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### **Optimization of Mass Rearing Protocols of Yellow Fever Mosquito, *Aedes aegypti* (Diptera: Culicidae)**

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#### **ABSTRACT**

An appropriate mass rearing system for the target insect is a pre-requisite for application of the sterile insect technique (SIT). To optimize the mass rearing protocol for *Aedes aegypti*, systematic research was undertaken. Efficiency of larval diet, amount of diet, larval density and the effect of an adult food supplement were evaluated. Three larval diets, viz., fish-feed, poultry-feed and biscuit were tested for their effect on the body size of adults and on developmental time of immature stages of *Ae. aegypti*. Among the three larval diets tested, fish-feed was found to be the most suitable rearing diet for the larvae of *Ae. aegypti*. Therefore, further studies were carried out to optimize the amount of fish-feed given to larvae and the larval density to get the largest possible adults along with high pupal and adult survival. A prolonged developmental period and smaller adults of both sexes were evident when insufficient larval food was provided. However, a daily feed of 0.7g of fish-feed diet was found to be adequate to raise 1000 larvae in a tray. This amount of diet was found to produce optimum sized adults and significantly shortened the developmental period relative to other feeding regimes. High pupal and adult survival and a high rate of female insemination were also observed when an adequate amount of larval diet was used. The longevity of males was evaluated with the provision of different adult supplements. A 10% sugar solution and a mixture of 10% sugar plus 10% honey were found to increase male longevity compared to males given only water and males that were completely starved. The sex ratio under our optimal rearing conditions was found to be male biased with 54.5 % males and 45.5% females, which would be helpful for the production of a male-only release generation in the application of the SIT.

**KEYWORDS:** *Aedes aegypti*, mass rearing, mosquito, larval diets, SIT

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## INTRODUCTION

*Aedes aegypti* is the major vector of dengue breeds in various types of indoor and outdoor water containing containers.<sup>1,2</sup> Besides, their eggs are resistant to desiccation and in the absence of water eggs may remain viable for up to one year.<sup>3</sup> Therefore, controls of *Aedes* spp. mosquitoes are very difficult with conventional control programmes. Chemical control is effective against most insect pests but the continuous application of chemical pesticides has some serious disadvantages.<sup>4</sup> Chemical pesticides are indiscriminate in nature, kill non-target beneficial insects, pollute soil and water, lead to outbreaks of secondary pests, can cause acute and chronic poisoning and accumulate in the food chain. To reduce health hazards and other disadvantages of using chemical pesticides, the sterile insect technique (SIT) may be an alternative for mosquito management as a part of integrated pest management programme. In SIT, large numbers of sterile insects are released into the environment to mate with the wild counterparts that are present in the environment.<sup>5</sup> SIT was first used successfully in 1958 in Florida to control the Screw worm fly (*Cochliomyia hominivorax*).<sup>6</sup> In 2004, eight weekly releases of sterile *Ae. aegypti* male pupae were conducted in parts of Italy to evaluate their performance in the field against a natural population. A significant difference in egg production and fertility was observed in the release area compared with the control area.<sup>7</sup>

The success of SIT application for insect control is highly depended on having a good mass rearing protocol. Insects are reared in many laboratories for different purposes including bioassay, physiological research, postharvest treatments and so on. For these purposes, the rearing protocols are mainly designed to maintain colonies and for the production of a small number of insects. However, in SIT research, insect rearing systems have to be optimized for mass production of high quality insects which would be able to compete with wild males upon release in the field. The success of the SIT will depend largely on whether released males can successfully compete for mates against wild males.<sup>8</sup> Evidence from laboratory experiments suggests that release of unfit sterile male results in failure of the SIT. For example, the general failure of mosquito control programmes launched in the 1970s that aimed to reduce vector populations by releasing sterile males can be largely attributed to their poor mating competitiveness.<sup>9</sup>

