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# Evaluation of a new device for measuring the appropriate food quantity required for optimal developmental time, adult body size, and reduced mortality in insectary-reared *Anopheles* mosquitoes

**Siriporn Phasomkusolsil, Kanchana Pantuwatana, Jaruwan Tawong, Yossasin Kertmanee, Nantaporn Monkanna, Elizabeth W Wanja and Silas A Davidson**

**Abstract**
As part of the rearing process for *Anopheline* mosquitoes, a new method for accurate delivery of food to mosquito larvae was evaluated by the Armed Forces Research Institute of Medical Sciences (AFRIMS) Thailand. Larvae were fed using AFRIMS spoons or a standard salt shaker, and larval developmental time, mortality, adult size, and food remnants remaining in the larval rearing water were evaluated. The food quantity delivered by the spoons to *Anopheles dirus* and *An. minums* larvae (0.27, 0.18 g/day, respectively) was less than that from the salt shaker (2.20, 0.36 g/day, respectively). Larval development time was not affected by the different amounts of daily food. However, using the AFRIMS spoons lowered the overall mortality rates, produced larger females, reduced food remnant contamination of the rearing water, and improved mosquito production in the insectary. This food delivery method may be of benefit to other insectaries seeking to maximize their mosquito production.

**Keywords:** *Anopheles*, larval diet, quantity, developmental time, mortality, adult size

## 1. Introduction
In Thailand, five *Anopheles* species are considered to be serious malaria vectors. These include *Anopheles dirus*, *An. baimaii*, *An. minimus*, *An. pseudowillmori* and *An. aconitus* [1-4]. Malaria is a serious public health problem, which for many decades has ranked high among the leading causes of morbidity and mortality in Thailand [5]. Two mosquito species found along Thai–Myanmar border [6], *An. dirus* (Peyton & Harrison) and *An. minimus* (Theobald), are the most extensively studied mosquitoes in Thailand. Their interactions with humans and the *Plasmodium* species that cause human malaria are well-studied [7-9]. Colonies of both of these mosquito vectors of malaria are maintained in Thai insectaries and are used for numerous studies.

The Mosquito Insectary at the Armed Forces Research Institute of Medical Sciences (AFRIMS) Bangkok, Thailand, has cultured various *Anopheles* mosquito colonies used to study vector biology and vector–parasite interactions. Mosquitoes are required in large numbers for experimental studies in different laboratories within AFRIMS and other outside institutions. Rearing mosquito colonies for production of 100,000 female mosquitoes monthly is a technically challenging exercise, not least because the quality of the experimental mosquitoes needs to be high and their production constant. This quality is influenced by many factors such as population density, food availability, temperature, and humidity. Therefore, adopting good mosquito rearing practices in an insectary is critically important for maintaining high-quality mosquito colonies and preventing colony collapse. For larval maintenance, various rearing methods have been developed over many years. To achieve maximum production, the larval diet is an important factor for the successful rearing of mosquitoes, and numerous larval diets have been tested and adopted for use [10-13]. Although larval feeding with these diets is mostly satisfactory for larval growth and development, extra care is essential to

ensure that excessive food is not fed to the larvae, as left-over food pollutes the breeding water. It has been reported that the formation of scum from excess food on the surface of the water has deleterious effects on larvae, and that one night's scum can kill early instar larvae and pupae [14]. Hence, the purpose of this study was to test a set of well-calibrated spoons developed at AFRIMS for accurately measuring the amount of food required for feeding mosquito larvae in an attempt to mitigate the problem of overfeeding. We also investigated the practicality of using these spoons for the daily maintenance of the mosquito larvae colonies. The following four parameters were investigated: larval developmental time, mortality rate, adult body size, and scum weight. Food delivery via the AFRIMS spoons was compared with food delivery via a salt shaker for the larval mass rearing of *An. dirus* and *An. minimus* under laboratory conditions.

Our experiments show that using these spoons to accurately measure larval food not only proved to be a convenient and cost-effective way to feed larvae, but also improved the larval survival rate and supported a healthy mosquito colony.

## 2. Materials and Methods

### 2.1 Mosquito colonies and rearing conditions

Colonies of *Anopheles dirus* and *An. minimus* were reared and maintained using a standard rearing procedure at AFRIMS in Bangkok, Thailand during March-September, 2018. Adults were provided with a 5*%* solution *(*v*/*v*)* of multivitamin syrup (Multilim®, Atlantic Laboratories Corporation Ltd., Bangkok, Thailand) mixed with 5*%* sucrose *(*w*/*v*)* (Lin®, Thai Roong Ruang Industry Co., Ltd., Kanchanaburi, Thailand) soaked in saturated cotton.

