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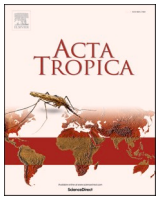

Optimizing and synchronizing *Aedes aegypti* colony for Sterile Insect Technique application: Egg hatching, larval development, and adult emergence rate

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ABSTRACT

Mosquito Sterile Insect Technique (SIT) programs can be developed in smaller agencies through synchronization of the colony development to take advantage of the natural male early emergence. This paper examined key aspects of *Ae. aegypti* colony synchronization work, including egg hatching, larval development, and adult emergence to produce sufficient numbers of adult male mosquitoes within a specific timeframe for irradiation and release. Our data indicated that a relatively low percentage of males are required for colony propagation. Additional results highlighted that fresher *Ae. aegypti* eggs could yield as high as a 93 % hatching success than older eggs when placed under vacuum pressure in yeast infused water for 1.5 h. Eggs that were one-month old hatched (93 %) better than older eggs (0–32 %). A higher egg density in the hatching flask was correlated to a lower hatch rate, and higher larval density was related to unsynchronized pupae and delayed adult emergence. By keeping *Ae. aegypti* larvae at reasonable density, over 95 % of adults emerged on the first two days of emergence – indicating a high synchronicity. A standardized colony maintenance protocol therefore renders a synchronized larval development and adult male emergence which are critical in SIT programs.

1. Introduction

Sterile Insect Technique (SIT) has emerged as a promising tool for controlling *Aedes aegypti* populations (Aldridge et al., 2024), which are vectors for deadly diseases such as dengue, Zika, and chikungunya (Olivia et al., 2021). While promising, SIT has also been intimidating for medium to small mosquito control agencies due to the cost of large-scale releases. For instance, the Mediterranean Fruit Fly eradication program required 500,000 sterile fly releases per square mile per week (USDA, 2012). Most mosquito abatement/control districts are unable to facilitate this scale of production and release. For a more targeted approach, colony maintenance, larval development and adult emergence must be carefully controlled. To optimize colony maintenance for SIT, several factors must be considered, including standardizing egg hatching, synchronizing larval development, timing adult emergence and separating males efficiently. By taking advantage of the natural emergence of males first, the colony can be synchronized to produce males on a specific timeframe for irradiation and release. Thus, there is a need for enhancing sustainable and effective mosquito production in the insectary using optimized workflow and standardized procedures.

Indeed, the primary undertaking in mosquito colony is ensuring effective egg hatching, larval development, and adult emergence. Hatching is induced in *Aedes* eggs by depletion of dissolved oxygen in the surrounding water (Zheng et al., 2015). To produce large numbers of mosquitoes for SIT purposes, a high hatching rate is necessary. However, water alone does not create the favorable medium for *Aedes* eggs to hatch. To hatch *Ae. aegypti* eggs, different labs use different techniques such as yeast solution (Byttebier et al., 2014), deionized water (Allan and Kline, 1998), boiled deionized water (Saifur et al., 2010), dechlorinated water (Dieng et al., 2016), 0.01 % solution of ascorbic acid (Barbosa and Peters, 1969) and bacterial broth (Wohl and McMeniman, 2023; Zheng et al., 2015). All these different techniques aim to deoxygenate the water – an important environmental cue that stimulates eggs to hatch. However, identifying the easiest and best hatching technique continues to be a challenge for insectaries in different settings and scales.

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The duration of storage of mosquito eggs is also key in determining the hatching success rate. Studies have reported the storage duration of *Ae. aegypti* eggs ranging from 3 months (McMeniman and O'Neill, 2010) to 5 years (Mayilsamy, 2019) depending on laboratory conditions, and associated reductions in hatching success with longer storage time. Determining the maximum longevity of *Ae. aegypti* eggs for good hatching is therefore essential.

Optimizing larval rearing conditions is key for promoting rapid growth and development while minimizing mortality rates. Proper larval growth helps enhance mosquito fitness and adult emergence rates. Several studies have noted that overcrowding should be avoided to prevent competition for resources and reduce stress on larvae (Barbosa et al., 1972; Barrera, 1996; Yadav et al., 2017). High mortality, long larval periods, and the small size of resulting adults were observed when the larval density was high, as well as when the amount of food provided was small (Wada, 1965). Larvae should therefore be reared in spacious containers with ample surface area to facilitate efficient feeding and ultimately result in better adult fitness.