Diet is the most important component of insect rearing. A balance is essential between large scale insect production and performance of the adults. Negative effects of inappropriate feeding regimes for

container breeder mosquitoes on population growth, individual growth, individual fecundity, survival to adulthood and developmental time has been reported.<sup>10, 11, 12, 13, 14</sup> Female mosquito body size, adult longevity and blood feeding success were likely to be influenced by larval nutrition and conditions during development.<sup>15, 16, 17</sup> Larval crowding and adult body size have strong, independent effects on the mating competitiveness of adult male *Anopheles gambiae*.<sup>18</sup>

In this paper, we describe a mass rearing protocol for the production of high quality male *Ae. aegypti*. However, data on females is also included in this paper, since the quality of females is also important for the maintenance of a productive colony. Selection of optimum larval diets, larval rearing density, amount of diet vs larval rearing density, pupal and adult survival, adult size, longevity, ability to inseminate and the adult sex ratio are discussed.

## **MATERIALS AND METHODS**

### ***Laboratory conditions for rearing of Aedes aegypti***

The mosquito colony maintenance and all experiments were conducted in two walk-in incubators each 3 x 3 x 2.5 m (W x L x H) in size. The temperature, relative humidity and light:dark cycle were constantly maintained at 27°C ( $\pm 2^\circ\text{C}$ ), 60-80% RH and 12hL:12hD. Adults were supplied with a fresh cotton ball soaked in 10% glucose solution daily.

### ***Larval diets***

To assess the performance of three diets namely, fish-feed, poultry-feed and biscuits, the diets were ground to fine powder and 0.2g of one diet was sprinkled on the water of the rearing trays daily depending on treatment. However, upon selecting fish-feed as the best diet among the three, all later experiments were conducted using fish-feed only.

### ***Collection of eggs***

Female mosquitoes were fed chicken blood by artificial membrane feeding following a modification of the technique of Mourya *et al.*<sup>19</sup> and Mishra *et al.*<sup>20</sup>. Chicken blood was collected in a blood bag containing anticoagulant (citrate phosphate dextrose) from the slaughter house in the market. About 20ml of blood was added to petriplates and the petriplates were placed upside down on top of adult mosquito cages. A bag containing warm water ( $\sim 37^\circ\text{C}$ ) was placed on the top of each petriplate to

keep the blood warm and females were allowed to blood feed for 30-40 minutes. The mosquitoes were blood fed for at least two consecutive days and females started egg laying after 3 days of blood feeding. To collect eggs, a strip of paper inside beaker (250ml) containing 100ml water was placed into the adult cages in such a manner that the lower part of the strip was submerged in water to keep it wet. Later the egg strips were collected, dried and kept in covered petriplates containing water vials plugged with cotton until use.

### ***Larval rearing***

A paper strip containing *Ae. aegypti* eggs was submerged in a tray (40cm × 27.30cm) containing 1.5cm depth of water and the larvae that hatched within six hours were used for experiments. Newly emerged 1<sup>st</sup> instars were collected using a dropper and placed into a rearing tray; sufficient water added to maintain the water level at 1.5 cm. For experiments to select the appropriate larval diet, glucose biscuit, fish-feed and poultry-feed were used independently in different treatments. In all other experiments larvae were fed fish-feed, finely ground in a blender and passed through a sieve to remove large particles. Periodically the rearing trays were cleaned to remove excess waste material by using a vacuum aspiration method (with the help of vacuum pump). During the cleaning process a bright light was shone on one corner of the rearing tray with a lamp to keep the larvae to the other side, since larvae show negative photo taxis. After cleaning sufficient water was added to restore the initial water level.

### ***Determination of optimum amount of diet and density of larvae for the production of high quality adults***

To observe the effects of larval density on growth in laboratory conditions we added 200, 300, 400, 500, 700 or 1000 larvae to trays containing 600ml (1.5cm) of water, to give larval densities of 0.33, 0.50, 0.66, 0.83, 1.17 and 1.67 larvae/ml respectively. These densities corresponded to 0.18, 0.27, 0.37, 0.46, 0.64 and 0.92 larvae/cm<sup>2</sup> in the trays used. To ensure the desired numbers of larvae in each tray, first instars were collected from the stock rearing tray using a plastic dropper and counted into the designated rearing tray. In these experiments, the amount of diet was maintained to 0.2g diet of fish-feed per tray daily. In the experiment to determine the optimum amount of diet 0.2, 0.5 and 0.7g fish-feed were fed daily to 1000 larvae per tray.