### 2.2 Rearing procedures

The insectary used regulated temperature and humidity control systems for optimal growth of Anopheline mosquitoes. Mosquito maintenance and all the experiments conducted herein were carried out at 25±2 ºC and 60–80% relative humidity with a photoperiod of 12-h light followed by 12-h dark (12L:12D). The rearing process was started by placing approximately 300 eggs on the water surface of a plastic cup (15-cm diameter, 8-cm high) filled with 600 ml of filtered tap water. After hatching, the larvae were transferred to larval rearing pans (30×35 cm, 5-cm high) containing 2.5 L of filtered tap water. Water was added as necessary to compensate for evaporation loss, and the volume was held constant. Fish food (HIPRO®, Perfect Company Group Co., Samutprakarn, Thailand) was ground in a motor and sieved through a 42-mesh sieve (355 *μm* US standard no.) and then used fed to the larvae until they all pupated.

### 2.3 Larval feeding using AFRIMS spoons

Larvae were separated into two experimental feeding groups. The first instar of *An. dirus* larvae (n=150) and *An. minimus* larvae (n=200) were manually counted in each group. More numbers of *An. minimus* were included in each group because of their smaller size. One treatment group received finely-ground fish food measured using the AFRIMS spoons. These spoons were of three sizes (Fig. 1): a) size 0.01 g, diameter 2.5 mm; b) size 0.02 g, diameter 4.0 mm; and c) size 0.05 g, diameter 6.0 mm. The second treatment group received the same type of fish food as the first group but from a salt shaker. Feeding followed the schedule shown in Table 1. Six replicates were conducted for each treatment group. The first to the fourth instar larvae of *An. dirus* and *An. minimus* were fed with different food amounts using the AFRIMS spoons or the salt shaker (Table 2).

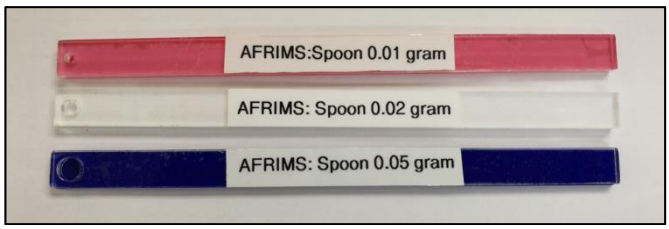


**Fig 1:** Design of the AFRIMS spoons used for larva food feeding

**Table 1:** Larval mosquito feeding schedule

| Mosquito age (Day) | Stage | Amount of food delivered by AFRIMS spoons | | | No. of shakes of the salt shaker (one=1 shake) | | |
|---|---|---|---|---|---|---|---|
| | | Morning at 0800 | Noon at 1200 | Afternoon at 1600 | Morning at 0800 | Noon at 1200 | Afternoon at 1600 |
| Day 0 | Eggs | - | - | - | - | - | - |
| Day 1 | Eggs | - | - | - | - | - | - |
| Day 2 | L1 | 0.01 g | - | - | One | - | - |
| Day 3 | L1 | 0.01 g | - | - | One | - | - |
| Day 4 | L2 | 0.02 g | - | - | One | - | - |
| Day 5 | L2 | 0.02 g | - | - | One | - | - |
| Day 6 | L2–L3 | 0.02 g | - | 0.02 g | One | - | One |
| Day 7 | L3 | 0.05 g | 0.05 g | 0.05 g | One | One | One |
| Day 8 | L3 | 0.05 g | 0.05 g | 0.05 g | One | One | One |
| Day 9 | L3-L4 | 0.05 g | 0.05 g | 0.05 g | One | One | One |
| Day 10 | L4 | 0.05 g | 0.05 g | 0.05 g | One | One | One |
| Day 11 | L4 / Pupae | 0.05 g | - | 0.05 g | One | - | One |
| Day 12 | L4 / Pupae | 0.05 g | - | - | One | - | - |
| Day 13 | Pupae | Pupal collection, debris removal and tray cleaning | | | | | |