Finally, for continued propagation of the colony, understanding the minimum number of males required to sustain the colony is critical. This is accomplished potentially by additional rounds of blood feeding for parous females (Xue et al., 1995). Since the SIT consumes almost all of the male mosquitoes, it is necessary to determine the minimum number of males needed to keep the routine colony maintenance.

Therefore, optimizing colony work protocols is central to ensure efficient scaled rearing of mosquitoes for SIT programs. This paper aimed to explore strategies for synchronizing and optimizing key aspects of *Ae. aegypti* colony work, including egg hatching, larval development, and adult emergence to enhance the collection of adult males for SIT.

2. Methods

2.1. Overall colony maintenance

A colony of locally obtained *Ae. aegypti* (L.) was established at the West Valley Mosquito and Vector Control District Laboratory in Ontario, California. Eggs were hatched using vacuum pressure. Larvae were reared in plastic tubs (35.9 × 19.4 × 12.4 cm) with 2 L of tap water and 0.44 g alfalfa rabbit food pellets. A further 0.44 g alfalfa pellets were provided to third and fourth instar larvae. Pupae were collected into 540 mL cylindrical plastic containers half-filled with water from larval tubs and placed in a cage (30 × 30 × 30 cm) for emergence. Approximately 2000 adults were maintained per cage and provided 10 % sucrose solution ad libitum. Room temperature was maintained at 27° ± 2 °C and relative humidity of 55–65 %, with a 12:12 hr light:dark photo period. One week after emergence, female mosquitoes were blood fed (bovine whole blood with 3.8 % NaCit) (Animal Technologies, Tyler, Tx) for three days using a Hemotek blood feeding apparatus (PS-6 System, Discovery Workshops, Accrington, UK) with chicken skin coverings. Sucrose solution was removed from the adult cages overnight before blood feeding. After blood feeding, adults were again provided with 10 % sucrose solution ad libitum. Egg collection was performed 5 days after blood feeding. A 540 mL plastic container lined with Whatman filter paper (9 cm diameter) was half-filled with tap water and 0.0620 g of brewer's yeast and placed in each mosquito cage. After 5 days, the plastic container was removed from the cage, and the filter paper was retrieved and left to desiccate at room temperature. Eggs were then manually counted under a microscope and placed in a storage bag kept at room temperature until needed.

2.2. Comparing Ae. aegypti egg hatching techniques

An experiment aiming at assessing the effect of using different vacuum pressures on egg hatching success and synchronicity was conducted. Three treatments were tested for egg hatching synchronicity: different vacuum pressures, the duration under vacuum pressure, and the addition of yeast. Batches of 200 eggs, in storage for <5 weeks, were hatched under varying conditions. A 1-L filtering flask filled with 400 mL of tap water was used for hatching. Flasks were set up separately for vacuum pressures of 500 mmHg (−67 kpa) and 600 mmHg (−80 kpa), with each vacuum pressure tested for a 1-hour period and a 1.5-hour period. Hatched larvae were counted under a microscope and then transferred into a larval rearing tub along with the egg filter paper. After 24 h, hatched larvae were counted again, and egg filter paper was removed. To determine the effect of brewer's yeast on hatching, 0.0620 g of brewer's yeast was added to the 400 mL hatching water under 600 mmHg for 1-hour and 1.5-hours. Hatched larvae were counted then transferred into a larval rearing tub with the egg filter paper and counted again after 24 h.

2.3. Hatching rate by egg age

Stored eggs were used to determine the effect of storage time on egg hatching rate. One hundred eggs (±5 %) from collections aged 1, 2, 4, 5, 8, and 9 months old, were hatched in triplicate in filter flasks under vacuum pressure of 600 mmHg for 1.5 h with 0.0620 g yeast infused in 400 mL tap water. Hatching room climate was maintained at 27 °C temperature and 65 % humidity. The contents of the flask were transferred into larval rearing tubs. Egg filter papers were removed after 24 h. Larvae were recorded at the third instar stage.

