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Research article

Effect of cage size on *Aedes albopictus* wing length, survival and egg production

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HIGHLIGHTS

- Adult cage size may influence the quality of mass-reared *Aedes albopictus* sterile males.
- Surprisingly, wing length, adult survival and egg production resulted somehow better in the smaller cages.
- Increasing trend of egg production was observed during 20 generation of colonization in the smaller cages.

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ABSTRACT

Background: *Aedes albopictus* is currently the most widespread invasive mosquito species in the world. It has paramount medical importance since females are efficient vectors of important viruses affecting humans. The development of alternative control strategies to complement control measures has become an imperative and involves the Sterile Insect Technique (SIT). Research to improve the productivity of mass-rearing, as well as the quality of mass-reared males is of essential importance for the success of SIT.

Methods: This study compared the influence of three differently sized cages for *Ae. albopictus* mass-rearing on wing length, adult survival and egg production during 20 generations of colonization. Plexiglas cages of 40x40x40 cm (C1), 100 × 20 × 100 cm (C2) and 100 × 65 × 100 cm (C3) were loaded with equal adult density, and sex ratio of 1:1. An open source image processing and analysis programme (ImageJ) was used for the wing measurement and egg counting.

Results: In all tested cages, we identified two periods separated by the generation showing the minimum value of each considered parameter (wing length, adult survival and egg production). The wing length and adult survival passed through the phases of initial decrease to about intermediate colonization time, and increased afterwards. Fecundity was steady during the first period and increased in the second one. Cage C1 demonstrated not only the best values for all parameters but also the smallest decrease in the initial phase. Recovering of the caged mosquitoes in the second half of the study was higher in cages C1 and C2, than in C3.

Conclusions: C1 provided the least negative selection pressure on wing length, adult survival and egg production for reared *Ae. albopictus*. Anyhow, since maximising mosquito density by exploiting the minimum space is a priority in mosquito mass-rearing, C2 might be a better choice for better fitting the space of mass-rearing rooms.

1. Introduction

Aedes (*Stegomyia*) *albopictus* (Skuse, 1895) (Diptera: Culicidae) is currently the most widespread invasive mosquito species in the world (Benedict et al., 2007). Having originated in tropical forests of South-East Asia this species is spreading globally to every continent except Antarctica (Benedict et al., 2007; Enserink, 2008; Schaffner et al., 2009; ECDC, 2019) via passive transport of drought and cold resistant eggs in used tyres or in lucky bamboo containers (Schaffner et al., 2009), as well as through different means of passenger vehicles (cars, buses, ferries, boats, yachts) where mosquito adults are "unauthorized passengers" (Petrić et al., 2006, 2009).

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Aedes albopictus females are aggressive, daytime biters and efficient vectors of more than 20 viral pathogens (including dengue, chikungunya and Yellow fever) and nematodes of genus *Dirofilaria* (such as dog heartworm) (Gratz, 2004; Paupy et al., 2009; Zhong et al., 2013). Recent outbreaks of chikungunya and dengue in Hawaii, Mauritius, Gabon, Madagascar and La Reunion (Rezza, 2012), as well as outbreaks of chikungunya and dengue in Europe (Rezza et al., 2007; WHO/EMCA, 2011; Venturi et al., 2017), further demonstrate the increasing public health importance of this species worldwide (Zhong et al., 2013).

Therefore, the development of alternative strategies to complement existing control measures has become imperative (Hamady et al., 2013) and a research program on the Sterile Insect Technique (SIT) aiming to the suppression of *Ae. albopictus* was started in 1999 (Bellini et al, 2007, 2010, 2013).

SIT program usually consists of four main phases: a) mass-rearing of the target species in specially designed facilities; b) sex separation in order to release males only; c) male sterilisation and d) release in nature (Dyck et al., 2005).

Quality of mass-reared sterile males is ultimately assessed by their ability to compete against wild males for mating with wild females in the field (Harris et al, 2011, 2012) thus inducing the desired population sterility rate. Assessing parameters of male ability in the laboratory is the first and necessary step before performing filed releases (Massonnet--Bruneel et al., 2013). Research to improve the productivity of mass-rearing as well as the quality of mass-reared males is of fundamental importance to the success of SIT strategy (Benedict et al., 2009).

Selective adaptation to artificial rearing conditions may strongly influence the fitness of reared strains (Hoffmann and Ross, 2018; Nunny, 2002; Reed and Frankham, 2003; Whitlock, 2002), which could lead to pheno- and genotypic changes (Bartlett, 1984). Laboratory adaptive phenomena may take place soon following colonization with visible impact in a few generations (Latter and Mulley, 1995; Montgomery et al., 2000; Woodworth et al., 2002). They might reduce the quality of the reared mosquitoes (Nunny, 2002) when these have to be released and effectively compete for and mate with wild females.

