

From production to pre-release: quality assessment of *Aedes albopictus* males produced following FAO/IAEA procedures and within an SIT program in La Reunion island

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Research Article

Keywords: Sterile Insect Technique, genetic control, mosquitoes, biological quality, mating competitiveness, operational procedures

Posted Date: June 20th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9893737/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

The success of the sterile insect technique (SIT) relies on the biological quality and field performance of released sterile males. However, multiple factors throughout the production to release workflow, may affect insect quality. This study evaluated the quality of sterile *Aedes albopictus* males produced following standardized FAO/IAEA guidelines at the Insect Pest Control Laboratory (IPCL), as well as those produced within an operational SIT program in La Réunion island.

Methods

Mosquitoes were mass-reared, sex-separated, irradiated, and subjected to a two-day simulated long-distance transport following FAO/IAEA operational workflow. Key biological quality indicators, including the post-treatment survival, escape rate, longevity and mating competitiveness were assessed.

Results

Male post-treatment survival rate remained high after irradiation ($97.5 \pm 0.14\%$) and after 48 hours of transport ($90.7 \pm 0.81\%$), particularly when the males were allowed a 24-hours recovery period with access to a 10% sucrose solution. In contrast, stress or complete starvation significantly reduced the survival rate to below 60%. The escape rate declined from $68.9 \pm 3.2\%$ in control group to $58.8 \pm 1.0\%$ and further to $19.2 \pm 2.3\%$ after irradiation and transport, respectively. However, it partially recovered to $33.1 \pm 1.6\%$ following a 24-hour recovery period post-transport. The mating competitiveness index of irradiated males at a sterile-to-fertile male ratio of 1:1 ranged from 0.76 to 1.15. However, it declined to 0.25 after a two-day transport, with corresponding induced sterility levels of 52.86 ± 6.45 and 13.03 ± 5.02 , respectively. Increasing the sterile-to-fertile male ratio from 1:1 to 5:1 or 10:1 effectively compensated for this reduction, increasing sterility levels to $80.35 \pm 8.07\%$. In La Réunion's field-based SIT program, results confirmed the robustness of the implemented protocols, with sterile males performing as competitively as wild-type males under semi-field conditions.

Conclusion

Sterile *Ae. albopictus* males produced following FAO/IAEA mass-rearing and irradiation procedures maintained a high biological quality, demonstrating their suitability for SIT implementation. However, a transport duration of 48 hours was unsuitable for effective field program deployment.

Background

Aedes albopictus (Skuse) (Diptera: Culicidae) is one of the most invasive species and plays a major role as a vector of dengue, chikungunya, and Zika viruses [1, 2]. Over recent decades, these arboviruses have expanded geographically, resulting in an increasing global burden of mortality and morbidity [3, 4]. The distribution of *Ae. albopictus* has also expanded significantly, particularly into temperate regions, increasing the risk of outbreak in Europe [5]. In 2024, a total of 169 countries were identified as having more than 10% of their population living in at-risk areas for dengue, chikungunya and Zika transmission [6]. *Aedes albopictus* is the predominant mosquito species in the island of La Réunion, and represents a persistent public health concern due to its high vector competence and remarkable adaptability to urban and peri-urban environments [7]. It is the principal vector responsible for recurrent dengue and chikungunya outbreaks on the island [8, 9]. From 2017 to March 2025, La Réunion experienced active and continuous circulation of the dengue virus. More recently, between August 2024 and June 2025, the island faced a notable outbreak of chikungunya virus, with more than 54,000 confirmed cases and 43 associated deaths [10], making the second major outbreak of this virus on the island.