2.4. Effect of egg density on hatching rate

To determine the effect of egg density on hatching rate, three egg densities (500, 750 and 1000) were hatched in triplicates. First, eggs were inspected under microscope for any deformity and those with deformed eggshells were excluded from hatching. For each cohort, hatching was done in filter flasks with 400 mL tap water infused with 0.0620 g brewer's yeast under vacuum pressure 600 mmHg for 1-hour in 27 °C room. The flask contents were then transferred to larval rearing tubs. Egg filter papers were removed after 24 hrs and inspected under microscope to count eggshells opened to ensure hatching. Hatched larvae were recorded by counting under microscope and hatched eggshells were inspected under microscope for opening for confirmation.

2.5. Effect of larval density on pupae synchronization and emergence rate

The effect of larval density on synchronization of pupation rate was studied. Eggs were hatched in the manner as the egg density tests above (500 eggs/flask). Three days after hatching, larvae were counted and divided into cohorts of 200, 250, 300 per larval rearing tub in triplicates. All larval rearing tubs were synchronously fed (Day 1, 4, and 6) with increasing proportional amounts of alfalfa depending on the density of larvae. Pupae were recorded daily. Adult emergence was monitored, and freshly emerged adults were recorded daily and removed from the cages.

2.6. Comparing fecundity in different male to female ratio experiment

To compare the fecundity of females in different male to female ratios, pupae were collected and placed in cups (177.4 ml) half-filled with water and covered with dome screen lids. Emerged adults were aspirated out of cups within 24 h of emergence and sexed. Adults were placed in separate cages with male to female ratios of 1:1, 1:2, 1:3, 1:4, and 1:5 with a total of 60 adults per cage. Each ratio cohort was prepared in triplicate. Adults were given 10 % sucrose solution ad libitum. Mosquitoes were provided with two cycles of blood feeding and eggs were collected after each blood feeding. Fecundity was compared across the ratio cohorts in the two blood feeds. The number of fed mosquitoes were counted before egg laying and these numbers were used to calculate fecundity for each cage. Resulting eggs were counted and recorded.

2.7. Effect of successive blood feeding on fecundity

Utilizing male to female ratio of 1:1 from previous experiment, female mosquitoes were provided a second blood meal one week after egg collection and additional eggs were collected the following week. Successive third and fourth rounds of blood feeding were conducted one week after the previous one for a total of 4 blood meals. The resulting eggs were dried, counted, and stored at room temperature. Eggs generated from each blood meal round were hatched and larvae recorded at the third instar stage.

2.8. Statistical analysis

Hatching rates were compared among the different hatching techniques. Differences between egg hatch rates were analyzed using generalized linear model (GLM) with binomial distribution where the response variable was the number of hatched eggs (number of larvae), and the explanatory variable was the hatching technique (500 mmHg, 600 mmHg and addition of yeast) and the timing of the egg filter paper removal (immediately vs after 24 hrs). To assess significant differences, post-hoc comparisons among cohorts were made with Fisher's LSD test on ranks, adjusting the significance of the test with the Holm–Bonferroni correction for multiple comparisons (Abdi, 2010).

Hatching rates of egg cohorts of different ages and egg densities were also compared using generalized linear model (GLM) with binomial distribution. Fecundity of different male-to-female ratios for the two blood feeding cycles was compared using two-way ANOVA. In this analysis, we examined the effect of male-to-female ratios, blood-feeding cycles and their interaction on the dependent variable, fecundity. Shapiro and Bartlett tests were done to test the normality and homoscedastic of the data. To assess significant differences, post-hoc tests (Tukey's HSD) were conducted.

Pupation rate was calculated as the number of pupae collected per day out of the total larvae in the previous day. The effect of larval density on pupation and emergence rate was compared using GLM with binomial distribution. Adjusting the significance of the test with the Holm–Bonferroni correction for multiple comparisons, the Post hoc comparisons among cohorts were made with Fisher's LSD test on ranks to test for significant differences among egg densities and storage durations.

Synchronization of pupation was defined as the proportion of pupation out of the total larvae in a given day. Similarly, synchronization of emergence was defined as the proportion of adults emerged in a given day out of the total pupae in the container. After ensuring normality of data, Repeated Measures ANOVA was applied to compare mosquito pupation and emergence rates over three consecutive days. The number of pupae and adults emerged each day were considered as dependent variables while days (Day 1, Day 2, and Day 3) were considered as independent variables.