### ***Determination of body size, sex ratio and insemination rate***

Adults from each experimental cage were collected using a glass aspirator and mosquitoes were anaesthetized with chloroform. After sexing, male and female mosquitoes were randomly chosen from each cage to measure the wing length, used as a proxy for overall body size.<sup>21, 22</sup> One wing was removed using forceps and a needle. Wings were measured using a stage meter with an ocular micrometer from the apical notch to maxillary margin, excluding the wing fringe.

The sex ratio was determined by counting all the male and female adults produced in each treatment of each experiment.

The insemination rate was determined by adding virgin males and females to adult cages for 5 to 10 days to allow mating, then collecting females from each cage for dissection under a microscope. Both the males and females were provided with a 10% sugar solution from the onset of emergence.

### ***Measuring longevity of male adults***

Pupae were collected from the tray of 1000 larvae reared with 0.7g fish-feed diet daily and kept in individual vials for emergence. Immediately after emergence, the males were taken for the longevity measurement. Longevity of males was determined by keeping single adults in paper cups covered with mosquito netting. Adults were daily supplied with a fresh cotton ball soaked in different adult supplements viz water, 10% sugar solution and 10% sugar solution with 10% honey. Control males were kept starved. Longevity data were averaged from 20 individuals of each group.

### ***Measuring time taken from pupation to adult emergence***

Larvae were reared at a density of 1000 per tray (1.67 larvae/ml) fed with 0.7g fish-feed diet per tray daily. Once pupation was underway, all pupae were removed from the tray at around 6pm, and after one hour all newly eclosed pupae were collected, kept in plastic vials and observed for adult emergence. Observations were made on an hourly basis starting 36 hours after pupation, and the hour in which each adult emerged was recorded. Three replicates were conducted, two of which consisted of 10 pupae and one included 9 individuals; average time to emergence was calculated for each replicate, and then an overall average was calculated.

### ***Statistical Analysis***

Sigma Plot 2000 was used to generate the graphs from experimental data and statistical analyses were conducted using Office XL 2007 software.

## **RESULTS AND DISCUSSION**

### ***Selection of optimum larval diet***

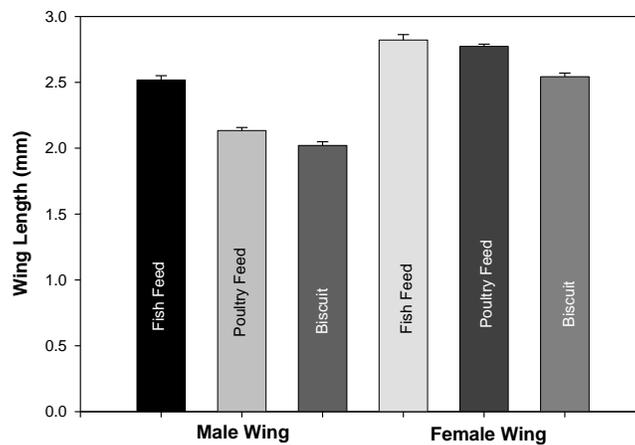
Our primary objective was to select an appropriate diet for the mass rearing of *Aedes aegypti* larvae. We have tested three larval diets *viz.*, poultry-feed, fish-feed and biscuits to evaluate the effect of each on the body size of the resulting adults. About 0.2g of each diet was provided to 300 larvae daily. Graph 1 shows the size of both males and females by treatment, represented by their wing size. Average wing sizes were 2.5( $\pm$ SE 0.03) & 2.8( $\pm$ SE 0.04) mm, 2.1( $\pm$ SE0.02) & 2.7( $\pm$ SE 0.01) mm, 2.0(SE  $\pm$ 0.02) & 2.5(SE $\pm$  0.02) mm, respectively, for male and female adults reared on fish-feed, poultry-feed and biscuit.