The effectiveness of conventional mosquito control methods, which rely primarily on chemical insecticides, is increasingly being undermined by insecticide resistance and public concerns about toxicity, limited specificity, and the contamination of food and the environment [11, 12]. As a result, there is a growing demand for alternative, more sustainable control strategies [13]. The sterile insect technique (SIT) is an eco-friendly, species-specific biocontrol method that involves releasing large numbers of sterile males, incapable of producing viable offspring when mating with wild females. This reduces the size of the targeted populations without harming non-target organisms or leaving residues. The SIT method can be effectively incorporated into area-wide integrated pest management (AW-IPM) programs [14], complementing source reduction and targeted adult control, particularly in densely populated urban settings with cryptic breeding sites for *Aedes* mosquitoes. While SIT offers significant advantages, its successful implementation hinges on maintaining the biological quality of the released sterile males throughout the entire production-to-release pipeline. During this process, male mosquitoes are exposed to multiple stressors associated with mass rearing, irradiation, transport and handling, which may individually or cumulatively affect their phenotypic (physiological and behavioral) quality. For example, the transition from small-scale laboratory colonies to industrial production (mass-rearing) has the potential to inadvertently select for domestication traits and a concomitant reduction in field performances [15]. Moreover, irradiation (achieved by gamma or X-ray exposure), which is an imperative step to induce sterility through dominant lethal mutations in germ cells, can cause somatic damage, impacting metabolic function, flight muscle integrity, and longevity [16] and ultimately reduce flight ability and mating success [17, 18]. Finally, transporting and handling processes from the production facility to the release site impose additional stress, through mechanical vibration, temperature fluctuations, and temporary food deprivation leading to increased mortality, reduced longevity and escape rate in surviving males [18].

The Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) have played a pivotal role in the global development of the SIT package against several insect pests of agricultural, medical and veterinary importance. Since 2004, the Joint FAO/IAEA Centre

of Nuclear Techniques in Food and Agriculture has led efforts to develop a comprehensive SIT package against mosquitoes, aimed at supporting Member States in implementing this environment-friendly control method within AW-IPM programs. Through continuous research and development, specialized equipment and standardized operating procedures have been established, and guidelines have been developed and published, covering mosquito colonization, mass rearing, sex-separation, irradiation, packing and transport, and mark-release-recapture methods [19–22]. Most of these guidelines have recently been updated to incorporate technological advances and optimized protocols. Notable developments include the adoption of capsule-based egg hatching method [23] optimized larval and adult mass-rearing methods [24, 25], automation of pupal sex-separation [26, 27], the transition from small-scale pupal irradiation to large-scale adult irradiation [28], and improved methods for handling, long-distance transport [18, 29] and release [30]. Collectively, these innovations support the large-scale production of high-quality sterile males for operational SIT programs against mosquito vectors implemented by Member States. Nevertheless, adaptations or slight deviations from of the standard FAO/IAEA procedures are sometime required due to ecological differences, mosquito strain variability, and logistical constraints such as larval diet availability, and transport conditions. In La Réunion, where the OPTIS (*Opérationnalisation de la Technique de l’Insecte Stérile contre les Aedes vecteurs de la dengue à La Réunion*) project is being implemented, most FAO/IAEA procedures are applied with minor adaptations, particularly adjustments in larval diet formulation and rearing density [24] to better suit local operational conditions.

Several field trials are currently evaluating the use of the SIT as a genetic approach for the suppression of *Aedes* mosquito populations [31], and 34 SIT pilot projects targeting mosquitoes are being implemented worldwide [32]. However, the success of these programs relies fundamentally on the implementation of efficient, standardized procedures for large-scale production and on maintaining the biological quality of the released sterile males [33]. To date, most quality assessment studies have focused on males produced under small-scale rearing and irradiation conditions [17, 34–40] and assessed insect quality using limited sample sizes, which may not accurately reflect operational conditions. Evaluating the quality of the sterile males produced under large-scale production, irradiation, and exposed to transport logistics over long distances is therefore essential for successful operational deployment. When validating new equipment or protocols, quality parameters such as adult emergence rate, adult survival rate, body size and male flight ability are commonly used as key indicators, providing valuable information on production consistency. However, a critical performance metric for program success is the mating competitiveness of the end product: release-ready sterile males. This metric must be evaluated prior to any large-scale implementation of the protocol.

The objective of this study was to evaluate the biological quality of *Ae. albopictus* males produced under realistic operational workflows. More specifically, we aimed to: (i) evaluate the quality of *Ae. albopictus* males produced according to the full FAO/IAEA operational production process, at the Insect Pest Control Laboratory (IPCL), in Seibersdorf, Austria, (ii) investigate the effect of overflooding sterile-to-fertile male ratios (OFR) on mating competitiveness, and (iii) evaluate the quality of *Ae. albopictus* males, reared and irradiated within an operational SIT program in the island of La Réunion.