To monitor any changes that may occur, easy to apply quality control tests have been proposed and are under evaluation (Balestrino et al., 2017; Carvalho et al., 2014; Culbert et al., 2018; Lenteren et al., 2009; Madakacherry et al., 2014). They involve regular measurements of pupal weight, mating and flight ability, adult male longevity, sex ratio (deviation from a colony's "normal" sex ratio may give an early indication of rearing problems), emergence rate, timing of emergence, sterility rate (FAO/IAEA/USDA, 2003).

Finding good and relatively simple indicators to assess for the colony quality, such as body size, wing length and adult longevity is essential to compare the performances of colonies and to follow their dynamics during long term mass-rearing programs (Marrelli et al., 2006).

We selected the wing length as the significant morphological variable being correlated with the body size in mosquitoes and their mating performances (Kelly and Edman, 1992; Menge et al., 2005; Ward, 1963). Mosquito size may influence survivorship and it may also have a positive correlation to fecundity which is a fitness trait important to evaluate the efficiency to convert the blood meal to eggs and the probability of offspring to survive and to become adults (Kelly and Edman, 1992; Menge et al., 2005). To our knowledge there are meagre data on the impact of the long-term colonization and the influence of mass-rearing cage shape and volume on wing size, adult survival and productivity of the colony. Previous studies on *Ae. aegypti* showed that colonization after seven and eleven sequential generations may have an effect on the genetic and phenotypic variation (Lorenz et al., 1984). Benedict et al. (2009) reported several studies on the differences between mass-produced and natural mosquitoes during colonization, one of which on two inbred strains of *Ae. triseriatus* undergoing full-sib mating for at least twelve generations (Matthews and Craig, 1989).

The aim of the study was to examine the influence of length of colonization and cage size and shape on *Ae. albopictus* wing length, adult survival and egg production during 20 generations of colonization. The observed differences would help in optimization of mass-rearing process and rearing facility design.

2. Methods

2.1. Mosquito stocks and rearing methods

The *Ae. albopictus* strain (named RER) used for this experiment was started from few thousands eggs collected in the field in different urban areas of Emilia-Romagna region, in northern Italy, during 2011. The wild eggs were hatched in the laboratory and reared to the adult stage. The adults were housed in 40 × 40 × 40 cm Plexiglas cages and blood fed to obtain the F_1 eggs which were used for the experiment. Mosquitoes coming from F_1 eggs were reared and placed as pupae in three different cages (1,280, 4,000 and 13,000 pupae in cage C1, C2 and C3 respectively) for 20 generations under laboratory conditions in a climate-controlled room (28 ± 1 °C, 80 ± 2% RH and a photoperiod of 14:10 L:D). Egg hatching, as well as larvae, pupae and adult rearing, were performed according to standard procedures (Bellini et al., 2007; Damiens et al., 2012; Puggioli et al., 2013).

2.2. Cage description and operation

Three Plexiglas cage sizes were used for the experiment. The cages had the following dimensions (length x width x height): 40 × 40 × 40 cm (volume 64 L – C1), 100 × 20 × 100 cm (volume 200 L – C2) and 100 × 65 × 100 cm (volume 650 L – C3). In each cage, components were organized to guarantee similar rate of accessibility to mosquitoes, such as:

- in C1: one pupal plate, two egg cups, one sugar feeder, one blood feeder;
- in C2: four pupal plates, six egg cups, three sugar feeders, three blood feeders;
- in C3: 13 pupal plates, 20 egg cups, ten sugar feeders, ten blood feeders.

Each cage had circular openings, which were covered with a net, and at least one of those openings was connected with tissue sleeve to allow access inside the cage (Balestrino et al., 2014). The cages were kept inside climate controlled chamber at quite stable environment condition as indicated above, and positioned at the same shelve-height during the experiment. In addition, to reduce "chamber door" effect and expose the cages to similar temperature and RH micro variations, the cages were rotated after each generation according to the distance to the door.

In all cages, the adult density was kept at 20 adults/litre of cage volume. Therefore in each generation, a total number of 1,280, 4,000 and 13,000 pupae were placed in cage C1, C2 and C3 respectively.

After emergence, adults were supplied with *ad libitum* 10% sucrose solution, while two blood meals were offered to the females at each generation (on day seven and eight from pupae introduction). Blood meal consisted of fresh, mechanically defibrinated, swine blood, heated at 37 °C and placed into each cage for 30 min by a unit feeding device.

