosquitoes of a particular region. As mosquito susceptibility changes in parallel with the larval habitat's population carrying capacity, the observations of this study can be used to plan appropriate vector control measures.

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