For all the tests, significance level of 5 % was set ($P < 0.05$). The dataset was analyzed using R software (IBM Corp, Version 27.0, Armonk, NY). Standard errors (±SE) were indicated on graphs.

3. Results

3.1. Comparing Ae. aegypti egg hatching techniques

Hatching rates of *Ae. aegypti* eggs were compared among different hatching techniques (Fig. 1). Raising the vacuum pressure from 500 to 600 mmHg (GLM: $\chi^2 = 22.15$; df = 2; $P < 0.05$) and the duration from 1 hr to 1.5 hr ($\chi^2 = 19.52$; df = 1; $P < 0.05$) significantly increased the hatching rate. Adding yeast further boosted the hatching rate from 44.5 to 85.2 % ($\chi^2 = 28.31$; df = 1; $P < 0.05$). Overall, adding yeast and retrieving the egg filter paper the following day yielded a high hatching rate (post-hoc: $F = 13.11$; $P < 0.05$).

3.2. Hatching rate by egg age

Hatching rates were compared among cohorts of eggs stored from one to nine months (Fig. 2). Eggs that were one- (93 %) and two-month (81 %) old hatched significantly (GLM: $\chi^2 = 36.52$; df = 3; $P < 0.05$) higher than older eggs (0–33 %). Eight- and nine-months old eggs did not hatch at all. Two-month-old eggs (81 %; post-hoc: $F = 9.31$; $P < 0.05$) hatched better than older eggs (ranging 0–33 %).

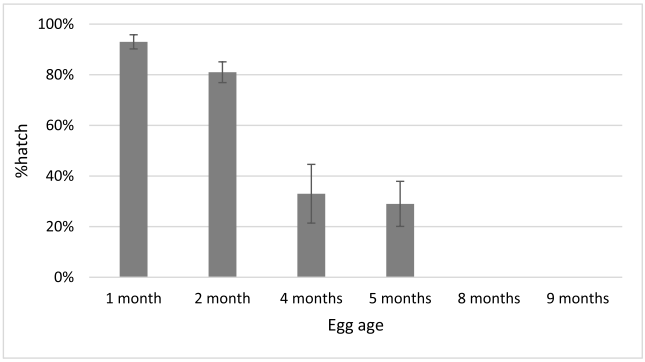

Fig. 2. Hatching rate (%) comparison of *Ae. aegypti* eggs stored for 1–9 months under room temperature. SE are indicated on the bars.

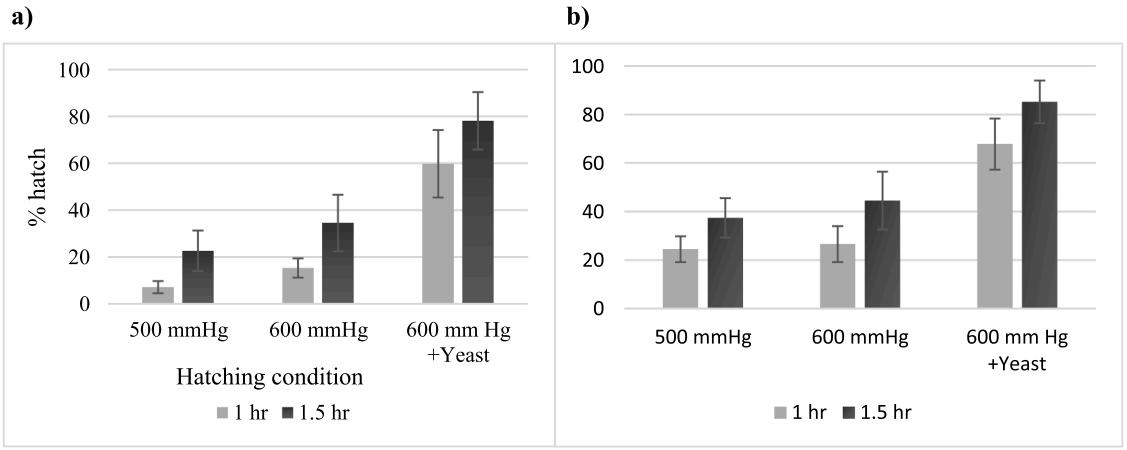

Fig. 1. Hatching rate (%) of *Ae. aegypti* eggs when under vacuum pressure and yeast for 1 hr and 1.5 hr. a) when egg filter paper strips with eggs left for 1 hr and removed; b) when egg filter paper strips left for 24 h and removed the following day. SE is indicated on the bars.