Wing size of males and females (proxy for body size) were both shown by Analysis of Variance (ANOVA) to be significantly different according to the larval diet they were reared on (males:  $F=73.84$ ,  $p<0.005$ ; females:  $F=24.08$ ,  $p<0.007$ ). Apart from the wing size, total developmental time from first instar larvae to adults was found to be shorter when reared on fish feed ( $15.6\pm 0.3$  days) or poultry-feed ( $15.3\pm 0.3$  days) diets than biscuit ( $28.3\pm 1.2$  days) fed larvae. During the experiments we observed that the water became turbid within two days when poultry-feed was used.

Larval diets for large scale rearing of insects may have an impact on adult competitiveness, which is particularly crucial for mass release programmes under the Sterile Insect Technique (SIT).<sup>23</sup> When laboratory-reared insects are released in the field, they need to compete with their wild counterparts. It is expected that larger insects would compete with their wild counterpart more efficiently and our experimental data on the optimum diet type shows that fish-feed diet was able to produce larger males as well females than other types of diets tested. The protein content of fish-feed (17%) and in poultry-feed (16.5%) were very similar, which was also the case with other nutrients, but in biscuit the protein content was lower (7.3%). The presence of a relatively higher amount of proteins in both fish-feed and poultry-feed was most likely responsible for the development of larger adults as well as the shorter

developmental period. Hence fish-feed was found to be the best of these three larval diets for rearing *Ae. aegypti* larvae.

In insects, larval feeding and nutrient intake plays an important role in their being able to reach their critical mass for metamorphosis and subsequent adult development.<sup>24</sup> In female mosquitoes, the larval reserves have a significant impact on egg production<sup>25</sup> and in males, the larval reserves are essential for enhancing mating performance.<sup>26</sup> As fish-feed was found to be a suitable larval diet, further studies were carried out using fish-feed diet only.



Graph.1: Effects of larval diet on the wing length of male and female *Ae. aegypti*

### ***The effect of larval density on developmental period, pupal survival and wing length***

The effects of various larval densities viz., 200, 300, 400, 500, 700, and 1000 larvae per tray were investigated when fed with 0.2g fish-feed diet per tray daily. Data on the developmental period (from 1st instar to emergence of the last adult of each larval density tested) and pupal survival are presented in Table 1. Developmental period were found to be significantly prolonged at higher larval densities ( $F=181.23$ ,  $p<0.007$ ) with the feeding regime tested. The shortest developmental period was  $14.25 (\pm 0.2)$  days for a density of 200 larvae per tray ( $0.33$  larvae/ml,  $0.18$  larvae/cm<sup>2</sup>) while the slowest development was  $33.6 (\pm 1.2)$  days for a density of 1000 larvae per tray ( $1.67$  larvae/ml,  $0.92$  larvae/cm<sup>2</sup>).

Nevertheless, the pupal survival from larvae reared at all densities ranged between 90.2 and 95.4% and no definite trend of pupal survival was observed from rearing at different larval densities. Nor did we observe any significant differences in adult survival from pupation.

The wing size of males and females which developed from larvae reared at different densities was found to be decrease with increasing larval density (Graph. 2); larval density had a significant effect on the mean wing length of both males and females (Male wing:  $F= 56.96$ ,  $p<0.002$ ; Female wing:  $F= 66.23$ ,  $p<0.001$ ).