**Table 2:** Mean food amounts (g) supplied to the larval stages of *An. dirus* and *An. Minimus*

| Mosquito sp. | Instars | Mean amount of food (g)/day* | | Mean amount of food (g)/instar | |
|---|---|---|---|---|---|
| | | AFRIMS spoons | Salt shaker | AFRIMS spoons | Salt shaker |
| *An. dirus* | L1 | 0.01±0.0 | 0.10+0.04 | 0.12±0.0 | 1.19±0.04 |
| | L2 | 0.04±0.0 | 0.19±0.05 | 0.48±0.0 | 2.34±0.05 |

| | | | | | |
|---|---|---|---|---|---|
| | L3 | 0.10±0.04 | 1.10±0.11 | 1.86±0.04 | 19.82±0.11 |
| | L4 | 0.12±0.04 | 0.81±0.49 | 2.65±0.04 | 19.36±0.49 |
| | Total | 0.27±0.06 | 2.20±0.52 | 5.11±0.06 | 42.71±0.52 |
| *An. minimus* | L1 | 0.01±0.0 | 0.02±0.01 | 0.18±0.0 | 0.25±0.01 |
| | L2 | 0.02±0.0 | 0.06±0.05 | 0.20±0.0 | 0.72±0.05 |
| | L3 | 0.05±0.02 | 0.15±0.07 | 0.74±0.02 | 2.48±0.07 |
| | L4 | 0.10±0.04 | 0.14±0.10 | 3.14±0.04 | 5.34±0.10 |
| | Total | 0.18±0.05 | 0.36±0.09 | 4.26±0.05 | 8.79±0.09 |

*Amount of food per tray.

### 2.4 Duration of larval development, pupation rate and mortality rate

All trays were checked daily at 08:00, 12:00, and 16:00 h and any water lost through evaporation was replaced. The stage of larval development was observed and the time from first instar (L1) to pupae was recorded. Pupae were collected daily using a dropper, counted, and then placed into water in the plastic cups. The cups containing the pupae from each treatment group were marked with the treatment group names until they emerged. The rate of pupation calculated for each treatment was based on the total number of pupae obtained at the end of the development period. The date of adult emergence and number of adults emerging were recorded daily. To assess the effects of the experimental food measuring device on mortality rates, dead larvae and pupae were counted daily. The larvae were touched gently with a glass rod and were considered dead when the signs of movement were absent. A pupa was considered dead if it did not move when prodded repeatedly with a soft brush. Any moribund larvae/pupae were added to the dead larvae/pupae for the purpose of calculating the mortality percentages. Dead larvae/pupae were removed from the rearing water whenever they were found. Missing larvae from each treatment were also recorded as dead.

The adequacy of a diet for mosquito larvae can be expressed via an index comprising the following two parameters: (1) the percentage of larvae that pupate and (2) the duration of larval development from the first instar to the fourth instar, the L1–L4 period. This index was calculated from the formula of Trager [15], as slightly modified below:

$$\text{Index} = \% \text{ Pupation} / \text{L1–L4 duration}$$

This index is useful for comparing the different amounts of food provided by the AFRIMS spoons and the salt shaker. When no pupation has occurred, the index value will be zero.

### 2.5 Wing length measurement

Mosquito wing length is known to be positively correlated with mosquito body size. Therefore, one day after emergence, 60 adults from each treatment (30 males, 30 females) were randomly selected for wing length measurement. The right wing was dissected from the body and placed into a drop of distilled water on a microscope slide and, using an eyepiece micrometer, the wing length was measured as the distance from the axillary incision to the apical margin (excluding fringes), as described by Nasci [16]. The mean wing lengths were calculated for each treatment group.

### 2.6 Food debris

On the last day of pupa collection, water in each tray was discarded by sieving it through a pre-weighed filter paper and a mesh. The solid materials collected on each filter paper were dried for 2 days at a constant temperature of 27 °C, and then weighed on an OHAUS® scale (Switzerland). The accuracy of the balance was tested by measuring the gram-weight fractions of an analytical standard from the US Department of the Army, US Army TMDE Region Pacific. The weight of the debris from each treatment group was recorded.

### 2.7 Data analysis

One way ANOVA was used to compare the effect of food provided to the larvae using the AFRIMS spoons with that of the salt shaker. A 95% confidence interval was used in Tukey's Honestly Significant Test to indicate a statistical difference for all possible pairs of means.