3.3. Effect of egg density on hatching rate

Hatching rates were compared among cohorts of different egg density (500, 750 and 1000 eggs per flask) (Fig. 3). The lowest egg density (500 eggs/flask) had the highest hatching rate (mean = 95 %) while the highest egg density (1000 eggs/flask) was associated with relatively low hatching rate (82 %). The difference in hatching rate among the three egg density cohorts was significant (GLM: $\chi^2 = 16.12$; df = 2; $P < 0.05$).

3.4. Effect of larval density on pupae synchronization

Pupation rate was compared among cohorts with different larval density (Table 1). Synchronization of pupae declined with increasing larval density (GLM: $\chi^2 = 34.88$; df = 2; $P < 0.05$). The lowest larval density cohort (200 larvae per tub) had the highest synchronized pupation (97.1 % in the first day; post-hoc; $F = 7.13$; $P < 0.05$). Pupation rate in cohorts with higher larval densities was generally less synchronized (71.3–83.7 % pupated in the first day) and pupation continued for four days.

3.5. Effect of larval density on adult emergence rate

Emergence rate was compared among the cohorts with different larval density (Fig. 4). The cohort with lowest larval density (200 larvae per tub) showed a higher synchronicity (ANOVA, $F_{(2, 7)} = 11.9$; $P < 0.05$) as 95.2 % of *Ae. aegypti* adults emerged in the first two day of emergence. In contrast, cohorts with higher larval density showed extended emergence as emergence continued for four days. About 90 % of males in the cohort with lowest larval density emerged in the first day and the remining emerged the following day while emergence in the cohorts with highest larval density continued for four days (Fig. 4).

3.6. Comparing fecundity in different male to female ratio cages

Fecundity of female mosquitoes was compared among cohorts with different male to female ratios (Table 2). Ratios of 1:1 and 1:2 had significantly higher ($F_{(4, 13)} = 7.18$; $P < 0.05$) egg production that the other ratios in both the first and second round blood feeding. The number of eggs laid in the first-round blood feeding was generally higher than the second blood feeds.

3.7. Effect of successive blood feeding on fecundity

Fecundity of mosquitoes and egg hatching rate showed the same trend across the four successive blood meals (Fig. 5). Eggs from the first blood meal had a higher hatch rate (mean = 88 ± 4.1 %; $F_{(3, 10)} = 33.43$, $P < 0.05$) while those from second to fourth blood meals had lower hatch rates (62 % – 66 %). Overall, both fecundity (ANOVA: $F_{(3, 10)} = 33.43$, $P < 0.05$) and hatching rate (GLM: $\chi^2 = 41.15$; df = 3; $P < 0.05$)

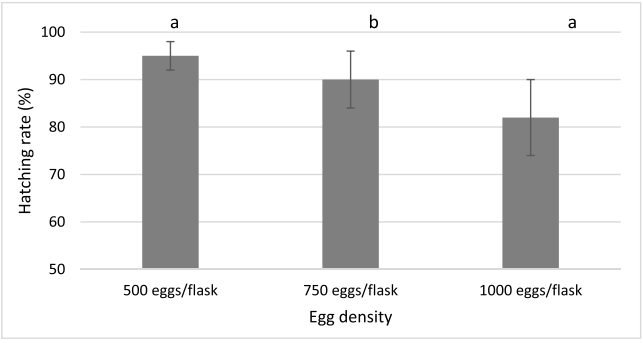

Fig. 3. Effect of *Ae. aegypti* egg density (500 eggs/flask, 750 eggs/flask and 1000 eggs/flask) on hatching rate. Same letters indicate significant difference ($P < 0.05$, One-way ANOVA, Tukey HSD test).

Table 1
Daily pupation rate comparison among the three cohorts with different larval density.