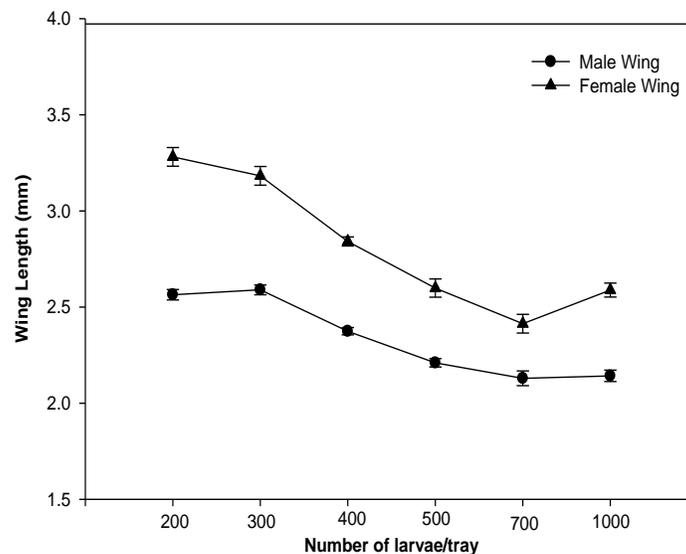
Our data along with other previous reports show that the developmental period extends as the larval density increases.<sup>13, 27, 28</sup> This signifies that either the larvae were in competition due to an insufficient amount of food being present in trays containing higher larval densities or that the available space in rearing trays were not enough for such high larval density rearing. Inherently strong larvae were able to compete for the food well and they grew more quickly than the weaker larvae. However, data on wing size of both sexes suggested that 0.2g diet per tray per day was insufficient for raising larvae at the higher densities (700 and 1000 larvae per tray), since wing length of both males and females was found to be reduced significantly as larval density increased. It has been reported that *Ae. aegypti* has stronger intraspecific completion than interspecific completion, which has an influence on life history traits.<sup>10, 29, 30</sup> Life-history traits of *Ae. aegypti* show strong phenotypic responses to larval competition and when larval competition become high developmental time increases and adult body weight, size decreases.<sup>31, 32, 33</sup> A strong negative correlation between wing size of *Ae. aegypti* and larval density or food concentrations have also been reported.<sup>34</sup> In mosquito control strategies, such as SIT which rely on the release of mass reared insects, the rearing protocol needs to be efficient and produce high quality mosquitoes which could compete for mating with the wild counterparts successfully and have a comparably long lifespan. Hence, adjustment of food availability and larval density is very crucial for mass rearing protocol optimization.

Data on pupal survival did not show any relationship with larval density, and though reduction of body size and delayed developmental period was observed at higher larval densities, we did not observe any such negative impact on pupation rate. Over 90% of larvae pupated in all experimental conditions. Some early studies have suggested that energy assimilated during early larval development converts into

structural growth of *Ae. Aegypti*.<sup>33</sup> A developmental threshold or a critical mass has been proposed which is required to initiate pupation.<sup>35, 36</sup> Based on the developmental period and the wing length, we conclude that 0.2g diet was insufficient for raising *Ae. aegypti* larvae at densities above 300 larvae per tray. However, more than 90% of larvae were able to pupate in all the densities. This result clearly indicated that the larvae from all the densities were achieved at least minimal reserve that allowed the last instars to go for pupation.

**Table: 1**Developmental period and pupal survival in *Ae. aegypti* reared at different larval densities and fed with 0.2gfish-feed diet per tray daily

Larvae/Tray (1500 ml)	Days (larva - adult) (SE)	Percent Pupal Survival (± SE)	Percent Adult Survival (± SE)
200	14.25 (SE ±0.2)	94.7(SE ±2.5)	99.0(SE ±0.2)
300	16 (SE±0.5)	93.5(SE ±2.8)	99.0(SE ±0.1)
400	20.5(SE ±1.5)	90.8 (SE ±2.8)	98.2(SE±0.4 )
500	26.3 (SE ±0.06)	93.7(SE ±0.6)	99.0(SE ±0.1)
700	33.0 (SE ±1.5)	95.4(SE ±1.5)	98.3(SE ±0.4)
1000	33.6 (SE ±1.2)	90.2(SE ±2.0)	97.9(SE ±0.7)



**Graph.2:** Wing length of male and female *Ae. aegypti* reared at different larval densities and fed daily with 0.2g fish-feed larval diet per tray