## 3. Results

The results of the food delivery experiments showed that the amount food delivered by AFRIMS spoons was less than that of the salt shaker (Table 2). The mean amount of food delivered to the *An. dirus* and *An. minimus* larvae by the AFRIMS spoons was low (0.27 and 0.18 g/day, respectively) compared with the mean value for the salt shaker, which was high (5.11 and 4.26 g/day, respectively).

### 3.1 Duration of larval development, pupation rate and mortality rate

The larval developmental periods, pupation rates, and adult emergence rates for *An. dirus* and *An. minimus* larvae fed by either AFRIMS spoons or the salt shaker are shown in Table 3. The development rates for the larval stages of both species are shown in Fig. 2. The results show that the developmental time of the first instar (L1) to the fourth instar (L4) for *An. dirus* was not affected by the different amounts of food provided daily ($P$=0.343). *An. dirus* larvae fed on the lower food amount using AFRIMS spoons showed a developmental time from L1–L4 of 10.8 d relative to those fed on the larger food amount from the salt shaker (11.0 d). The developmental time (L1–L4) for *An. minimus* larvae differed significantly when fed using the two different devices ($P$=0.047). Larvae feeding using AFRIMS spoons produced significantly faster development (12.8 d) than larvae fed using the salt shaker (13.7 d). After the beginning of pupation and adult emergence, significant differences between the two different feeding devices were detected in the proportion of both *An. dirus* and *An. minimus* pupae and adults ($P$<0.05). When fed using AFRIMS spoons, the *An. dirus* larvae produced a significantly higher proportion of pupae (93.9%) and adults (92.8%), compared with the group fed using the salt shaker (82.9% for pupae and 73.1% for adults). Also, the *An. minimus* pupation rate and adults emergence were 95.7% and 92.3% when using AFRIMS spoons and 82.5% and 69.6% using the salt shaker, respectively. The value of the index gave some indication of the extent of metamorphosis into the adult stage. A longer developmental period would have been

of little value. In this experiment, the index values for the AFRIMS spoons and the salt shaker were 8.69 and 7.54 for *An. dirus*, and 7.47 and 6.02 for *An. minimus*. Most of the larvae became adults in around 2 weeks. The effect of food quantity on length to pupation was also apparent (Fig. 3) in *An. minimus*. The length of the *An. dirus* pupal stage showed no significant difference between the lower-amount diet provided by using AFRIMS spoons (3.8 d) and the higher-amount diet provided by the salt shaker (4.8 d) (*P* value 0.073), but there was a significant difference in the time taken by *An. minimus* to pupate (*P* value 0.001).

Table 4 shows the mortality rates for each stage from L1 to adult emergence. The mean mortality rate for *An. dirus* larvae when fed using AFRIMS spoons was 6.11% whereas the rate for the salt shaker was 17.11%, a statistically significant difference (*P* value 0.014). However, the mortality rate for the pupal stage was not influenced by feeding using AFRIMS spoons or the salt shaker. Notably, the adult mortality rate was found to significantly decrease with decreasing amounts of food delivered by the AFRIMS spoons (*P* value 0.001). Furthermore, mortality from the first instar (L1) to adult *An. dirus* was significantly lower for the group fed using AFRIMS spoons (7.22%) compared with the salt shaker (26.89%; *P* value, 0.002). Additionally, the mortality rates for *An. minimus* larvae and pupae were significantly influenced by the feeding method, with the rates for AFRIMS spoons at 4.25 and 2.17% and the salt shaker at 17.50 and 11.67%, respectively. For *An. minimus* L1 to adult mosquitoes, feeding using the AFRIMS spoons resulted in substantially lower mortality (7.67%) than that observed when the salt shaker was used (30.42%; *P* value, 0.003). These results indicate that larvae fed on the smaller amounts of food delivered by the AFRIMS spoons could survive for longer than those fed on the larger amounts of food delivered by the salt shaker.