The eggs were laid on white egg paper (white creped papers IF C140, Industrial Filtro S.r.l., Cologno Monzese, Italy), positioned in 250 ml plastic egg cup, containing 100 ml of water. Five days after the second blood meal, the egg papers were collected, left to dry in the climatic chamber for 24–48 h, scanned and counted automatically by using an open source image processing and analysis programme, ImageJ (U.S. National Institutes of Health, Bethesda, MD) (Bellini et al., 2007).

2.3. Wing measurement, adult survival and egg production

From each cage at each generation, the 50 males and 50 females were randomly collected, killed by freezing and stored at -20 °C for wing measurement. The right wing was removed from mosquito mesothorax above

the alula notch, using fine forceps. After dissection, the wing was transferred to the mount slide in a small drop of deionised water (ten wings were placed per slide). After water evaporation, the image of every wing was taken by a digital camera mounted on a TV2/3″C 0.63 phototube, using 10x eyepiece, 2.5x objective stereomicroscope. The software used to take the photo was Eye Demo (IDS Imaging Development Systems GmbH). Every photo was saved as a.jpg format with 96 dpi resolution.

The wing length was defined as the length from the axillary incision (or alula notch - Al) to the tip of the wing (excluding the fringe scales) between veins R_3 and R_{4+5}. The software ImageJ was used for wing length measurement by linear method (Mains, 2007). Prior to measurement, the calibration of the system was performed, using a micrometer slide.

The mean wing length for both males and females, for each cage, was calculated for the generations from F_1 to F_7, F_9, F_{11}, F_{13}, F_{15}, F_{17}, F_{18} and F_{20}.

To check adult survival, at day 15 after introduction of the pupae in the cages, all mosquitoes alive were collected by an aspirator and counted. The survival rate was estimated by comparison of the number of mosquitoes alive and the total number of mosquitoes introduced in each cage for generations F_2 to F_5, and consecutive odd generations till the end of the study: F_7, F_9, F_{11}, F_{13}, F_{15}, F_{17} and F_{19}.

The sex ratio was observed at the beginning of the F_2 to F_7, F_9, F_{11} and F_{12} to F_{20}, by taking extra sample of about 1,000 pupae from the same trays used to supply the cages.

The total number of eggs was observed by the procedure described, and the mean fecundity was calculated for each cage based on the estimated number of females introduced in each cage.

2.4. Statistical analysis

Wing length, adult survival and egg production in each cage size were analysed by Statistica 12.6, during the 20 generation period (overall period) as well as the first and the second period.

The first period has been distinguished from the second by the generation characterised by the minimum value of the considered parameters. For wing length the first (decrease) and the second (increase) periods were: F2 to F11 and F12 to F20; for adult survival: F2 to F9 and F10 to F19; and for egg production: F2 to F14 and F15 to F20 respectively.

These periods were used when performing analysis of variance (ANOVA) and Duncan's multiple range test (for analyzing parameter differences among cages) and t-test (to analyze parameter differences between two periods in each cage) as well as in testing the significance of the slope of regression lines (for each of observed parameters) in all cages and homogeneity of regression line slopes (parallelism test) among cages.

The different division into periods was performed in the case of Pearson's correlation analysis (used to test the possible association between investigated parameters) in order to provide an equal number of generations in each period. To do this, generation F11 was chosen to be the breaking point between two periods. This breaking point corresponds to the division of the periods of a shift in the tendency of the wing length, the parameter most frequently used in testing correlations with adult survival and egg production.

3. Results

3.1. Wing length

As expected (Virginio et al., 2015), mean wing length (all generations, and cages combined) was significantly higher in females (2.55 ± 0.19 mm) compared to males (2.10 ± 0.14 mm) ($p < 0.01$). The wing lengths of both males and females showed remarkable fluctuations during the 20 generation period in all cages, as well as the slight initial tendency of increase, followed by a decrease to generation F_{11}, and then increase again to generation F_{20} (Figure 1). The mean wing length of generation F_{20} was not different from the initial values (F_1) in the case of males in C1 (2.21 ± 0.09 mm at F_{20} against 2.22 ± 0.07 mm in F_1), while it was significantly higher in females (2.73 ± 0.12 mm at F_{20} against 2.67 ± 0.07 mm in F_1 ($p < 0.01$) (Figure 1). In cages C2 and C3 at F_{20}, in both sexes, the mean wing length was significantly lower ($p < 0.01$) comparing to F_1 and also against C1. In males belonging to F_{20} it was 2.11 ± 0.11 mm in C2 and 2.16 ± 0.08 mm in C3 against 2.22 ± 0.07 mm in F_1, with significant statistical difference ($p < 0.01$) between C2 and C3 at F_{20}. In females at F_{20} it was 2.59 ± 0.11 mm in C2 and 2.58 ± 0.12 mm in C3 against 2.67 ± 0.07 mm in F_1 with no significant difference between C2 and C3 at F_{20} (Figure 1).