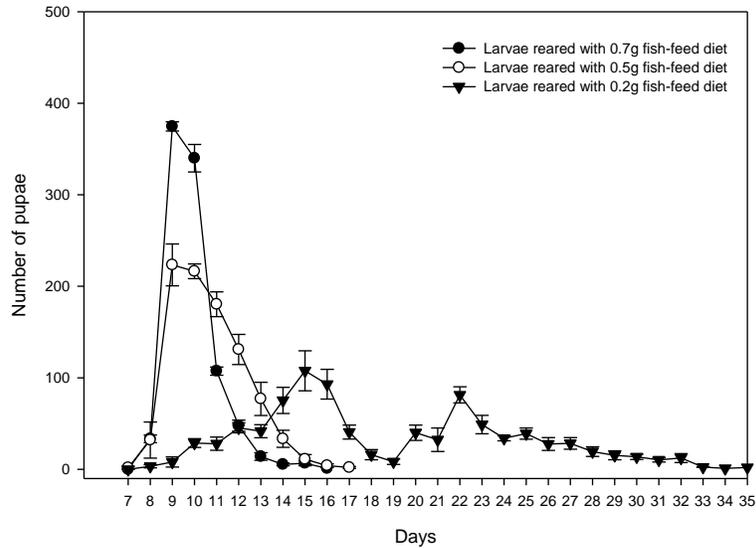
### ***Effect of availability of larval diet on developmental period and adult body size***

Prolonged larval development and reduced wing lengths were observed with larval density (Table 1), when larvae were reared with 0.2g diet per tray daily. As the effects were much more pronounced at the highest density, we attempted another sets of experiments providing 0.5 and 0.7g diet per tray daily to trays of 1000 larvae. Total developmental time was recorded as 16.6 ( $\pm 0.6$ ) and 15.3 ( $\pm 0.3$ ) days for rearing 1000 larvae with daily fish-feed provision of 0.5 and 0.7g respectively compared to a prolonged development time of 33.6 ( $\pm 1.2$ ) days (Table 1) when we provided 0.2g diet for 1000 larvae. During the larval stage if not enough nutrients can be accumulated, the larvae can wait for 2-3 weeks until they finally pupate or die.<sup>37</sup> The 14-33 days taken for pupation to occur when larvae were fed 0.2g fish-feed diet daily (Table 1) shows that this was insufficient to rear even 200-1000 larvae in our present rearing conditions. This is supported by the reduced wing length with increased larval density (Graph. 2). Our results showed a narrower peak of pupation when the amount of larval diet increased (Graph.3). However, a prolonged pupation with two minor peaks of pupation was observed when only 0.2g diet was provided for 1000 larvae per tray. Curves of pupation spanning 8.3 ( $\pm 0.3$ ), 9.6 ( $\pm 0.8$ ) and 26.3 ( $\pm 1.3$ ) days were recorded when providing 0.7, 0.5 and 0.2g diet for 1000 larvae respectively. From the onset of pupation to the 6<sup>th</sup> day, 97.1%, 86.2% and 12.6% pupae were collected from the colony reared with daily provision of fish-feed larval diet of 0.7g, 0.5g and 0.2g diet for 1000 larvae, respectively.

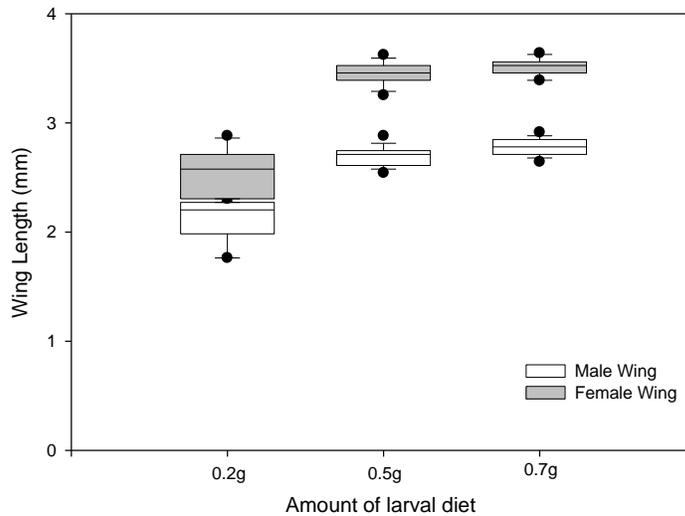
Body size (wing length) of both the male and female mosquito was found to be significantly dependent on the amount of larval diet provided when reared at the higher larval densities studied (Graph. 4). The Analysis of variance showed that increasing the amount of larval diet could produce larger adults of both sexes (male wing:  $F=507.69$ ,  $P<0.003$ ; female wing:  $F=799.93$ ,  $P<0.006$ ).