**Table 3:** Effects of larval diet delivery via AFRIMS spoons or the salt shaker on developmental time, pupation and adult emergence in *An. dirus* and *An. minimus* mosquitoes. Mean values within a column followed by the same letter are not significantly different (*P*>0.05, Tukey's honestly significant test).

| Mosquito sp. | | Developmental period (L1 – L4) (days) ±SD | % Pupation ±SD | % Adult emergence ±SD | Index |
|---|---|---|---|---|---|
| *An. dirus* | AFRIMS spoons | 10.8±0.4a | 93.9±3.3a | 92.8±4.5a | 8.69a |
| | Salt shaker | 11.0±0.0a | 82.9±8.5b | 73.1±9.7b | 7.54b |
| | *P*-value | 0.343 | 0.0144 | 0.002 | 0.012 |
| *An. minimus* | AFRIMS spoons | 12.8±0.4b | 95.7±1.1a | 92.3±1.9a | 7.47a |
| | Salt shaker | 13.7±0.8a | 82.5±7.6b | 69.6±13.6b | 6.02b |
| | *P*-value | 0.047 | 0.0021 | 0.0026 | 0.001 |

**Table 4:** *An. dirus* and *An. minimus* mortality rates (%) at different stages. Mean values within a row followed by the same letter are not significantly different (*P*>0.05, Tukey's honestly significant test).

| Mosquito sp. | *An. dirus* | | | | *An. minimus* | | |
|---|---|---|---|---|---|---|---|
| | AFRIMS spoons | Salt shaker | *P*-value | | AFRIMS spoons | Salt shaker | *P*-value |
| Larval mortality ±SD (%) | 6.11±3.33b | 17.11±8.54a | 0.014 | | 4.25±1.13b | 17.50±7.60a | 0.002 |
| Pupal mortality ±SD (%) | 0.44±0.54a | 1.67±2.49a | 0.266 | | 2.17±0.61b | 11.67±5.65a | 0.002 |
| Adult mortality ±SD (%) | 0.67±1.03b | 8.11±3.14a | 0.001 | | 1.25±0.82 | 1.25±0.61 | - |
| L1 to adult mortality ±SD (%) | 7.22±4.53b | 26.89±9.74a | 0.002 | | 7.67±1.94b | 30.42±13.61a | 0.003 |

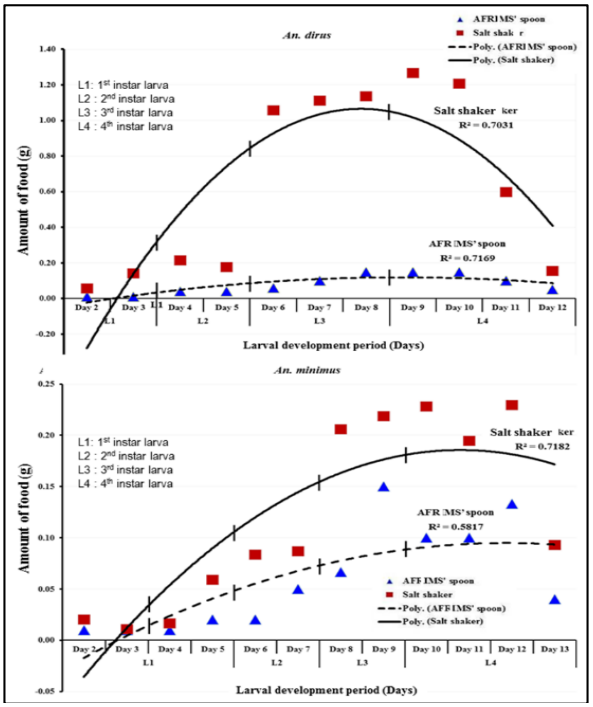


**Fig 2:** Amount of food and average instar duration of *An. dirus* and *An. minimus* larvae fed using AFRIMS spoons or the salt shaker.

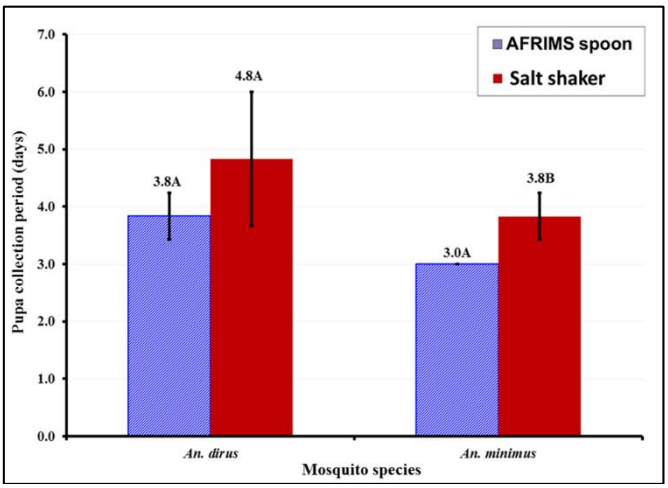


**Fig 3:** Length of time to pupation for *An. dirus* and *An. minimus* from larvae fed using AFRIMS spoons or the salt shaker. Mean values followed by the same letter are not significantly different from one another ($P$>0.05, Tukey's honestly significant test).