Larval density cohort		Total pupae collected	Mean±SE no pupae in each tub daily	% collected out of the total pupae
200 larvae/tub	Day 1	545	181.7 ± 8.2	97.1 %
	Day 2	13	4.3 ± 2.1	2.3 %
	Day 3	2	0.7 ± 0.3	0.4 %
	Day 4	1	0.3 ± 0.2	0.2 %
250 larvae/tub	Day 1	427	142.3 ± 18.4	83.7 %
	Day 2	51	17.0 ± 4.6	10.0 %
	Day 3	21	7.0 ± 3.2	4.1 %
	Day 4	10	3.3 ± 2.1	2.0 %
300 larvae/tub	Day 1	353	117.7 ± 21.4	71.3 %
	Day 2	73	24.3 ± 6.3	14.7 %
	Day 3	35	11.7 ± 4.9	7.1 %
	Day 4	34	11.3 ± 3.5	6.9 %

significantly declined with successive blood feeding (

4. Discussion

Establishing a standardized and synchronized mosquito rearing protocol to produce substantial numbers of competent male mosquitoes in a certain time period is key in scaled SIT application. This requires optimized egg hatching and larval rearing procedures. Our study highlighted that fresher *Ae. aegypti* eggs could yield as high as a 93 % hatching success than older eggs when placed under vacuum pressure in yeast infused water for 1.5 h. This technique is simple and adoptable by small-scale insectaries that are looking into establishing *Ae. aegypti* colony. Our study also underscored that egg density in hatching flasks and larval density in tubs could influence larval growth synchronization, pupation and emergence of *Ae. aegypti* mosquitoes in the insectary.

4.1. Factors affecting egg hatching rate

With lower levels of dissolved oxygen being a key factor in hatching *Aedes* eggs (Gjullin, 1941; Zheng et al., 2015), egg hatching technique plays an important role to ensure a synchronized larval development and emergence of *Ae. aegypti* for SIT application. The present study indicated that eggs placed under vacuum of 600 mmHg for the duration of 1.5 hour in hatching media containing brewer's yeast resulted in a high hatching rate. Similarly, previous studies have shown a boost in *Ae. aegypti* egg hatching when yeast (Byttebier et al., 2014), bacteria broth (Zheng et al., 2015) or partial vacuum (Barbosa and Peters, 1969) were utilized for hatching. The higher proportion of egg hatch in the yeast solution is coincident with expectations based on results of previous studies (Ponnusamy et al., 2011; Byttebier et al., 2014) because yeast simulates the bacterial bloom, which in turn triggers egg hatch in natural conditions. On the other hand, egg density has been shown to affect hatching rate in this study and elsewhere (Livdahl et al., 1984). Density-dependent competition in insects is often associated with delayed maturity and increased juvenile mortality (Couret et al., 2014; Schmidt et al., 2018). This may occur because ammonia influences the microbe populations on which mosquito larvae feed, or it may be a stressor to developing larvae as indicated by Moore and Fisher (1969).

