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Optimization of *Aedes albopictus* rearing procedures: Preliminary steps towards large-scale rearing of the species within the laboratory in Mauritius

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Abstract
As preliminary steps towards a large-scale production of *Aedes albopictus,* egg hatching, blood feeding and larval rearing procedures were optimized in the national entomological laboratory in Mauritius. A mixture consisting of 28% tuna meal, 36% bovine liver powder and 36% brewer's yeast in dechlorinated water, induced more hatching in eggs of *A. albopictus* (mean of 99.3%) than in four other media. Blood source (human and bovine) did not have a major effect on female survival, fecundity and egg hatchability. However, egg production was highest in cages where blood was provided on the roof top using Hemotek feeders as compared to cages where Hemotek feeders or blood-filled sausages were placed inside the cage. Furthermore, two locally-manufactured animal feeds costing approximately 1 USD per kilogram, Aquatro Tilapia Pre Grower™ and Chewli™, were as effective in rearing *A. albopictus* larvae as the IAEA diet (a diet optimized in Vienna for rearing the species).

Keywords: *Aedes albopictus*, hatching, blood feeding, larval diet

1. Introduction
The possibility of using the Sterile Insect Technique (SIT) as a major component of an Integrated Vector Management Strategy to control *Aedes albopictus* mosquitoes is currently being investigated in Pointe des Lascars and Panchvati, two villages in Mauritius [1]. The SIT in this particular context, involves a sustained massive release of laboratory-produced radio-sterilized *A. albopictus* males which must be fit enough to survive, disperse and compete for females within the target zone so as to reduce the reproductive potential of its wild population in the long term [2]. While rearing and irradiation processes should be optimized to minimize their negative effects on mosquito fitness, it is equally important especially within an SIT production unit, to ensure a synchronized development of the mosquito and to use materials and procedures that are cost effective, easily available and reproducible [3, 4]. As a first step towards a large-scale rearing of *A. albopictus* within the laboratory of the Vector Biology and Control Division, Mauritius, the purpose of this study was to optimize production of the mosquito using inexpensive, readily available materials. Protocols for inducing egg hatch in *A. albopictus* mosquitoes were conceived and compared with other published procedures. The impact of two easily available blood sources and three blood feeding methods were assessed on female fitness and fecundity. Furthermore, with the aim of finding a relatively inexpensive but equally effective substitute to the 'IAEA' larval diet (a diet specifically conceived for rearing *A. albopictus* at the FOA/IAEA Insect Pest Control Laboratory [5]), nine locally-available food components were assessed in their ability to rear the species' larv*ae.*

2. Materials and Methods
2.1. Experiment 1: Hatching media
This experiment was carried out to evaluate the efficacy of 6 media used for inducing egg hatch in *A. albopictus*. Hatching medium 1 was a 0.01% solution of ascorbic acid [6] (UPSA-C, Bristol-MyersSquibb, France; henceforth referred as 'Vit C') while medium 2 consisted of 83% CM0001 Nutrient Broth (Oxoid, Hampshire, England) and 17% brewer's yeast [7] (henceforth referred as 'NB'). Hatching media 3 and 4 (henceforth referred as IAEA1 and IAEA2), consisted of 50% tuna meal (Livestock Feeds Ltd, Mauritius), 36% bovine liver powder (MP Biomedicals, Santa Ana, CA) and 14% brewer's yeast [5] (MP Biomedicals,

Santa Ana, CA) except that in the latter medium, 0.01% wt: vol vitamin mix (Vanderzant Vitamin Mix, Bio-Serv, Frenchtown, NJ) was also added. The proportion of brewer's yeast was increased and vitamin mix not added in hatching medium 5 (henceforth referred as IAEA3) which consisted of 28% tuna meal, 36% bovine liver powder and 36% brewer's yeast.

For each treatment, five opaque hermetic jars (12 cm in height and 12 cm in diameter) each containing 1 L of warm dechlorinated tap water (26 °C) and one strip of egg paper with 200 *A. albopictus* eggs (aged 5 to 6 days old), were used. Egg papers were immersed in solutions containing 0.4 g/L of the respective food mixture. Larvae were counted 17 h after egg immersion and the egg papers subsequently bleached [8] to determine the total number of viable eggs. Hatch rate was calculated by dividing the number of larvae by the total number of viable eggs.

2.2. Experiment 2: Concentration of hatching media

This experiment was carried out to evaluate the hatching efficacy of six diluted concentrations (0.125, 0.25, 0.5, 1.0, 2.0, 4.0 g/L) of the IAEA3 food mix consisting of 28% tuna meal, 36% bovine liver powder and 36% brewer's yeast. Dechlorinated tap water was used as a control. The materials and methods used in this experiement, were similar to that described in Experiment 1 except that 10 replicates of 100 eggs (11-days old) were used for each dilution.

2.3. Experiment 3: Blood source

In this experiment, the effect of two sources of blood (human and bovine) on the survival, fecundity and egg hatching rates of F11 *A. albopictus* females were investigated. Human blood (11-days-old) from blood packs obtained from the blood bank of a nearby hospital (Victoria Hospital, Candos, S-20 16' 37" E-57 28' 19") and bovine blood (4-days-old) originating from a slaughter house in Roche Bois (Mauritius Meat Authority, S-20 8' 55" E-57 30' 31"), were used.

2.3.1. Female mortality

For each treatment, 5 Bugdorm cages (30 by 30 by 30 cm, MegaView, Taichung, Taiwan), each containing 50 virgin females (3-days-old) with constant access to a 10% sucrose solution, were set up. 5 ml of warm blood (37 °C) were provided to each cage for 30 minutes daily on 12 consecutive days using the Hemotek system (Hemotek PS6A/220) following the procedure outlined by Damiens *et al.* [9]. Female mortality was read at the end of the experiment.

2.3.2. Female fecundity and egg viability

For each treatment, 150 virgin females (3-days-old) were caged with 150 males (4 to 5-days-old) in Bugdorm cages for 48 hours. On the third morning, cages were blood fed following procedures outlined in the above section. For each treatment, 35 blood gorged females were individually transferred to plastic tubes (2 cm diameter, 10 cm long) lined with moist filter paper (Sartorius Stedium Biotech, Göttingen GmbH, Germany) for oviposition. Oviposition tubes were checked daily and ovipositing females were allowed 3 days to complete oviposition before the oviposition papers were removed from the tubes and left to maturate for 5 days. For each treatment, 20 oviposition papers containing maturated eggs, were counted and immersed in a hatching solution containing 0.125 g/L IAEA3 food mix. Newly hatched larvae (L1) were counted 17 hours after immersion and the egg papers were then bleached [8] to determine the total number of viable eggs. Hatch rate was calculated by dividing the number of L1 larvae by the total number of viable eggs laid per female.