Methods

The study was divided into two distinct and complementary experiments. Experiment 1 was conducted at the IPCL, Seibersdorf, Austria, and followed the full FAO/IAEA workflow, including mass-rearing, irradiation and transport. The aim of this experiment was to evaluate the quality of the produced sterile males and to assess the effect of overflooding sterile-to-fertile male ratio on mating competitiveness. Experiment 2 was carried out in La Reunion as part of an ongoing operational SIT program. In this setting, FAO/IAEA procedures were implemented, with minor adaptations to local conditions, such as the absence of brewer's yeast in the larval diet and a shorter transport duration. The quality of the produced sterile males was evaluated, with particular focus on their ability to compete with wild males for mating with wild females.

Experiment 1: Evaluating the quality of sterile *Aedes albopictus* males produced following FAO/IAEA standard procedures and the effect of overflooding sterile-to-fertile male ratios on mating competitiveness

Mosquito strain and rearing

The *Ae. albopictus* colony used in this experiment originated from Rimini, Italy, and has been maintained at the IPCL since 2018 under the environmental conditions described in the FAO/IAEA guidelines [21]. For this experiment, dissolvable capsules containing eggs and diet were individually introduced into mass-rearing trays [23]. Each capsule contained 135 mg of eggs (approximately 18,000 larvae) and 212 mg of diet, reaching a total weight of 347 mg. The entire rack which contains thirty mass-rearing trays, was used for each experiment and the overall experiment was repeated three times. The larvae were fed a 4% liquid diet consisting of 50% tuna meal, 35% black soldier fly powder, and 15% brewer's yeast, following a feeding regime that excluded the provision of food on days 2 and 3 [24]. Following pupae separation on day 6 after egg hatching (24h after first pupation) using the Wolbaki automated pupae sex sorter [26, 27], the male pupae batch was immersed in a 15% NaCl solution for one hour to eliminate any contaminating larvae [27]. Both male and female pupae were then volumetrically estimated [18]. The male pupae were subsequently divided into aliquots of 3,000 and transferred into 30 × 30 × 30 cm cages (BugDorm, DP1000, Taichung, Taiwan) with access to a 10% sucrose solution.

Irradiation, packing and long-distance transport

Three- to four-day-old emerged adults were chilled in a cold room (4 °C) for approximately 10 minutes [26]. Then, they were loaded into a 3D printed, coolable canister [28] and irradiated with 55 Gy using a Gammacell 220 irradiator (Nordion Ltd., Kanata, Ontario, Canada). The actual dose received by the mosquitoes was assessed using Gafchromic films dosimetry [41, 42] and resulted in 99% sterility.

Immediately after irradiation, batches of 3,000 sterile males were kept in cages for use in subsequent evaluations. The remaining males were returned to the cold room, and placed in square plastic boxes

($2.4 \times 2.1 \times 2.3 \text{ cm}^3$ for a compaction density of 86 mosquitoes/ cm^3), then placed inside a vacuum-insulated transport/shipment box (IAEA packing method V.2.0 as described by Mamai et al. [18]). The transport/shipment box was kept under laboratory conditions for 48 hours and regularly shaken and rotated to simulate a long-distance transport and movement during shipping [18, 43].

Quality assessment

Survival rate

The transport/shipment box was opened following the completion of the 48-hour transport simulation, as outlined in the study of Mamai et al. [18]. Subsamples of approximately 500 mosquitoes (aged 5–6 days) were randomly selected from square boxes and placed into open petri dishes (diameter $10 \text{ cm} \times 1 \text{ cm}$), which were placed inside cages ($17.5 \times 17.5 \times 17.5 \text{ cm}^3$, BugDorm, BD4M1515, Taichung, Taiwan). The survival rate, representing the proportion of sterile males that survived irradiation and transportation and successfully flew out of the petri dish, was evaluated after a recovery time of two hours without sugar and 24 hours with access to a 10% sucrose solution. On the day of irradiation, samples consisting of approximately 500 irradiated males each (aged 3–4 days) were placed in cages ($17.5 \times 17.5 \times 17.5 \text{ cm}^3$, BugDorm, BD4M1515, Taichung, Taiwan). The cages were divided into two groups. One group was provided with a 10% sucrose solution, while the other group was not given access to sugar and water (also referred to as “under stress”). The survival rate was recorded after 24 hours. The same survival rate was also observed in the control non-irradiated males (aged 4–5 days). Four technical replicates were conducted for each group and each time interval, and the experiment was repeated three times.