A shorter developmental period is advantageous for mass production of target insects for SIT applications, and so is a signifier of suitability of a rearing diet. Daily feeding of an increased amount of diet (0.7g) was sufficient to rear upto 1000 larvae without compromising the quality of adults of either sex. The wing size (a proxy for body size) was found to increase even more than the size of wild male and females of *Ae. aegypti* measured by Sumanochitrapon *et al.* the average wing size of wild *Ae. aegypti* males reported was between 2.2mm and 2.7mm, while the wing length of our lab-produced males ranged between 2.7mm and 3.0mm.<sup>38</sup>

Our experimental data on the larval density and the amount of diet clearly showed that the larvae of *Ae. aegypti* could be reared at a higher density than has been used in previous rearing (1000 larvae per tray) if a sufficient amount of an appropriate larval food was provided. Though larger males and shorter developmental time are obviously advantageous for mass production of mosquito for SIT applications, male performance and longevity are also very important.



Graph.3: Pupation curves for trays of 1000 larvae fed 0.2, 0.5 or 0.7g of fish-feed diet per day



Graph.4: Wing length of male and female adults reared on different amounts of larval diet. Trays of 1000 larvae were fed 0.2, 0.5 or 0.7g fish-feed diet daily, and the wings of resulting adults were measured and averaged by sex

### ***Longevity of males in respect to adult diets***

The longevity of the males was measured when they were provided with water only, 10% sugar solution, 10% sugar solution with 10% honey and under conditions of complete starvation. Single adult males were kept in individual paper cups, and the results are presented in Table 2. The average longevity of starved males, males provided with water, 10% sugar and 10% sugar plus 10% honey were 3.1 (SE±0.1), 11.2(SE±0.7), 17.1(SE±0.5) and 17.8(SE±0.3) days, respectively. The *analysis of variance* showed that adult diet had a significant effect on the longevity of male adults (F= 194.13, p<0.001); the longevity of the males supplied with 10% sugar or with 10% sugar with 10% honey did not differ significantly.

The variation in biological features of mosquito larvae and adults depends on local conditions in the field and on a range of other factors. For example, the mean male longevity varies from 15.7 days in the Orán strain to 33.8 days in the Rockefeller strain.<sup>39</sup> Adult body size has been reported to be positively associated with survival in *An. gambiae*.<sup>40</sup> Laboratory reared male *Ae. aegypti* were reported to survive an average of 14 days in the laboratory when provided with a 10% sugar solution at 28C° and 60-90% humidity.<sup>41</sup> Our laboratory produced males could survive 17.1 days when supplied with a 10% sugar solution under similar climatic conditions, and our findings clearly show that male adults require this sugar supplement for longer survival.

### ***Insemination***

The purpose of our study on the insemination rate was to evaluate the mating success of males reared under conditions found by the experiments above to be optimal in a laboratory setting, without considering the many other potentially important factors that may have significant impact on mating success. For this purpose we randomly chose 50 virgin males and 50 virgin females (1:1 sex ratio) from the population resulting from the 1000 larvae per tray, 0.7g fish-feed per day treatment in the experiment above conditions we showed to be the most suitable of those tested and allowed them to mate in a cage of 1m<sup>3</sup> for 5 to 10 days. This was done according to Helinski *et al.* and Matthews *et al.*<sup>42, 43</sup> Helinski *et al.*<sup>42</sup> allowed males and females to mate for 10 days and Matthews *et al.* allowed adults 5 days to mate in their experiment to investigate the effect of irradiation on insemination.<sup>43</sup> The insemination rate was determined by dissecting randomly selected females 5 to 10 days after introducing 50 virgin females into a cage containing 50 males. The presence of sperm observed under a microscope was scored as