### 3.2 Wing length

The mean wing length of *An. dirus* females differed significantly after with larval feeding using AFRIMS spoons (3.91 mm) or the salt shaker (3.65 mm, $P$<0.05). The wing length in males was significantly affected after use of the feeding device ($P$<0.05); the mean wing length for adults that emerged from larvae fed via AFRIMS spoons (3.61 mm) was longer than the wing length (3.37 mm) for the males fed via the salt shaker (Table 5). Similarly, the wing length for *An. minimus* females whose larvae were fed via AFRIMS spoons (2.93 mm) was significantly longer than in females that were fed via the salt shaker (2.81 mm, $P$<0.05). The mean wing lengths for the *An. minimus* males that emerged from the larvae fed via the AFRIMS spoons were slightly longer (2.89 mm) than those fed via the salt shaker (2.83 mm; $P$ value 0.045).

**Table 5:** Wing lengths (mm) for adult male and female *An. dirus* and *An. minimus* after emerging from the larvae fed using AFRIMS spoons or the salt shaker. Mean values within a row followed by the same letter are not significantly different ($P$>0.05, Tukey's honestly significant test).

| Mosquito sp. | *An. dirus* | | | *An. minimus* | | |
|---|---|---|---|---|---|---|
| | **AFRIMS spoons** | **Salt shaker** | ***P*-value** | **AFRIMS spoons** | **Salt shaker** | ***P*-value** |
| Adult size's female ±SD (mm) | 3.91±0.08a | 3.65±0.06b | 0.000 | 2.93±0.09a | 2.81±0.09b | 0.000 |
| Adult size's male ±SD (mm) | 3.61±0.07a | 3.37±0.08b | 0.000 | 2.89±0.11a | 2.83±0.10b | 0.045 |

### 3.3 Food debris

The weight obtained for the dried food remains from the larval rearing water trays for *An. dirus* and *An. minimus* was 188.1 and 120.2 mg for the salt shaker, respectively, the values of which were significantly higher than those obtained for the AFRIMS spoons at 81.9 and 71.2 mg, respectively; $P$ values 0.0007 and 0.0006, respectively (Fig. 4).

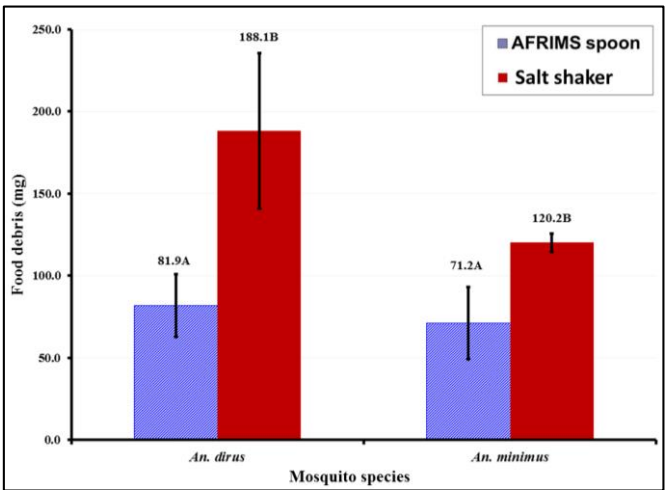


**Fig 4:** Weight of food debris obtained from the larval rearing water. Mean values followed by the same letter are not significantly different from one another ($P$>0.05, Tukey's honestly significant test).

## 4. Discussion

Mosquito larval rearing is affected by several factors including rearing density [17], water temperature [18] and food quality and quantity [19]. Studies have shown that providing the appropriate quality and quantity of larval food is important for mosquito development and survival [20], adult emergence [21] and body size [22]. In larval mosquito rearing, underfeeding can cause as much developmental delay as overfeeding, but will likely be evident later on, especially in the adult stage. Several studies have investigated the relationships between food quantity, larval development and adult production. When *An. maculipennis* larvae were fed an improper diet, they displayed delayed larval development and decreased survival up to the fourth instar [23]. There is also evidence that mosquito developmental time and mortality increase and adult body size decreases when food is scarce [24-26]. Chambers and Klowden [27] observed that *Aedes aegypti* larvae that are starved after reaching their critical weight will generally molt to pupae, but if they are starved before they reach this weight they eventually die without reaching the pupal stage. In insectary-based rearing systems, artificial larval diets, which are often a mix of ingredients such as fish food, bovine liver powder, squid liver powder, soy meal, dog biscuits, and brewer's yeast, are known to be effective food items for mosquito rearing [12, 28-30]. Food quantities are generally specified by weight or by volume. However, most laboratories do not specify exact quantities, but use informal measurements such as a pinch, a drop or even coverage on the water surface. The ideal quantity of larval food required for Anopheline larval stages is extremely difficult to gauge. Technicians generally provide too much food, so much that