2.4. Experiment 4: Blood feeding method

In this experiment three artificial blood feeding methods were investigated using F17 *A. albopictus* females and human blood (10-days-old) obtained from the blood bank of a nearby hospital (Victoria Hospital, Candos) was used. In the first method, blood was given by placing one aluminium blood plate of the Hemotek system (3.7 cm in diameter, containing 5 ml of warm blood covered with collagen membrane) on the top of each cage (Fig. 1a) while in the second method, a similar blood plate was placed in an inverted position (i.e with the collagen membrane facing the roof) inside each cage (Fig. 1b). The third method was relatively similar to that used by Deng *et al.* [10] and Balestrino *et al.* [11] where a collagen sausage (Edicas 23NC, FIBRAN, S.A., Girona, Spain) filled with 10 ml of warm blood was vertically inserted inside each cage (Fig. 1c).

For each treatment, 5 Bugdorm cages each containing 250 virgin females and 250 males were set-up with constant access to a 10% sucrose solution. Each cage was blood fed for 30 minutes daily during five days. At the end of the five days, a plastic beaker (8 cm in diameter) half-filled with dechlorinated tap water and lined with filter paper (Sartorius Stedium Biotech, Göttingen GmbH, Germany) for oviposition was inserted in each cage. Oviposition papers were replaced with fresh ones every two days until no more eggs were obtained from the cages. Fecundity was expressed as the total number of eggs collected from the treatment cages at the end of the experiment.

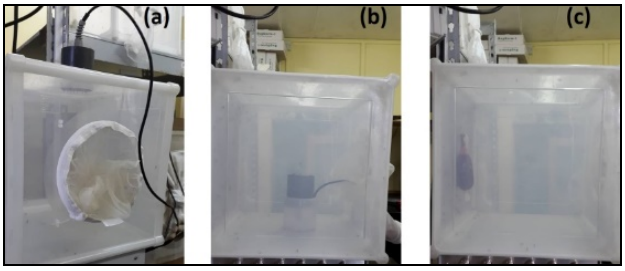

Fig 1: Three blood feeding methods with **(a)** Hemotek feeder on the roof top of a cage **(b)** Hemotek feeder in an inverted position inside a cage and **(c)** Blood-filled collagen sausage inside a cage.

2.5. Experiment 5: Larval diet optimization

Two experiments were carried out to optimize diet formulation for *A. albopictus* larvae. The IAEA diet conceived by the FAO/IAEA Insect Pest Control Laboratory in Vienna [5] (henceforth referred as the 'IAEA_Thai' diet), was used as a reference diet. In the first part of the experiment (Experiment 5A), 8 locally available diets (Aqu A, Aqu B, Chewli, Dog V., Koi, Aquarium, Rabbit and Veg) as well as a local substitute for the tuna meal powder used in the IAEA_Thai diet (henceforth referred as 'IAEA_Mau' diet), were tested. The two best candidate diets were selected for the second part of the experiment (Experiment 5B) and the effects of 4 additives (bovine liver powder, chickpea, brewer's yeast and vitamin mix) were tested to further optimize the diets.

The IAEA_Thai and IAEA_Mau diets consisted of 50% tuna meal, 36% bovine liver powder, 14% brewer's yeast and 0.4% wt:vol vitamin mix [5] while the 'Veg' diet is a combination of locally available grains consisting of equal proportions of red bean, corn, wheat, chickpea, rice, and bovine liver powder [12]. Table 1 shows the cost and supplier of the diet components as well as their abbreviated names henceforth used.

Table 1: Cost, supplier and abbreviations of raw materials used in larval diets for *A. albopictus* mosquitoes.

Diet / Diet components	Abbreviation	Supplier	Price/Kg (USD)
Bovine Liver Powder	BLP	MP BIOMEDICALS, LLC	114.4
Brewer's yeast	BY	MP BIOMEDICALS, LLC	24.1
Vanderzant Vitamin mix	Vit mix	Bioserv, US	56.0
Aquatro Tilapia Pre Grower (30% protein)	Aqu A	Livestock Feed Limited, Mauritius	0.7
Aquatro Tilapia Pre Grower (35% protein)	Aqu B	Livestock Feed Limited, Mauritius	0.9
Rabbit Production Feed	Rabbit	Livestock Feed Limited, Mauritius	0.6
Dog Food Vital	Dog V.	Livestock Feed Limited, Mauritius	1.1
Chewli Dog food	Chewli	Meaders Feeds Limited, Mauritius	0.9
Koi Granules Croissance	Koi	Marks Aquarium, Mauritius	6.0
Kora Aquarium food	Aquarium	Marks Aquarium, Mauritius	13.3
Tuna Meal (used by the IAEA)	-	T.C. Union Agrotech, Bukkalo Thailand	14.7
Tuna Meal (locally available)	-	Livestock Feed Limited, Mauritius	1.5
Red beans	-	Local grocery, Mauritius	1.8
Corn	-	Local grocery, Mauritius	2.1
Chick pea	-	Local grocery, Mauritius	1.7
Brown rice	-	Local grocery, Mauritius	3.2
Wheat	-	Local grocery, Mauritius	1.6

2.5.1. Experiment 5A

Raw materials were grinded and coarse particles removed using a sieve of 149 microns. A 7.5% wt: vol slurry of each diet was prepared by mixing the solid components in tap water. The mixtures were stored in the refrigerator at 4 °C and used daily for larval feeding. For each diet, 20 newly hatched larvae (L1), were manually counted and added to each of five containers (5 cm in diameter) filled with 30 ml of dechlorinated tap water so as to obtain a larval density of 0.7 L1 per ml in a water depth of 1.5 cm. On days 1, 2, 3, 4 and 5, respectively 53 µL, 107 µL, 160 µL, 213 µL and 107 µL of each diet were pipetted in the corresponding container. This amounted to 2.4 mg of solid food diet being given per larva over the 5 days of feeding.

All containers were checked daily at noon for pupae and the latter once formed, were transferred into transparent plastic tubes (2 cm diameter, 10 cm height) containing 8 ml of tap water. Each tube was covered with a netting and checked for adult emergence on a daily basis. Upon emergence, adults were sexed and their emergence date recorded. Time to pupation and time to emergence were respectively calculated as the development duration between L1 to pupa and L1 to adult stages. Survival to pupation and survival to adult emergence were calculated as the proportion of larvae that survived from L1 to the pupal stage and from L1 to the adult stage, respectively.

In a mass rearing facility, a diet that can accentuate protandry and produce males distinctively smaller than females (sexual dimorphism) is desirable since that help to increase the efficiency of male separation which is usually done mechanically using sieves. Often, only pupae formed in the first 24 h of pupation (which are usually mostly males) are harvested and mechanically sieved [13]. Hence, in this study, male pupae production rate at 24 h was defined as the number of male pupae formed in the first 24 h (from the beginning of pupation) divided by the total number of male pupae collected from each diet treatment. The sex ratio of pupae formed in the first 24 h (sex ratio at 24 h) was calculated as the proportion of male pupae to total pupae produced in the first 24 h from pupation onset. Overall sex ratio was calculated as the proportion of male pupae over total pupae produced throughout the experiment.