positive while absence was scored as negative. The insemination rates were averaged across five replicate cages to give a value of 92.4% ( $\pm$ SE1.1). We did not observe any significant difference in insemination rate of the females dissected at day 5, 7 or 10 (data not shown). Many physical traits influence mating behavior and reproductive success in insects, among which body size, age etc. are well documented for many dipteran insects including mosquitoes.<sup>44</sup> Larger individuals have greater reproductive success<sup>18, 45, 46</sup> and the density of adults in a cage also has significant effects on mating success.<sup>47</sup> Our results indicated that laboratory males reared at a relatively high larval density and provided with sufficient larval diet were able to mate and transfer their sperm to females with a high level of success.

### ***Sex ratio***

In all experiments, the sex ratio of lab reared *Ae. aegypti* was found to be male biased under all rearing conditions tested. However, if we include only the results that were obtained from the high density rearing conditions supplemented with sufficient amount of larval diets the sex ratio was 54.5% male to 45.5% female (SE $\pm$  0.9). In some populations of *Ae. aegypti* a 1:1 sex ratio has previously been reported.<sup>48</sup> However this is not always the case. A laboratory population reared from material collected in Córdoba city, Argentina was reported to have a slight to high female preponderance if reared during summer time.<sup>49</sup> A genetic factor distorter causing abnormal segregation ratios, resulting in the excess survival of males in *Ae. aegypti* has been reported.<sup>50, 51</sup> Strains that produced a high proportion of male progeny would be an advantage in mass rearing facilities for SIT applications or other mosquito control programmes which rely on the release of a male-only population. So, this slight male preponderance would increase rearing efficiency to produce male mosquitoes.

### ***Time to adult emergence from pupation***

The time taken from pupation to adult emergence of a sample of pupae reared with 0.7g fish-feed diet at a density of 1000 larvae per tray was measured. The minimum and maximum time for adult emergence ranged between 40 and 49 hours. On average, adults were found to emerge from pupae after 44.5 (SE $\pm$ 0.4) hrs (44.3, 44.9 and 44.5 hours in replicates 1 to 3, respectively). Previous studies have shown that the pupation period of several subpopulations of *Ae. aegypti* from Argentina ranged from 1.87 to 2.41 days (44.88 to 57.84 hours) at a mean laboratory temperature of 22.9°C.<sup>39</sup> However, in our laboratory experiments we have maintained laboratory temperature at 27 $\pm$ 2°C. In general, at a

higher temperature insects require less time to complete their development, temperature may be a factor in this shorter pupation period. In addition, other rearing conditions namely diet and larval density may also affect the length of the pupal development. In this experiment less than 1% pupae were found to be unable to emerge as adults, though in the experiments as a whole this value was above 2% in more stringent rearing conditions (Table 1).

## **CONCLUSION**

This study clearly demonstrated that fish-feed larval diet of 0.7g daily could be used to rear 1000 *Ae. aegypti* larvae in a tray of 40 x 27.3 cm for producing large males with a short developmental period that are capable of inseminating females with a high success rate. A 10% sugar solution was found to be required to prolong male survival. It has been reported that older *Ae. aegypti* males under field conditions transfer the greatest number of sperm to females.<sup>47</sup> Another study showed that larger *Anopheles freeborni* males could mate with more females than smaller males.<sup>46</sup> Hence, the efficiency of mosquito population suppression methods such as SIT which rely on release of males which must compete for females in the field may be increased if larger males with a prolonged life could be produced.

Within the range of conditions tested within the setting of our laboratory rearing we determined those that produced the largest males with the longest lifespan. The tray used in this experiment can harbor 1000 larvae and produce satisfactory male quality. However, more research could be done with larger trays to rear increased numbers of larvae. The deployment of some automation such as for moving trays for dispensing food, collecting pupae and adding water to trays may be very helpful to reduce the manipulation time required in a mass production facility.

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