Mosquito wing lengths for both males and females were significantly different between cages. When observing overall period (F_2 to F_{20}), males reared in C1 had significantly longer wings (2.13 ± 0.13 mm) compared to males from C2 (2.08 ± 0.15 mm) and C3 (2.10 ± 0.14 mm) ($p < 0.01$) (Table 1).

A significant statistical difference in male wing length in the overall period was also observed between C2 and C3 cages ($p < 0.01$). A similar situation was noticed in overall period in females, where mean wing length from C1 resulted higher (2.59 ± 0.16 mm) and significantly different from wing length of females reared in C2 (2.52 ± 0.20 mm) and C3 (2.54 ± 0.18 mm) ($p < 0.01$) (Table 1). Statistical difference in female wing length in overall period between cages C2 and C3 was evident as well ($p < 0.05$).

Trend analysis of the wing lengths for both sexes in all cages showed differences between the two periods. A decreasing trend in wing length was detected in all cages up to generations F_{11} when the lowest values were observed (apart from F_{17}), followed by an increasing trend in the last generations. In the first period strong negative linear trend was observed in all cages for both males and females. The significance of the slope of the regression lines was $p < 0.01$ (males in all cages and females in C2 and C3) and $p < 0.05$ (females in C1). A positive linear trend was

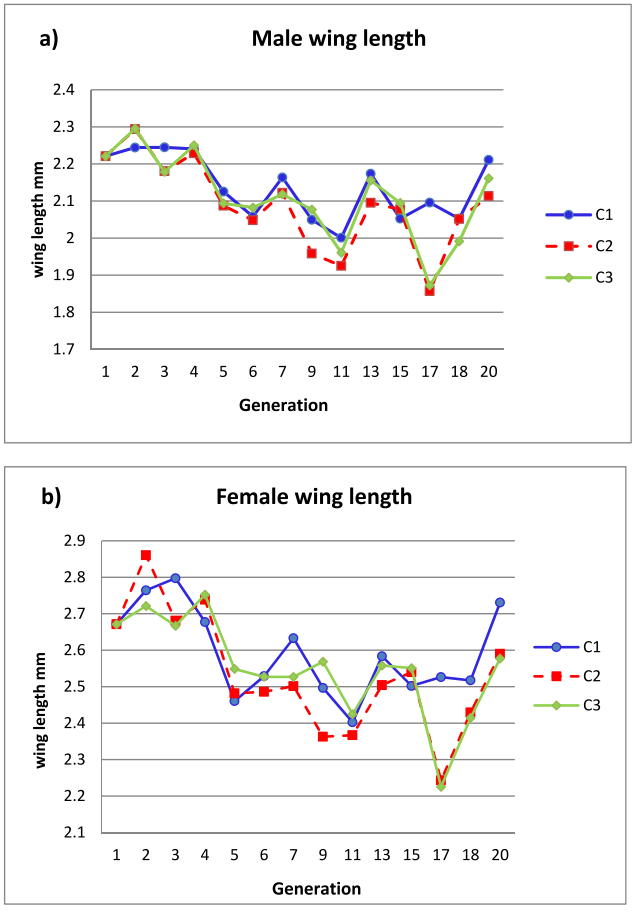

Figure 1. Wing length of *Aedes albopictus* males (a) and females (b) reared in the three cages.

Table 1. Wing length (mean ± SD) of *Aedes albopictus* males and females in the three cages.

Cage	Mean wing length (mm)					
	Males			Females		
	F_2 to F_{20}	F_2 to F_{11}	F_{12} to F_{20}	F_2 to F_{20}	F_2 to F_{11}	F_{12} to F_{20}
C1	2.13 ± 0.13 a	2.14 ± 0.13 a	2.12 ± 0.12 a	2.59 ± 0.16 a	2.59 ± 0.18 a	2.57 ± 0.14 a
C2	2.08 ± 0.15 b	2.11 ± 0.15 b	2.04 ± 0.13 b	2.52 ± 0.20 b	2.56 ± 0.21 b	2.46 ± 0.18 b
C3	2.10 ± 0.14 c	2.13 ± 0.14 ac	2.06 ± 0.14 b	2.54 ± 0.18 c	2.59 ± 0.16 ac	2.47 ± 0.18 b

Numbers in the same column followed by the same letter are not significantly different for $p < 0.05$.

observed during the second period in all cages but without achieving significancy. In the overall period negative slopes were detected in all cages for both males and females, but they were significant ($p < 0.05$) only in C2 and C3.