For each diet treatment, the right wing from a sample of 20 male and 20 female individuals were removed under a dissecting microscope. Wings removed were measured from the distal edge of the alula to the end of the radius vein excluding fringe scales (Packer and Corbet, 1989). A digital image of the wing was made using an Axiocam ICc5 camera (Carl Zeiss Microscopy GmbH, Germany) mounted on a Stemi 2000-C stereomicroscope (Carl Zeiss Microscopy GmbH, Germany), and measurements were performed with Zen 2012 SP1 analySIS B software (Carl Zeiss Microscopy GmbH, Germany).

2.5.2. Experiment 5B

The two best candidate diets (in terms of effectiveness and cost) from Experiment 5A were selected and IAEA_Thai was kept as the reference diet. To further optimize the two chosen diets (Aqu B and Chewli), the effects of 4 additives (bovine liver powder, chickpea, brewer's yeast and vitamin mix) were evaluated. 20% of the respective additives were added to each of the two candidate diets. A mixture consisting of 10% bovine liver powder, 10% brewer's yeast and 80% candidate diet was also tested. The 10 combination diets were referred to as Aqu B/BLP, Aqu B/pea, Aqu B/BY, Aqu B/BLP/BY, Aqu B/Vit, Chewli/BLP, Chewli/pea, Chewli/BY, Chewli/BLP/BY, Chewli/Vit. The experimental set up and recording of growth parameters were similar to those described in Experiment 5A.

2.6. Data analysis

Frequency data were angle transformed (arcsine sqrt) while count data log transformed (Log_{10} (n+1) before testing for normality (Anderson-Darling test) and homogeneity of variances (Levene's test) prior to statistical analysis. Excluding Experiment 5, differences in measured parameters among treatment groups were analyzed using one-way ANOVAs. For Experiment 5, General Linear Models [14] were used to evaluate the impact of larval diet on growth parameters. Means were separated by Tukey's Post hoc test. For pairwise comparison, the Student *t* tests or the non-parametric Mann-Whitney U tests were used. All statistical analyses were performed using Minitab 16 (Minitab Inc., State College, PA) with alpha level of 0.05. Unless otherwise stated, back-transformed values (mean and 95% confidence interval (CI)) are presented in the text and figures to aid interpretability.

3. Results

3.1. Experiment 1: Hatching media

Hatching media had a significant effect on the hatching of *A. albopictus* eggs (F = 107.49; df = 4, 20; P<0.0001). Hatch

rate (mean, 95% CI) in IAEA3 (99.3%, 95.7% - 99.9%) was significantly greater than in the Vit C, IAEA1 and NB media (Post hoc Tukey tests, $P<0.05$) while no significant differences in egg hatch was noted among NB, IAEA1 and IAEA2 (Post hoc Tukey tests, $P>0.05$). The results are illustrated in Fig. 2.

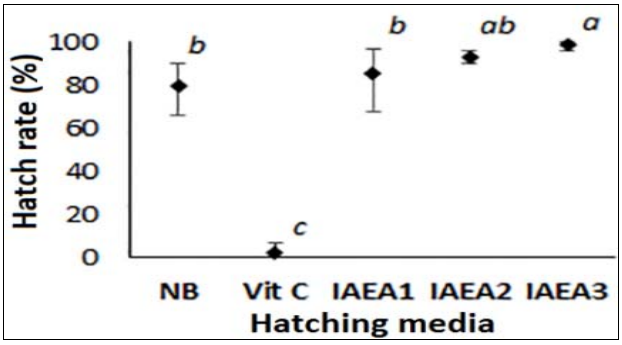

Fig 2: Hatch rate (mean, 95% CI) of *A. albopictus* eggs 17 hours after immersion in five hatching media (NB, nutrient broth; Vit C, 0.01% ascorbic acid; IAEA 1, a solution of tuna meal, brewer's yeast and bovine liver powder; IAEA 2, a mixture of IAEA and vitamins; IAEA 3, IAEA1 with thrice the yeast concentration). Different letters represent statistical differences between treatments (Post hoc Tukey tests, $P<0.05$).

3.2. Experiment 2: Concentration of hatching media

Hatch rate in the control solution consisting of water only (mean ± SEM, 4.1 ± 2.5%) was not included in the statistical analysis. No significant differences in hatch rate were noted among the different dilutions of IAEA3 food mix solution ($F = 0.76$; df = 5, 54; $P = 0.584$). The results are illustrated in Fig. 3.

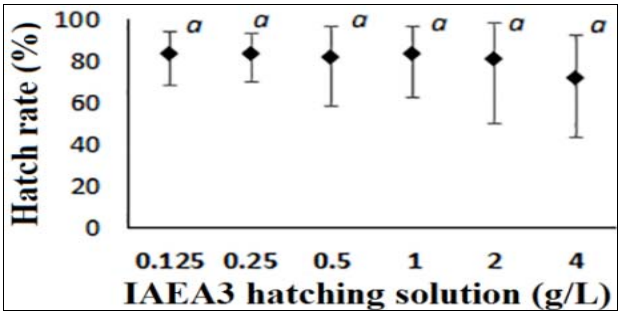

Fig 3: Hatch rate (mean, 95% CI) of *A. albopictus* eggs 17 hours after immersion in six diluted concentrations of IAEA 3 food mix (consisting of 40% tuna meal, 30% bovine liver powder, 30% brewer's yeast). Different letters represent statistical differences between treatments (Post hoc Tukey tests, $P<0.05$).

3.3. Experiment 3: Blood source

No significant differences in female mortality ($t = 2.19$; df = 5; $P = 0.080$), female fecundity (Mann-Whitney U = 135.5; $P = 0.082$) and egg hatchability (Mann-Whitney U = 153.5; $P = 0.212$) were noted between the two blood sources (Table 2).

Table 2: Mortality, fecundity and egg hatchability (mean ± SEM) of *A. albopictus* females fed on human or bovine blood.

Parameters	Blood Source	
	Bovine	Human
Female mortality (%)	31.2 ± 7.1[a]	15.6 ± 2.3[a]
Female fecundity (No. eggs/female)	42.7 ± 5.1[a]	28.6 ± 3.6[a]
Egg hatchability (%)	78.7 ± 5.4[a]	78.7 ± 7.4[a]

Means within a row followed by the same letter are not significantly different ($P>0.05$).