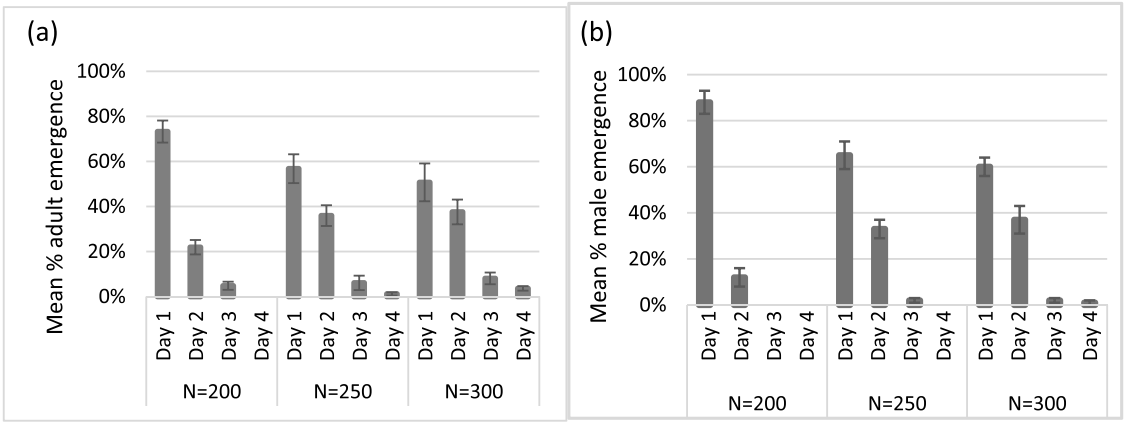

Fig. 4. Effect of *Ae. aegypti* larval density on synchronization of mosquito emergence. (a) total mosquito emergence; (b) male mosquito emergence. N represents larval density per tub for each cohort. SE indicated on the bars.

Table 2
Effect of male to female ratios and successive blood meal feeding on fecundity of female *Ae. aegypti* mosquitoes. (Different letters indicate statistical significance, ANOVA, $P < 0.05$ among the group).

M:F ratio	Mean ± SE no eggs/female		
	First round blood feed	Second round blood feed	Difference
1:1	105.63±14.8[a]	76.47±12.25[a]	$P = 0.085$
1:2	111.15±17.2[a]	61.80±8.7[a]	$P = 0.071$
1:3	61.13±8.9[b]	42.85±9.3[b]	$P = 0.002$
1:4	52.67±11.1[b]	28.18±10.8[b]	$P < 0.001$
1:5	38.68±5.9[b]	23.67±7.2[b]	$P = 0.004$

Growth retardant factor is also produced by overcrowded larvae, an effect that is not species-specific (Moore and Fisher, 1969). Growth retardant factor has been shown to lengthen development and prevent pupation in some Culicine mosquito species (Roberts, 1998). Further studies are needed to demonstrate what exactly causes low egg hatching in high density eggs.

One of the factors that could affect the viability of eggs is the duration of storage. Mayilsamy (2019) noted that while *Ae. aegypti* eggs stored for up to five years could somehow hatch, there was a remarkable reduction in the hatching success with an increase in storage duration. In our study, fresher eggs (one month old) rendered the highest hatching rate while older eggs, stored 5–9 months, yielded less or no hatch at all. Factors such as the immersion temperature, hatching medium, and physiological conditions of eggs determine the hatching response of *Ae. aegypti* eggs (Byttebier et al., 2014; De Majo et al., 2017; Mohammed and Chadee, 2011). The lower hatching rate of older eggs is probably caused by the loss of water in the eggs as a result of transpiration (Meola 1964). Thus, to hatch, these eggs will need repeated stimuli to first compensate for the loss of water as suggested by Byttebier et al. (2014). Egg hatching success could influence the production of mosquitoes during SIT. While older eggs could still hatch at a lower rate, the number of eggs needed to hatch to mass harvest mosquitoes for SIT program would be compromised by the low hatch.

4.2. Factors affecting mosquito fecundity in the lab

Several factors such as male to female ratio could affect female mosquitoes' fecundity in the insectary. Our data indicated that the higher the male to female ratio the higher the fecundity rate. Male *Ae. aegypti* are considered to be polygynous, potentially mating with multiple females within one day, while females are considered to be generally monandrous over their lifetime (Carrasquilla and Lounibos, 2015; Lounibos et al., 2002). This could be beneficial for *Ae aegypti* colony maintenance in SIT colony work since substantial number of eggs can be harvested even with very low ratio of males to females in the cages. Additionally, this indicates that a small proportion of males would be sufficient in a cage to maintain *Ae aegypti* colony in the insectary while using the majority for SIT.

Our study showed that fecundity of female *Ae. aegypti* decreased with consecutive blood meals (Table 2). Helinski and Harrington (2011) reported $a > 50$ % reduction in fecundity of female *Ae. aegypti* mosquitoes that after the fourth cycles of blood feeding. This is because multiple blood feeding depletes the sperms females stored from copulation.