Despite the fact of a positive linear trend within the second period, the average wing lengths were shorter compared to the first period. The differences in wing lengths between the first (F_2 to F_{11}) and the second (F_{12}–F_{20}) period were the least expressed in C1 (males 2.14 ± 0.13 mm and 2.12 ± 0.12 mm – at the very edge of significance ($p = 0.0499$); females 2.59 ± 0.18 mm and 2.57 ± 0.14 mm - no significant differences) (Table 1). Significant differences ($p < 0.01$) were recorded in both C2 (males 2.11 ± 0.15 mm and 2.04 ± 0.13 mm; females 2.56 ± 0.21 mm and 2.46 ± 0.18 mm) and C3 (males 2.13 ± 0.14 mm and 2.06 ± 0.14 mm; females 2.59 ± 0.16 mm and 2.47 ± 0.18 mm) (Table 1). Wing length in the first period was significantly shorter in cage C2 vs. cages C1 and C3 in both sexes (in males ($p < 0.01$), while in females in C2 vs C1 ($p < 0.01$) and in C2 vs C3 ($p < 0.05$)). In the second period, both males and females reared in C1 had significantly longer wings compared to C2 and C3 ($p < 0.01$) (Table 1).

3.2. Adult survival

The sex ratio (% female) of the introduced pupae was 0.41 (±0.11 SD) in cage C1; 0.41 (±0.12 SD) in cage C2; and 0.44 (±0.09 SD) in cage C3.

As expected (Liles and Delong, 1960; Puggioli et al., 2013), the average male survival rate in all cages (0.41 ± 0.22), was significantly lower than female survival rate (0.68 ± 0.18) ($p < 0.01$). Adult survival demonstrated strong fluctuations and general decline up to generation F_9, when the shortest survival of both sexes was observed in most of the cages (Figure 2 a, b). After that, the adult survival showed an increase up to the generation F_{19}, while fluctuations became less expressed particularly for C1 cage.

Male survival rate in overall period (F_2 to F_{19}) was highest in C1 (0.54 ± 0.24) followed by C2 (0.40 ± 0.16) and C3 (0.28 ± 0.18) (Table 2). Female survival rate in overall period showed a similar trend according to the cage size: maximum value was recorded in C1 (0.78 ± 0.16), intermediate in C2 (0.70 ± 0.18) while the minimum was observed in C3 (0.56 ± 0.14). Significant differences between cages C1 and C3 ($p < 0.01$) were found for both male and female survival, while female survival showed significant differences between cages C2 and C3 as well ($p < 0.05$).

Average male and female survival rates in the first period (F_2 to F_9) were lower than in the second period (F_{10} to F_{19}) in all cages, except for females reared in C3 where survival during the second period was lower than during the first one (Table 2). Significant differences between the two periods were observed only in males in C2 ($p < 0.05$) and C3 ($p < 0.01$).

Survival rate for both males and females, during the first period did not show any significant differences between cages. In the second period female survival was significantly higher in C1 vs C3 ($p < 0.05$).

The significant positive ($p < 0.05$) trend of survival rate was observed only in three cases: in C1 in females (in overall and the second period) and C3 in males (in overall period). In all other cases (overall and the second period) positive, but not significant linear trend was observed. In the first-period male survival rate trends in C1 and C2 were negative, but not significant, as well as in C2 for the females. Positive, but not significant slopes were recorded in C3 (male and female survival) and C1 in female survival.

3.3. Egg production

The number of eggs per female (fecundity) showed a robust, increasing trend during the 20 generation period in cages C1 and C2, while in C3 it was almost unchanged from the beginning. As in the previously described parameters, in egg production, extreme fluctuations were noticed in all cages (Figure 3).

In the overall period (F_2 to F_{20}) the egg production did not express any statistical differences among cages (Table 3). Nevertheless, it was highest in C1 (19.26 ± 7.88 eggs/female), followed by C2 (16.62 ± 7.19) and lowest in C3 (13.54 ± 4.73) (Table 3). Still, trend analysis of egg production showed that although the linear regressions were positive in all cages, they were significant ($p < 0.01$) only in C1 and C2.