3.4. Experiment 4: Blood feeding method

Blood feeding methods had a significant effect on egg production ($F = 4.89$; df = 2, 12; $P = 0.028$). Significantly more eggs were collected from cages with Hemotek feeder on the roof top than from cages blood fed on collagen sausages (Post hoc Tukey test, $P<0.05$). On the other hand, the position of the Hemotek feeders (on the roof outside the cage vs inside the cage facing the roof), did not have a significant impact on egg production (Post hoc Tukey test, $P>0.05$). The results are illustrated in Fig. 4.

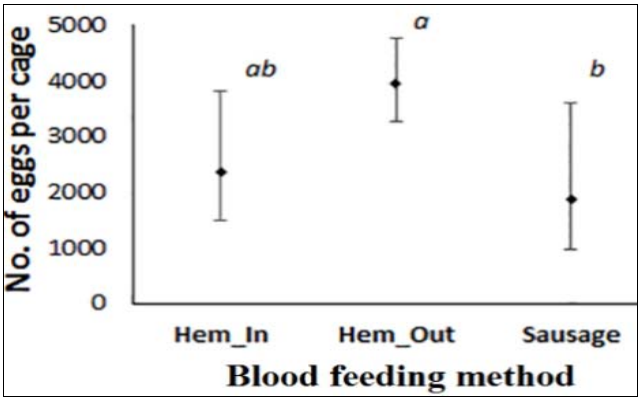

Fig 4: Total egg production (mean, 95% CI) from cages containing 250 male and female *A. albopictus* mosquitoes supplied with human blood using three blood feeding methods (Hem_In, one Hemotek feeder inside the cage; Hem-Out, one Hemotek feeder on the cage's roof top; Sausage, a collagen sausage inside the cage). Different letters represent statistical differences between treatments (Post hoc Tukey tests, $P<0.05$).

3.5. Formulation of larval diets

3.5.1. Experiment 5A: Mono-diets

Survival to pupation differed significantly among the diets ($F = 8.85$; df = 9, 40; $P<0.001$) with significantly fewer L1 reaching the pupal stage when fed on the Rabbit diet.

Time to male pupation ($F = 31.53$; df = 9, 40; $P<0.001$) and time to male emergence diets ($F = 34.17$; df = 9, 40; $P<0.001$) differed significantly among diets. Male larvae fed on Rabbit, Dog V. and Aqu A took significantly more time to pupate and to become adults than those fed on the IAEA_Thai diet. Male pupae production rate 24 h from pupation onset (calculated by dividing the number of mP produced in the first 24 h by the total number of mP produced throughout the experiment) differed significantly among the diets ($F = 3.86$; df = 9, 40; $P = 0.001$). Male larvae fed on Koi and Aqu A took significantly more time to pupate than those fed on the IAEA_Thai diet.

While sex ratio of pupae formed within 24 h from pupation onset did not differ significantly among diets ($F = 2.35$; df = 9, 40; $P = 0.033$), a significant difference in sex ratio was noted from the total pupae formed throughout the experiment ($F = 9.82$; df = 9, 40; $P<0.001$). Unlike the other diets, when larvae were fed on the Rabbit diet, mostly males pupated and reached the adult stage (Post hoc Tukey tests, $P<0.05$).

Diet had a significant influence on the wing length of *A. albopictus* males ($F = 44.33$; df = 9, 228; $P<0.001$) and females ($F = 19.34$; df = 9, 176; $P<0.001$). Males that fed on Koi, Aquarium, Veg, Dog V. or Rabbit diet, had significantly smaller wings than those reared on the IAEA_Thai diet (Post hoc Tukey tests, $P<0.05$). Wing of females reared on Chewli, Aqu B, IAEA_Mau and Koi did not differ significantly in length with those reared on the IAEA_Thai diet (Post hoc Tukey tests, $P>0.05$). The results are illustrated in Table 3.

Table 3: Development parameters (mean, 95% CI) of *A. albopictus* fed on 10 larval diets.

Parameters	Larval diet									
	Aquarium	**Aqu A**	**Aqu B**	**Chewli**	**Dog V.**	**IAEA_Thai**	**Koi**	**IAEA_Mau**	**Rabbit**	**Veg**
% L1 reaching pupation	90.0^{a}	92.8^{a}	99.8^{a}	97.6^{a}	92.2^{a}	99.2^{a}	93.1^{a}	94.5^{a}	57.1^{b}	91.6^{a}
	(79.2-97.1)	(79.8-99.4)	(97.1-99.4)	(89.1-99.9)	(88.3-95.3)	(94.2-99.6)	(78.7-99.7)	(76.0-99.8)	(44.1-69.9)	(84.0-96.9)
Time from L1 to mP (days)	6.6^{bcd}	7.0^{b}	6.6^{bcd}	6.5^{cd}	7.7^{a}	6.5^{cd}	6.9^{bc}	6.4^{d}	7.7^{a}	6.7^{bcd}
	(6.6-6.6)	(6.7-7.2)	(6.4-6.8)	(6.4-6.7)	(7.2-8.1)	(6.3-6.7)	(6.7-7.1)	(6.1-6.6)	(7.6-7.9)	(6.6-6.9)
Rate of male pupation in the first 24 h *	41.0^{abc}	7.3^{c}	44.2^{abc}	47.8^{abc}	30.8^{abc}	50.9^{ab}	12.9^{bc}	69.9^{a}	39.1^{abc}	30.2^{abc}
	(37.9-44.2)	(0.3-31.7)	(16.8-73.6)	(33.9-61.9)	(0.3-80.9)	(29.2-72.5)	(0.6-37.2)	(29.5-97.3)	(23.4-56.1)	(17.5-44.7)
Sex ratio 24 h from pupation onset **	1.0^{a}	1.0^{a}	0.9^{a}	1.0^{a}	0.9^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
	(1.0-1.0)	(1.0-1.0)	(0.6-1.0)	(0.9-1.0)	(0.5-1.0)	(0.8-1.0)	(1.0-1.0)	(0.8-1.0)	(0.9-1.0)	(1.0-1.0)
Sex ratio***	0.6^{a}	0.5^{a}	0.6^{a}	0.6^{a}	0.6^{a}	0.6^{a}	0.6^{a}	0.6^{a}	0.9^{b}	0.5^{a}
	(0.5-0.6)	(0.5-0.6)	(0.4-0.7)	(0.5-0.7)	(0.4-0.8)	(0.4-0.7)	(0.5-0.8)	(0.5-0.7)	(0.8-1.0)	(0.4-0.6)
% L1 reaching adult stage	86.4^{a}	87.7^{a}	97.6^{a}	94.4^{a}	86.2^{a}	89.5^{a}	93.7^{a}	90.4^{a}	57.2^{b}	87.1^{a}
	(71.6-96.4)	(77.4-95.2)	(89.1-99.4)	(84.0-99.6)	(80.7-90.9)	(82.2-95.1)	(81.5-99.6)	(72.7-99.4)	(43.7-70.2)	(73.8-96.2)
Time from L1 to male adults (days)	8.5^{c}	8.9^{b}	8.6^{bc}	8.5^{c}	9.8^{a}	8.4^{c}	8.8^{bc}	8.4^{c}	9.9^{a}	8.7^{bc}
	(8.3-8.6)	(8.6-9.3)	(8.4-8.8)	(8.3-8.7)	(9.4-10.3)	(8.1-8.7)	(8.6-8.9)	(8.1-8.6)	(9.6-10.1)	(8.6-8.9)
Male wing length (mm)	2.1^{cde}	2.2^{abcd}	2.2^{a}	2.2^{abc}	2^{e}	2.2^{a}	2.1^{bcd}	2.2^{ab}	1.9^{f}	2.1^{de}
	(2.1-2.1)	(2.1-2.2)	(2.2-2.3)	(2.2-2.2)	(2.0-2.1)	(2.2-2.3)	(2.1-2.2)	(2.2-2.2)	(1.8-1.9)	(2.0-2.1)
Female wing length (mm)	2.4^{de}	2.4^{cde}	2.6^{ab}	2.6^{bc}	2.3^{e}	2.6^{ab}	2.5^{bcd}	2.9^{a}	2.3^{e}	2.5^{cde}
	(2.4-2.5)	(2.4-2.5)	(2.6-2.7)	(2.5-2.6)	(2.3-2.3)	(2.6-2.7)	(2.5-2.6)	(2.7-2.8)	(2.2-2.4)	(2.4-2.5)