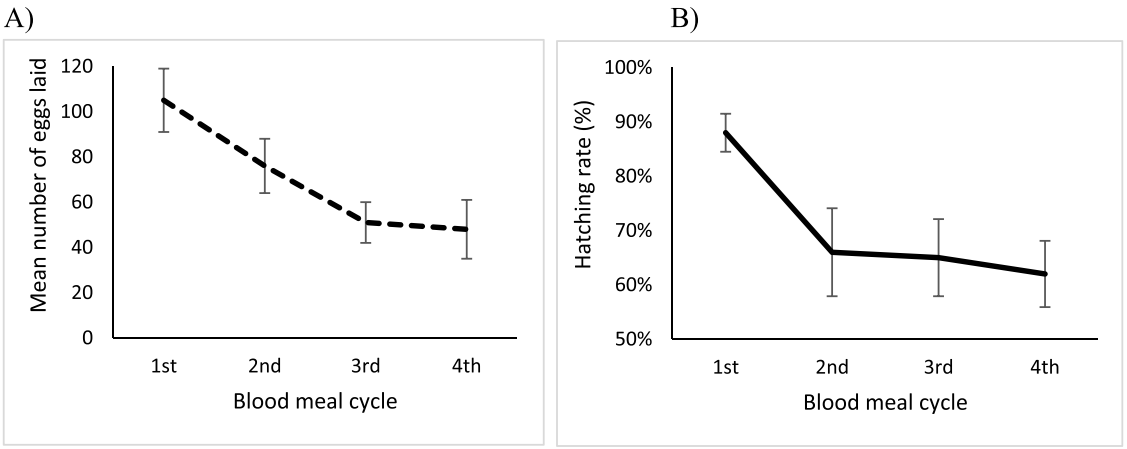

Fig. 5. Comparison of fecundity (A) and hatching rate (B) of *Ae. aegypti* following successive blood meals.

Similarly, hatching rate dropped from 88 % in eggs from first blood meal to 62 % in those from the fourth blood meal. This may suggest the natural factor that older mosquitoes would become less effective in producing eggs. Likewise, Petersen et al. (2018) indicated that the mean number of eggs laid by a female *Ae. aegypti* decreased from 63.2 in the first blood meal to 42.3 in the second blood meal. Female *Ae. aegypti* mosquitoes store and maintain sperm internally in the spermathecae (Gullan and Cranston, 2010) and continue fertilizing their eggs in successive blood meals for prolonged periods of time (Carrasquilla and Lounibos, 2015). Our study demonstrated that while additional blood meals can produce additional eggs for colony propagation, this must be balanced with the reduced hatching observed in these eggs.

4.3. Factors affecting larval synchronization and adult emergence

While many studies show the effects of larval density on health and synchronization of mosquito development (Barbosa et al., 1972; Livdahl, 1984; McLean-Cooper, 2008; Wada, 1965; Yadav, 2017), it leaves the question of whether colony synchronization would be affected at the earliest stage by egg density during hatching. Our study indicated that a higher egg density in the hatching flask results in lower hatching rate. This would indeed ultimately impact the synchronicity of mosquito emergence by affecting the larval growth as shown in this study (Fig. 4). Similarly, higher larval density influences adult emergence synchronization. Scaled SIT requires healthy and synchronized larval development and adult emergence so that a required number of mosquitoes can be collected in specific timeframe (one or two days). By keeping a reasonable larval density, over 95 % of the males emerged on the first day of emergence. Such high synchronization is particularly essential especially to smaller agencies as it helps to efficiently utilize the limited manpower and resources for SIT related colony work.

In conclusion, this study demonstrated simple but efficient *Ae. aegypti* egg hatching techniques and how egg and larval densities affect the synchronization of larval development and adult emergence. A standardized colony maintenance protocol that renders synchronized larval development and adult male emergence is therefore required in SIT programs. Without being intimidated by the resources SIT require for area-wide application by large agencies, small- and medium-size vector control agencies could plan targeted applications using optimized techniques that help synchronize mosquito development to produce adult males in a specific timeframe for SIT mosquito releases.

CRediT authorship contribution statement

Jennifer Thieme Castellon: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Solomon Kibret Birhanie:** Writing – original draft, Writing – review & editing, Methodology, Investigation, Validation, Formal analysis, Data curation, Conceptualization, Supervision. **Ale Macias:** Writing – review & editing, Methodology, Investigation, Data curation. **Rubi Casas:** Writing – review & editing, Methodology, Investigation. **Jacob Hans:** Writing – review & editing, Methodology, Investigation. **Michelle Q. Brown:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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