In the first period (F_2 to F_{14}) egg production did not show any significant differences between cages, and it was highest in C1 (15.46 ± 5.89), followed by C3 (13.46 ± 5.50) and C2 (12.84 ± 3.81). During the second period (F_{15} to F_{20}) egg production in C3 (13.69 ± 3.30) was significantly lower than in cages C1 (26.24 ± 6.25) and C2 (23.57 ± 6.86) ($p < 0.01$). No significant difference was detected between cages C1 and C2 in the same period (Table 3).

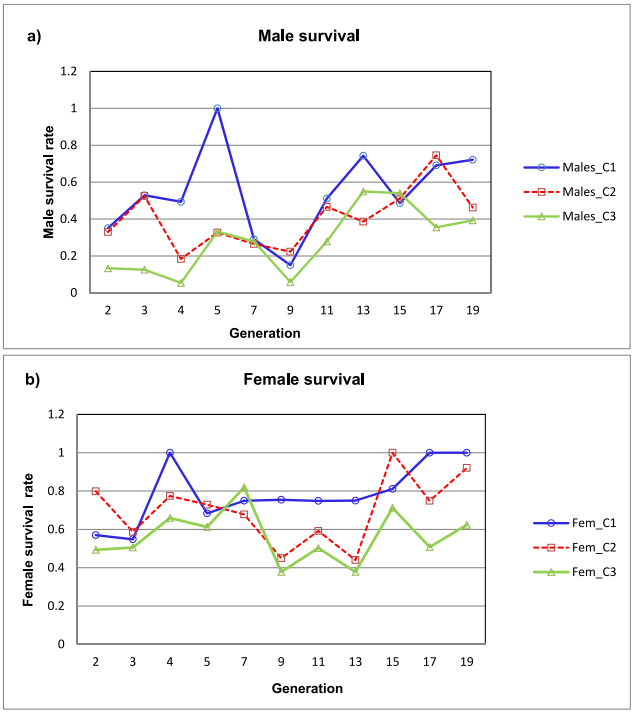

Figure 2. a. Survival rate of *Aedes albopictus* males reared in the three cages. b. Survival rate of *Aedes albopictus* females reared in the three cages.

Table 2. Survival rate (mean ± SD) of *Aedes albopictus* males and females in the three cages.

Cage	Survival rate					
	Males			Females		
	F_2 to F_{19}	F_2 to F_9	F_{10} to F_{19}	F_2 to F_{19}	F_2 to F_9	F_{10} to F_{19}
C1	0.54 ± 0.24 a	0.47 ± 0.29 a	0.63 ± 0.12 a	0.78 ± 0.16 a	0.72 ± 0.16 a	0.86 ± 0.13 a
C2	0.40 ± 0.16 ab	0.31 ± 0.12 a	0.51 ± 0.14 a	0.70 ± 18 ab	0.67 ± 0.13 a	0.74 ± 0.23 ab
C3	0.28 ± 0.18 b	0.16 ± 0.12 a	0.42 ± 0.12 a	0.56 ± 0.14 c	0.58 ± 0.15 a	0.54 ± 0.13 b

Numbers in the same column followed by the same letter are not significantly different for $p < 0.05$.

Significant differences in egg production between higher yield period (F_{15} to F_{20}) and lower yield period (F_2 to F_{14}) were recorded in cages C1 (increase was 10.78 eggs/female, $p < 0.01$) and C2 (10.73 eggs/female, $p < 0.01$), while in C3 that difference was negligible and not significant (0.23 eggs/female).

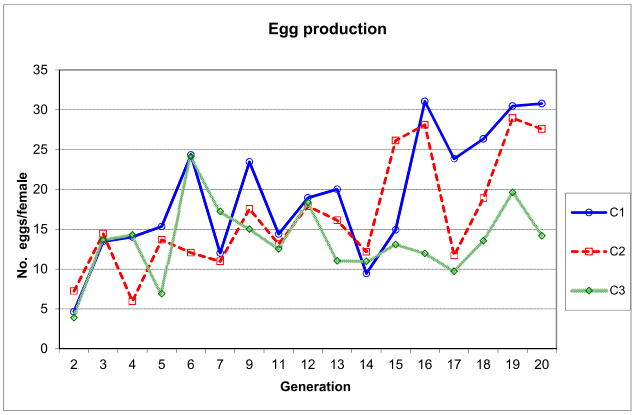

Figure 3. Egg production of *Aedes albopictus* reared in the three cages.

3.4. Correlation between parameters

Homogeneity of slopes assumption was met in all investigated parameters, except in egg production in the overall period where statistically significant evidence of slope heterogeneity was observed among C3 vs C1 and C2 ($p < 0.05$).