Means (95% CI) within a row followed by the same letter are not significantly different (Post hoc Tukey tests, $P>0.05$).

L1, newly hatched larvae; P, pupae; mP, male pupae.

* Number of male pupae obtained in the first 24 h from the beginning of pupation divided by the total number of male pupae produced for each diet treatment.

** Sex ratio (M / (F+M)) of pupae formed in the first 24 h from the beginning of pupation.

*** Sex ratio (M / (F+M)) of pupae formed throughout the experiment.

3.5.2. Experiment 5B: Composite diets

No significant differences in survival to pupation ($F = 1.02$; df = 12, 52; $P = 0.447$), survival to adult emergence ($F = 1.50$; df = 12, 52; $P = 0.154$) and overall sex ratio ($F = 1.50$; df = 12, 52; $P = 0.441$) were noted among diets. For both Aqu B and Chewli, the addition of brewer's yeast, bovine liver powder, pea or vitamin mix to the mono diets, did not have a significant effect on time to male pupation, time to adult male emergence, male pupae production rate and sex ratio 24 h from pupation onset when compared to their respective mono diet (Post hoc Tukey tests, $P>0.05$). Moreover, no significant differences in the above mentioned parameters were observed among larvae fed on Aqu B, Chewli and IAEA_Thai diets (Post hoc Tukey tests, $P>0.05$). Furthermore, male larvae fed on a diet consisting of 80% Chewli and 20% brewer's yeast, took significantly less time to reach the adult stage than those fed on the IAEA_Thai diet.

Excluding the diet composed of 80% Aqu B, 10% brewer's yeast and 10% bovine liver powder; male wing length did not significantly differ among the other diets (Post hoc Tukey tests, $P>0.05$). Males reared on diet made up of 80% Aqu B, 10% brewer's yeast and 10% bovine liver powder, had significantly bigger wings than those fed on the IAEA_Thai (Post hoc Tukey tests, $P<0.05$) and the Aqu B mono diet (Post hoc Tukey tests, $P<0.05$). Wings of females reared on the 80% Chewli and 20% bovine liver powder diet were significantly smaller than the other diets (Post hoc Tukey tests, $P<0.05$). Moreover, excluding the latter diet, female wing length did not differ significantly among the other diets (Post hoc Tukey tests, $P>0.05$). The results are illustrated in Table 4.

Table 4: Development parameters (mean, 95% CI) of *A. albopictus* fed on 13 larval diets.

	Larval diet												
	Aqu B	**Aqu B + BLP + BY**	**Aqu B + BLP**	**Aqu B + pea**	**Aqu B + BY**	**Aqu B + Vit mix**	**Chewli**	**Chewli + BLP+ BY**	**Chewli + BLP**	**Chewli + Pea**	**Chewli + BY**	**Chewli + Vit mix**	**IAEA_ Thai**
% L1 reaching pupation	97.6^{a}	96.7^{a}	100.0^{a}	98.8^{a}	98.8^{a}	96.1^{a}	99.8^{a}	95.4^{a}	100.0^{a}	99.2^{a}	99.8^{a}	99.2^{a}	98.4^{a}
	(89.1-99.9)	(72.6-96.5)	(100.0-100.0)	(91.2-99.3)	(91.2-99.3)	(88.4-99.7)	(97.1-99.4)	(73.7-98.9)	(100.0-100.0)	(94.2-99.6)	(97.1-99.4)	(94.2-99.6)	(88.4-99.2)
Time from L1 to mP (days)	6.3^{ab}	6.7^{ab}	6.6^{ab}	6.6^{ab}	6.3^{ab}	6.3^{b}	6.6^{ab}	6.8^{a}	6.5^{ab}	6.7^{a}	6.2^{b}	6.6^{ab}	6.7^{ab}
	(6.1-6.5)	(6.6-6.8)	(6.5-6.7)	(6.4-6.9)	(6.1-6.6)	(6.1-6.4)	(6.4-6.9)	(6.4-7.2)	(7.6-7.9)	(6.6-6.9)	(6.1-6.4)	(6.2-7.0)	(6.1-7.3)
Rate of male pupation in the first 24 h *	69.3^{ab}	29.4^{ab}	41.2^{ab}	33.1^{ab}	67.4^{ab}	77.4^{ab}	34.0^{ab}	30.6^{ab}	54.2^{ab}	27.0^{b}	81.0^{a}	35.6^{ab}	41.4^{ab}
	(48.9-86.3)	(18.3-41.8)	(29.0-53.9)	(8.1-64.9)	(41.4-88.7)	(62.1-89.7)	(9.5-64.6)	(5.1-65.7)	(27.5-79.6)	(12.0-45.5)	(51.5-98.3)	(1.6-83.3)	(3.8-87.0)
Sex ratio 24 h from pupation onset **	0.8^{abc}	1.0^{a}	0.7^{bc}	1.0^{abc}	0.7^{bc}	0.9^{abc}	1.0^{abc}	1.0^{ab}	0.7^{bc}	1.0^{abc}	1.0^{abc}	0.7^{c}	1.0^{abc}
	(0.5-1.0)	(1.0-1.0)	(0.4-1.0)	(0.8-1.0)	(0.6-0.9)	(0.7-1.0)	(0.8-1.0)	(0.9-1.0)	(0.5-0.9)	(0.8-1.0)	(0.7-1.0)	(0.3-0.9)	(0.8-1.0)
Sex ratio***	0.5^{a}	0.6^{a}	0.4^{a}	0.4^{a}	0.5^{a}	0.5^{a}	0.6^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.5^{a}	0.4^{a}	0.5^{a}
	(0.4-0.6)	(0.5-0.7)	(0.3-0.6)	(0.3-0.5)	(0.4-0.6)	(0.4-0.6)	(0.4-0.7)	(0.3-0.7)	(0.3-0.6)	(0.3-0.7)	(0.4-0.6)	(0.3-0.6)	(0.4-0.6)
% L1	90.5^{a}	90.1^{a}	99.8^{a}	95.9^{a}	95.3^{a}	88.8^{a}	98.8^{a}	93.3^{a}	98.2^{a}	96.1^{a}	93.8^{a}	98.2^{a}	98.4^{a}