the water becomes brown and murky and a film potentially forms on the surface reducing the amount of oxygen available for the larvae. Therefore, over or underfeeding are common problems seen with mosquito rearing.

In this study, we studied the effects of larval food quantity by using a carefully calibrated new device (named AFRIMS spoons) for assessing the amount of food required for the optimal growth and development of *Anopheles* mosquitoes in a laboratory setting. The spoons were used to measure a specific amount of larval food to identify the amount that best supports larval development. The spoon was designed in three sizes and is made of poly methyl methacrylate (also known as acrylic plastic). The spoons are lightweight, impact resistant, easy to clean, making them a good choice for larval feeding. To make the spoons, three strips, each 1 cm wide and 15 cm long, were cut from a 4-mm-thick acrylic sheet. A hole was drilled on the top of one side far from the edge to prevent cracking the edge of the acrylic sheet, and we ensured that the drill bit did not penetrate the sheet.

Mass production of *An. dirus* and *An. minimus* in an insectary requires a balanced diet that favors high survivorship, consistently good larval development and body size, and produces healthy, high quality adults. Thus, the amount of food provided to Anopheline larvae is clearly a critical component of mosquito rearing. The preliminary studies indicate 0.01-0.12 g diet/day/tray is an effective feeding amount by an AFRIMS spoon, is the optimal quantity for *An. dirus* and *An. minimus* development, in terms of achieving a faster time to pupation, higher survival rate, and larger adult size. Our experiments have also shown that the amount of larval food provided to larvae using the AFRIMS spoon strongly affected larval development. We found that it was very difficult to gauge the optimal amount of food when using the salt shaker, and its inconsistency of measurement led to either over or under feeding of the larvae. This is of concern because higher survivorship during the larval stages as well as the available energy reserves that influence flight potential [31] are related to competitive advantages at the adult stage.

Wing length has been used as a morphometric measurement of body size and weight in mosquitoes [32], a feature that is more important for females because it determines lifetime fecundity [33]. Previous studies have reported that well-nourished, larger adult females have greater survival potential, blood-feeding success [16, 34, 35], vectorial capacity [36] and produce more eggs during their lifetimes than their smaller counterparts [37]. Hence, deficiency in larval food is expected to produce smaller adults. The mean value of the wing length in the female *Anopheles* that developed from larvae fed using the AFRIMS spoons was larger than that of *Anopheles* females from some other laboratories [38, 39]. Some studies have reported that supplying an improper larval mosquito food quantity decreases the vectorial capacity of *Anopheles* females infected with pathogens [40, 41], in terms of survival, length of the gonotrophic cycle, and the number of emergent adults. Additionally, laboratory studies have shown that the larval diet is strongly impacted by quantity in terms of adult blood feeding and reproductive success [42].

Our experimental results showed that overfeeding with the salt shaker contaminated the larval rearing water, which may have resulted in larval suffocation. The mortality rate data showed that the larvae did not survive as well in water with excess food from the salt shaker as in water where the larvae were fed via AFRIMS spoons. Scum from food remnants from the salt shaker was evidently a problem because the immature stages of *Anopheles* were found dead at the bottom of the rearing trays. The lethal effect of an overabundance of food has been attributed to scum formation on the surface of the breeding medium in other mosquito species [14].

## 5. Conclusion

An optimal larval diet is a critical requirement for successful mass rearing of immature *An. dirus* and *An. minimus* larvae. To ensure optimal development, the quality and quantity of artificial larval diets should be taken into account for mass rearing [43]. Our results emphasize the importance of ensuring that the optimal quantity of larval diet is provided. This study showed that a precise and measured method of delivering larval food is better than the salt shaker method. Further experiments are needed to determine the optimal feeding amount for each Anopheline as it likely varies from species to species.

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