Correlation between wing length and survival was significant only in males, in C2 in the second period and it was very high and negative ($r = -0.953$; $p < 0.05$). When comparing wing length and egg production significant correlation ($p < 0.05$) was observed only in cage C2 in the first period both for males and females, and it was high and negative ($r = -0.745$ and $r = -0.751$ respectively). Correlation between survival and egg production was significant only in females: in C1 in the overall period and it was moderate and positive ($r = 0.650$; $p < 0.05$), while in C2 in the first period was high and negative ($r = -0.881$; $p < 0.05$).

4. Discussion

This study analyzed the influence of cage size on *Ae. albopictus* wing length, adult survival and egg production, during 20 generations from the beginning of colonization.

Considering the trends in the observed parameters the study period can be divided into two sub-periods. In the first period, mosquitoes expressed a significant decreasing trend in wing length, as well as in adult survival due to the negative influence of the new environment (colonization pressure). In the second period, the situation reversed, expressing an increasing trend in the parameters suggesting progressive adaptation. The length of the two periods resulted different by the considered parameters. Adult survival started to recover first (after generation F_9), followed by the wing length (after generation F_{11}). Egg production showed a steady increase but was significantly lower in the first than in the second period (in cages C1 and C2).

Despite that positive trend of wing length recovery recorded in the second period, the average values were lower compared to the first period. Only the wing lengths of the last generation approached the values of the initial population. On the other hand, adult survival and egg production resulted higher in the second period.

Mosquitoes reared in cage C1 had longest wings in both sexes (overall, the first and the second periods). So, the negative influence of colonization pressure and cage dimensions affected mosquitoes from C1 in the lowest degree. In the overall period and the first period wing length of both sexes in cage C2 was significantly shorter than in C3. In the second period, the situation changed, and the difference to C3 was not significant indicating that mosquitoes in C2 probably recovered faster than in C3, which was also shown in survival and egg production.

Mosquitoes reared in cage C1 lived significantly longer than in C3, no significant difference between C1 and C2 was found, while females from C3 lived significantly shorter compared to those from C2. Even though egg production did not express any significant difference among cages in the overall period and the first period, in the second period C3 resulted significantly lower than cages C1 and C2. Actually, in the second period C1 and C2 manifested a very high increase in egg production suggesting good adaptation to new conditions similarly as presented by Hoffmann and Ross (2018).

Adult body size (expressed through wing length) is a central life history character in mosquito fitness studies (Koenraadt, 2008), while possible factors for male mating success are considered to be: male body size (Huho et al., 2007; Maïga et al., 2012; Voordouw and Koella, 2007; Yuval et al.,

Table 3. Egg production (mean ± SD) of *Aedes albopictus* in the three cages.

Cage	Egg production (No. of eggs/female)		
	F_2 to F_{20}	F_2 to F_{14}	F_{15} to F_{20}
C1	19.26 ± 7.88 a	15.46 ± 5.89 a	26.24 ± 6.25 a
C2	16.62 ± 7.19 a	12.84 ± 3.81 a	23.57 ± 6.86 a
C3	13.54 ± 4.73 a	13.46 ± 5.50 a	13.69 ± 3.30 b

Numbers in the same column followed by the same letter are not significantly different for $p < 0.05$.

1993), age (Chambers and Klowden, 2001; Huho et al, 2006, 2007; Verhoek and Takken, 1994), genetics (Voordouw and Koella, 2007), sperm length (Klowden and Chambers, 2004; Voordouw et al., 2008) and energetic reserves (Huho et al., 2007; Maïga et al., 2012; Yuval et al., 1994).

Correlation between wing length, adult survival and egg production was lower than expected, probably due to the high variability of the parameters, seemingly different timing of their reaction to colonization, and different recovery speed of the parameters followed. Correlation between wing length and both survival and egg production was weak and, if significant, negative. Mosquitoes reared in C3 had significantly longer wings than mosquitoes from C2 (in both sexes), but adult survival and egg production in C3 expressed lower values than in C2. This might indicate that time needed for recovery of survival (second period) is longer than for the wing length, and that male wing length is not directly correlated to the mating success in cages. Following this, it seems that wing length might not always contribute to the increased adult survival and egg production in the colony; although in our experiments the males with longest wings (C1) lived significantly longer than C3. Similar was found by Hamady et al. (2013) who observed that *Ae. albopictus* females derived from wild and laboratory pupae of the similar size showed significant differences in egg production. Contrary, Blackmore and Lord (2000) reported that large females produce more eggs than small ones. It is also documented that females are more attracted to bigger males who have better fitness and greater reproductive capacity (Yuval et al., 1993). The larger *Ae. aegypti* males not only lived longer than smaller ones (Maciel-De-Freitas et al., 2007), but also the total number of spermatozoa recovered from testes and seminal vesicles was significantly higher in large vs small males within the same age group indicating a higher reproductive capacity and fitness of large males (Ponlawat and Harrington, 2007).