reaching adult stage	(83.3-63.8)	(63.8-100.0)	(97.1-99.4)	(81.0-99.8)	(78.2-99.7)	(78.7-95.9)	(91.2-99.3)	(73.0-99.9)	(91.9-100.0)	(88.5-99.7)	(81.7-99.6)	(92.0-100.0)	(88.4-99.2)
Time from L1 to male adults (days)	8.8^{abcd}	9.0^{ab}	8.7^{abcd}	8.8^{abc}	8.6^{bcd}	8.4^{cd}	8.7^{abcd}	9.1^{a}	8.6^{bcd}	9.0^{ab}	8.3^{d}	8.7^{abcd}	8.9^{abc}
	(8.7-8.9)	(8.7-9.3)	(8.6-8.8)	(8.6-9.0)	(8.4-8.7)	(8.2-8.7)	(8.4-9.1)	(8.9-9.4)	(8.4-8.7)	(8.6-9.3)	(8.2-8.3)	(8.5-9.0)	(8.3-9.6)
Male wing length (mm)	2.2^{b}	2.3^{a}	2.2^{ab}	2.3^{ab}	2.3^{ab}	2.3^{ab}	2.2^{ab}	2.2^{ab}	2.2^{ab}	2.2^{ab}	2.2^{ab}	2.2^{ab}	2.2^{b}
	(2.2-2.2)	(2.3-2.3)	(2.2-2.3)	(2.2-2.3)	(2.2-2.3)	(2.2-2.3)	(2.2-2.2)	(2.2-2.3)	(2.2-2.2)	(2.2-2.3)	(2.2-2.2)	(2.2-2.3)	(2.2-2.2)
Female wing length (mm)	2.8^{ab}	2.8^{ab}	2.8^{a}	2.7^{ab}	2.8^{a}	2.8^{a}	2.7^{ab}	2.8^{a}	1.9^{c}	2.6^{b}	2.8^{a}	2.7^{ab}	2.7^{ab}
	(2.7-2.8)	(2.7-2.8)	(2.8-2.9)	(2.7-2.8)	(2.8-2.8)	(2.8-2.9)	(2.7-2.8)	(2.8-2.8)	(1.8-2.1)	(2.6-2.7)	(2.8-2.8)	(2.7-2.8)	(2.7-2.8)

Means (95% CI) within a row followed by the same letter are not significantly different (Post hoc Tukey tests, $P>0.05$).
L1, newly hatched larvae; P, pupae; mP, male pupae.
* Number of male pupae obtained in the first 24 h from the beginning of pupation divided by the total number of male pupae produced for each diet treatment.
** Sex ratio (M / (F+M)) of pupae formed in the first 24 h from the beginning of pupation.
*** Sex ratio (M / (F+M)) of pupae formed throughout the experiment.

4. Discussion

Because hatching of eggs in *Aedes* mosquitoes is usually induced in anoxic condition [15, 16], several methods have, in the past, been used to remove oxygen from the hatching medium, including boiling the water [16], bubbling nitrogen gas through the water [17], adding ascorbic acid [6, 18], yeast [19, 20] or Nutrient Broth [13] to the hatching medium. While synchronous hatching of *A. albopictus* eggs was achieved in a diluted solution of Nutrient Broth at the Centro Agricoltura Ambiente in Bologna [13], this medium was further optimized with the addition of yeast at the FAO/IAEA Insect Pest Control Laboratory in Vienna and found to induce 10 to 18 times more hatching in *A. albopictus* eggs that in a medium of boiled deionized water [7]. In our study, mean hatch rate of *A. albopictus* eggs in a 0.01% solution of ascorbic acid was 2.4%, which is significantly lower than that recorded for *A. aegypti* (89%) [18] and *A. sierrensis* (33.5%) [6]. Moreover, a mixture consisting of 28% tuna meal, 36% bovine liver powder and 36% brewer's yeast (i.e. the IAEA3 food mix), which is 158 times cheaper than the Nutrient Broth medium used in Vienna [7], induced significantly more hatching in *A. albopictus* eggs than the latter solution. Hatch rate in diluted solutions of IAEA3 food mix was still optimum at the lowest tested concentration which indicates that the dissolved oxygen concentration required to induce hatching in *A. albopictus* eggs, was achieved when only 0.125g of the IAEA3 food mix was added to one litre of dechlorinated tap water. Besides its relatively low cost, components constituting the IAEA3 food mix are identical to that used in the IAEA diet conceived by the FAO/IAEA Insect Pest Control Laboratory for mass rearing larvae of the species [5], hence making the IAEA3 food mix an attractive hatching medium for use in a mass production facility.

Blood source (human and bovine blood) did not have a significant impact on female survival rate, female fecundity and egg hatchability. Although female engorgement rate was not investigated in this study, it was observed during 20 field studies carried out across the world that *A. albopictus* females preferentially fed on humans rather than on other available animals [21, 22]. The traceability of human blood packs, the high standards associated with its collection, pathogen screening and storage [23, 24] coupled with an unreliable supply of bovine blood due to recent outbreaks of foot and mouth disease in the country [25], makes the use of human blood a more attractive option for colony blood feeding in the Vector Biology and Control Division national laboratory. Female fecundity (mean ± SE) was low (36 ± 3 eggs per female) as compared to other studies investigating the first gonotrophic cycle of the mosquito (mean value ranging from 42 to 143 eggs per female [10, 26, 27, 28, 29] but was higher than that reported by Balestrino *et al.* (13 ± 1 eggs per female) [11]. An interplay of several factors could have contributed to the high variation in female fecundity observed among the studies including, size of male and female mosquito known to be modulated by the colonization process or the larval rearing conditions, blood meal source, blood feeding method, availability of carbohydrates, age of mosquito, mating conditions and suitability of oviposition sites [10, 30-39].

While *A. albopictus* females are known to be difficult in imbibing blood within laboratory set-ups, especially when artificial membrane feeders are used [10, 36, 40], this study confirms the observation made by Lyski *et al.* [36] who noted that blood meal presentation could significantly influence the blood feeding success of *A. albopictus* females. However, while Lyski *et al.* [36] observed higher engorgement rate in cages where females were fed on collagen sausage membranes in a vertical feeding position and subsequently argued that it was easier and less risky for engorged females with a heavy abdomen to maintain a nose-up posture relative to an upside-down one, in our study, egg production was significantly higher in cages where Hemotek feeders were placed on the roof top with females feeding in an upside-down position. Females used in our study are from the 17th generation that have originated from parental colonies which have been established and blood fed in an upside-down position using Hemotek feeders. It is therefore highly probable that the observation made in our study could be the result of adaptation or pre-selection of females breeding in an upside down position during the process of colonization. During a study by Deng *et al.* [10], an adaption of lab-bred *A. albopictus* to a blood feeding method was in fact suggested when significant differences in female engorgement rate was observed in 1st and 2nd generations females blood fed using two methods of blood feeding while such a difference was not noted in 3rd generation females.

While the IAEA_Thai diet was conceived at the IAEA/FOA laboratory in Vienna with the aim of mass producing high quality adult mosquitoes using 'cheap, globally available ingredients of consistent quality' [5, 14, 41], several set-backs are often encountered in its procurement in Mauritius, including its non-availability in the country, the high importation cost involved and the uncertainty of obtaining custom clearance for some of the diet components because of the associated risk of bacterial contamination. Nine locally available diet/diet

components were hence investigated in this study using the IAEA_Thai as the reference diet. Larval and pupal growth parameters as well as wing length of *A. albopictus* mosquitoes reared on three of those diets, namely, Aquatro B, Chewli and IAEA_Mau, did not differ significantly from those fed on the IAEA_Thai diet. When additives, notably, vitamin mix, pea, brewer's yeast or bovine liver powder, were added to Aquatro B and Chewli, the above-mentioned parameters did not significantly differ when compared to their respective mono diet except when a mixture of brewer's yeast and bovine liver powder was added to Aquatro B which resulted in a slight (0.01 mm) but significant increase in the length of males' wings.

Although the effect of diet on adult longevity and female fecundity was not investigated in this study, wing length of *A. albopictus* mosquito was found to be a good indicator of fecundity [32, 42]. Furthermore, while Koenraadt [43] found that pupal cephalothorax length in *A. aegypti* could predict adult male body size, it was observed in *A. albopictus*, that adult size had a positive effect on adult longevity [44] and that reproduction was negatively affected in smaller males due to reduced spermatogenesis and testis size [45]. Hence, during this preliminary study, Chewli™ and Aquatro B (Aquatro Tilapia Pre Grower™ 35% protein), two locally manufactured diets which are 57 times cheaper than the IAEA_Thai diet, were found as to be as effective in rearing *A. albopictus* larvae as the latter diet. Further studies will however need to be carried out to test the suitability of the diets in rearing the mosquito at a larger scale where important parameters such as synchronicity at the onset of pupation, size homogeneity and female contamination during male harvesting procedures, will be investigated.

5. Conclusion

As part of an SIT feasibility study against *A. albopictus* in Mauritius, egg hatching, blood feeding and larval rearing procedures were evaluated in terms of cost, effectiveness and availability of materials, which are essential components to consider during a large-scale production of the species. Optimal hatching of *A. albopictus* eggs were noted in a mixture consisting of 28% tuna meal, 36% bovine liver powder and 36% brewer's yeast at a concentration of 0.125 g per litre of dechlorinated water. Moreover, while blood source (human and bovine blood) did not have a major impact on the survival rate and fecundity of female *A. albopictus*, egg production was highest in cages where blood was provided on the roof top using Hemotek feeders as compared to blood being provided inside the cage. Furthermore, two locally-manufactured animal feeds, Aquatro Tilapia Pre Grower™ and Chewli™, were as effective in rearing *A. albopictus* larvae as the IAEA diet (a diet conceived in Vienna for an optimal rearing of the species) and were approximately 57 times cheaper than the latter diet. The suitability of both diets to rear *A. albopictus* at a larger scale will be investigated in future studies.

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7. References

1. Iyaloo DP, Elahee KB, Bheecarry A, Lees RS. Guidelines to site selection for population surveillance and mosquito control trials: A case study from Mauritius. Acta Tropica. 2014; 132:140-149.
2. Knipling EF. Possibilities of insect control or eradication through the use of sexually sterile males. Journal of Economic Entomology. 1955; 48:459-462.
3. Benedict MQ, Knols BG, Bossin HC, Howell PI, Mialhe E, Caceres C *et al.* Colonisation and mass rearing: learning from others. Malaria Journal. 2009; 8(2):S4.
4. Alphey L, Benedict M, Bellini R, Clark GG, Dame DA, Service MW *et al*. Sterile-insect methods for control of mosquito-borne diseases: an analysis. Vector-Borne and Zoonotic Diseases. 2010; 10(3):295-311.
5. Balestrino F, Puggioli A, Gilles JRL, Bellini R. Validation of a New Larval Rearing Unit for *Aedes albopictus* (Diptera: Culicidae) Mass Rearing. PloS one. 2014; 9(3):e91914.
6. Schwan TG, Anderson JR. A comparison of egg hatching techniques for the western treehole mosquito, *Aedes sierrensis*. Mosquito News. 1980; 40(1):263-9.
7. Zheng ML, Zhang DJ, Damiens DD, Lees RS, Gilles JRL. Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae)-II-Egg storage and hatching. Parasites & Vectors. 2015; 8(1):1.
8. Trpis M. A new bleaching and decalcifying method for general use in zoology. Canadian Journal of Zoology. 1970; 48(4):892-893.
9. Damiens D, Soliban SM, Balestrino F, Alsir R, Vreysen MJB, Gilles JRL. Different blood and sugar feeding regimes affect the productivity of *Anopheles arabiensis* colonies (Diptera: Culicidae). Journal of Medical Entomology. 2013; 50(2):336-343.
10. Deng L, Koou SY, Png AB, Ng LC, Lam-Phua SG. A novel mosquito feeding system for routine blood-feeding of *Aedes aegypti* and *Aedes albopictus*. Tropical Biomedicine. 2012; 29:169-174.
11. Balestrino F, Puggioli A, Bellini R, Petric D, Gilles JRL. Mass production cage for *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology. 2014; 51(1):155-163.
12. Khan I, Farid A, Zeb A. Development of inexpensive and globally available larval diet for rearing *Anopheles stephensi* (Diptera: Culicidae) mosquitoes. Parasites & Vectors. 2013; 6(1):1.
13. Bellini R, Calvitti M, Medici A, Carrieri M, Celli G, Maini S. Use of the sterile insect technique against *Aedes albopictus* in Italy: First results of a pilot trial. In: Area-Wide Control of Insect Pests. Springer, Netherlands. 2007, 505-515.
14. Puggioli A, Balestrino F, Damiens D, Lees RS, Soliban SM, Madakacherry O *et al.* Efficiency of three diets for larval development in mass rearing *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology. 2013; 50(4):819-825.
15. Borg AF, Horsfall WR. Eggs of floodwater mosquitoes II. Hatching stimulus. Annals of the Entomological Society of America. 1953; 46(4):472-478.
16. Judson CL. The physiology of hatching of aedine mosquito eggs: hatching stimulus. Annals of the Entomological Society of America 1960; 53(5): 688-691.
17. Fallis SP, Snow KR. The hatching stimulus for eggs of *Aedes punctor* (Diptera: Culicidae). Ecological Entomology. 1983; 8(1):23-28.