According to Maïga et al. (2012) *An. gambiae* males caught during mating were significantly bigger than free-flying males in swarms. Contrary to that, Charlwood et al. (2002) did not find any differences in body size between mated and non-mated *An. gambiae*, while according to Crompton et al. (2003) and Ng'habi et al. (2008) the intermediately sized males of *An. gambiae* mated more frequently than others, probably because of better agility in flight and quicker contact with females. Later was also found for average sized males of the mayfly *Baetis bicaudatus* (Peckarsky et al., 2002). So, the fact that smaller males could, in some cases, be better in finding mates, would possibly explain lower egg production in C3 compared to C2, despite significantly longer wings in C3.

Although it would be expected that males from larger cages have significantly longer wings during colonization because having more space to fly, the males from C1 resulted with longest wings. It seems that wing length under the conditions of same mosquito/volume density is not influenced by potential space availability but other, yet unknown factor/s. Also, adult survival and egg production were higher in smaller cages (C1 and C2).

Information gained during this study could be applied in the optimizing of mass–rearing facility exploitation, equipment construction/purchase and climate chamber design. During no-release periods in temperate regions (when there is no need for *Ae. albopictus* mass production) C1 cages can be used for maintaining colonies of the different strains. Using C1 cages will ensure the best fitness of the colony in the intermittent periods. For the mass production, few generations prior to the first planned release, C2 cages might be used, since their volume and shape are more convenient for high production and fitting the mass-rearing chambers. The switching of C1 and C2 cages relative to sterile male non-release and release periods might have beneficial influence on mosquito colonies' quality and productivity.

Further research should be done with increased sample size to compensate for high variability of the parameters and over more than 20 generations to determine duration and steadiness of the recovery trends. It should be useful to supplement observation of egg production with daily insemination rate as a more precise estimator of male mating performance. Besides, it would also be beneficial to investigate changes in wing symmetry and its correlation to other parameters.

5. Conclusions

This study highlights the level of cage size influence on *Ae. albopictus* fitness indicators (wing length, adult survival and egg production). Obtained results indicated the shape and volume of the cage most beneficial to colony productivity and fitness. Further, the results could be used to increase the yield of males in the mass-rearing facilities and feedback the optimisation of space, material, time and labour.

The smallest cage tested (C1 = 40 × 40 × 40 cm) demonstrated not only the best values for all observed parameters but also their smallest decrease during the first period. Recovering of mosquito population in the second period was much better in cages C1 and C2, than in C3. Additionally, larger size and greater weight make C3 (100 × 65 × 100 cm) not convenient for practical use. In the C3 cage wing length and adult survival (of both sexes) were significantly lower than in C1, while egg production did not show any recovering in the second period (typical for cages C1 and C2). Both sexes from C2 had significantly shorter wings, but their survival and egg production capacity did not show any significant difference compared to adults in C1.

We believe that mosquito rearing in cage C1 provides the least negative selection pressure on wing length, adult survival and egg production of the initial population of *Ae. albopictus* RER strain. Anyhow, if space saving is a priority in mosquito mass-rearing, C2 might be a better choice than C1. Cage C2 has a more convenient shape (upright cuboid) and higher volume than C1, which is more practical and economical for mass production of mosquitoes needed for SIT implementation.

Testing the other combinations of cage volume and shape (small upright cuboids, large cube etc.) might further contribute to the understanding of behaviour of the colonies and sustainability of mass-production.

To determine duration and steadiness of the recovery trends extended evaluation should be done following the parameters for more than 20 generations. Also, including the measurement of daily insemination rate might contribute to the estimation of male mating performance.

Declarations

Author contribution statement

Dubravka Pudar: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Arianna Puggioli: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Fabrizio Balestrino: Conceived and designed the experiments; Performed the experiments.

Victoria Sy: Performed the experiments.

Marco Carrieri: Analyzed and interpreted the data.

Romeo Bellini, Dušan Petrić: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

All data publicly available upon request in Sanitary Entomology & Zoology Department, Centro Agricoltura Ambiente "G. Nicoli" - IAEA Collaborating Centre, Crevalcore (Bologna), Italy.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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