18. Mulla MS, Chaudhury MF. Release of new mosquito larvicides into water from granular formulations. In: Proceedings and papers of the annual conference of the California Mosquito Control Association. California. 1968, 86.
19. Morlan HB, Hayes RO, Schoof HF. Methods for mass rearing of *Aedes aegypti* (L.). Public Health Reports. 1963; 78(8):711.
20. Farnesi LC, Martins AJ, Valle D, Rezende GL. Embryonic development of *Aedes aegypti* (Diptera: Culicidae): influence of different constant temperatures. Memórias do Instituto Oswaldo Cruz. 2009; 104(1):124-126.
21. Egizi A, Healy SP, Fonseca DM. Rapid blood meal scoring in anthropophilic *Aedes albopictus* and application of PCR blocking to avoid pseudogenes. Infection, Genetics and Evolution. 2013; 16:122-128.
22. Faraji A, Egizi A, Fonseca DM, Unlu I, Crepeau T, Healy SP *et al*. Comparative host feeding patterns of the Asian tiger mosquito, *Aedes albopictus*, in urban and suburban Northeastern USA and implications for disease transmission. PLoS Neglected Tropical Diseases. 2014; 8:e3037.
23. Mega blood donation 1825 pints of blood collected. http://www.govmu.org/English/News/Pages/Mega-Blood-Donation-1-825-pints-of-Blood-Collected.aspx. 2012.
24. Donor Testing. http://bts.health.govmu.org/English/Activities/Pages/Donor-Testing.aspx. 2007.
25. Foot and mouth disease outbreak: threats for the local livestock. http://defimedia.info/foot-and-mouth-disease-outbreak-threats-local-livestock. 2016.
26. Del Rosario A. Studies on the biology of Philippine mosquitoes. II. Observations on the life and behaviour of *Aedes albopictus* (Skuse) in the laboratory. Phillipine Journal of Science. 1963; 92:89-103.
27. Hawley WA. The biology of *Aedes albopictus*. Journal of the American Mosquito Control Association. 1988; 1(1):1-39.
28. Oliva CF, Damiens D, Vreysen MJ, Lemperière G, Gilles J. Reproductive strategies of *Aedes albopictus* (Diptera: Culicidae) and implications for the sterile insect technique. PLoS One. 2013; 8(11):e78884.
29. Zhang D, Lees RS, Xi Z, Bourtzis K, Gilles JR. Combining the Sterile Insect Technique with the Incompatible Insect Technique: III-Robust Mating Competitiveness of Irradiated Triple *Wolbachia*-Infected *Aedes albopictus* Males under Semi-Field Conditions. PloS One. 2016; 11(3):e0151864.
30. Hien DS. Biology of *Aedes aegypti* (L., 1762) and *Aedes albopictus* (Skuse, 1895) (Diptera, Culicidae). V. The gonotrophic cycle and oviposition. Acta Parasitologica Polonica. 1976; 24(6):37-55.
31. Yap HH, Lee CY, Chong NL, Foo AES, Lim MP. Oviposition site preference of *Aedes albopictus* in the laboratory. Journal of the American Mosquito Control Association-Mosquito News. 1995; 11(1):128-132.
32. Blackmore MS, Lord CC. The relationship between size and fecundity in *Aedes albopictus*. Journal of Vector Ecology. 2000; 25:212-217.
33. Armbruster P, Hutchinson RA. Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). Journal of Medical Entomology. 2002; 39(4):699-704.
34. Braks MAH, Juliano SA, Lounibos LP. Superior reproductive success on human blood without sugar is not limited to highly anthropophilic mosquito species. Medical and Veterinary Entomology. 2006; 20(1):53-59.
35. Mccann S, Day JF, Allan S, Lord CC. Age modifies the effect of body size on fecundity in *Culex quinquefasciatus* Say (Diptera: Culicidae). Journal of Vector Ecology. 2009; 34(2):174-181.
36. Lyski ZL, Saredy JJ, Ciano KA, Stem J, Bowers DF. Blood feeding position increases success of recalcitrant mosquitoes. Vector-Borne and Zoonotic Diseases. 2011; 11(8):1165-1171.
37. Phasomkusolsil S, Tawong J, Monkanna N, Pantuwatana K, Damdangdee N, Khongtak W *et al*. Maintenance of mosquito vectors: effects of blood source on feeding, survival, fecundity, and egg hatching rates. Journal of Vector Ecology. 2013; 38(1):38-45.
38. Panigrahi SK, Barik TK, Mohanty S, Tripathy NK. Laboratory evaluation of oviposition behavior of field collected *Aedes* mosquitoes. Journal of Insects. 2014, 1.
39. De Jesus CE, Reiskind MH. The importance of male body size on sperm uptake and usage, and female fecundity in *Aedes aegypti* and *Aedes albopictus*. Parasites & Vectors. 2016, 9:1.
40. Alto BW, Lounibos LP, Juliano SA. Age-dependent bloodfeeding of *Aedes aegypti* and *Aedes albopictus* on artificial and living hosts. Journal of the American Mosquito Control Association. 2003; 19(4):347-352.
41. Damiens D, Benedict MQ, Wille M, Gilles JRL. An inexpensive and effective larval diet for *Anopheles arabiensis* (Diptera: Culicidae): eat like a horse, a bird, or a fish? Journal of Medical Entomology. 2012; 49(5):1001-1011.
42. Armbruster P, Hutchinson RA. Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). Journal of Medical Entomology. 2002; 39(4):699-704.
43. Koenraadt CJM. Pupal dimensions as predictors of adult size in fitness studies of *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology. 2008; 45(2):331-336.
44. Xue R, Barnard DR. Effects of body size and nutritional regimen on survival in adult *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology. 2010; 47(5):778-782.
45. Medici A, Carrieri M, Scholte EJ, Maccagnani B, Dindo ML, Bellini R. Studies on *Aedes albopictus* larval mass-rearing optimization. Journal of Economic Entomology. 2011; 104(1): 266-273.