Escape rate

The assessment of adult male flight ability, referred to as the “escape rate,” was conducted using the FAO/IAEA- developed and standardized flight test device (FTD) [44–46]. Fifty adult males were introduced into the FTD, and the escape rate was calculated as the percentage of individuals that successfully exited the tubes. Escape rate was evaluated across four distinct groups of males: (i) non-irradiated males with access to a 10% sucrose solution, (ii) irradiated males that underwent a 24-hour recovery period with access to a 10% sucrose solution, (iii) post-transported irradiated males after a 2-hour recovery period without access to a 10% sucrose, and iv) post-transported irradiated males after a 24-hour recovery period with access to a 10% sucrose solution.

Mating competitiveness under semi-field conditions

Mating competitiveness experiments were performed within a climate-controlled ecosphere with an average temperature of $22.42 \pm 0.02^\circ\text{C}$, and a relative humidity of $58.57 \pm 0.05\%$ measured using the ONSET HOBO U12-012 data logger; Onset Computer Corporation, MA, USA. The ecosphere is setup to work within natural photoperiod. Semi-field cages ($1.75 \times 1.75 \times 1.75 \text{ m}^3$, Live Monarch. Boca Raton.

USA) were used for the competition experiment. A larval tray ($40 \times 30 \times 7 \text{ cm}^3$) containing water and two 150 mL sample cups with a 10% (w/v) sucrose solution fitted with a cellulose sponge cloth was placed in each cage. The trays provided humidity and attractive resting surfaces and visual cues that facilitated the mosquitoes' access to the sugar sources. For each competitiveness assay, three technical replicates of 25 fertile males along with the required number of sterile males were released into the semi-field cage first. Then, 25 fertile females were added to each cage, and the mosquitoes were allowed to mate for 24 hours. The age mosquitoes ranged from 4 to 6 days, depending on the treatment group (irradiated only or irradiated and transported).

The following treatments with sterile-to-fertile (S:F) male ratios were used:

- 1) Irradiated males, 4 h recovery, S:F ratio 1:1
- 2) Irradiated males, 24 h recovery, S:F ratio 1:1
- 3) Irradiated males, 24 h recovery, S:F ratio 5:1
- 4) Irradiated males, 24 h recovery, S:F ratio 10:1
- 5) Irradiated and transported (48 h) males, 4 h recovery, S:F ratio 1:1
- 6) Irradiated and transported (48 h) males, 24 h recovery, S:F ratio 1:1
- 7) Irradiated and transported (48 h) males, 24 h recovery, S:F ratio 5:1
- 8) Irradiated and transported (48 h) males, 24 h recovery, S:F ratio 10:1

After a 24-hour mating period, the adults were collected with a battery-powered hand-held aspirator and transferred into cages ($17.5 \times 17.5 \times 17.5 \text{ cm}^3$; BugDorm BD4M1515, Taichung, Taiwan). Only females were isolated in a cold room (4°C) to immobilize the mosquitoes, and transferred into separate cages ($17.5 \times 17.5 \times 17.5 \text{ cm}^3$; BugDorm BD4M1515, Taichung, Taiwan). Control assays were also conducted in $17.5 \times 17.5 \times 17.5 \text{ cm}^3$ cages (BugDorm, Taichung, Taiwan), each containing 25 fertile females and one of the following male groups: (i) 25 fertile males or (ii) 25 sterile males. The cages were maintained under standard laboratory conditions and provided with a 10% sucrose solution. Females were fed twice (one the same day and a second two days later) with fresh porcine blood, collected with the anticoagulant Phosphate (concentration 5g/L), using collagen sausage casings (Edicas 28NU, FIBRAN S.A., Girona, Spain). The blood-fed females were subsequently allowed to oviposit en masse in plastic cups filled one-third with deionized water and lined with germination paper. The collected eggs were air-dried and hatched using standardized procedures to determine the hatch rate and calculate the competitiveness index and induced sterility.

Experiment 2: Quality assessment of sterile *Aedes albopictus* males within an SIT program in La Réunion

Mosquito strain and rearing

The *Ae. albopictus* strain used in this experiment originated from the eggs collected in Sainte Marie, in the northern district of the island of La Reunion, and has been maintained under laboratory conditions since 2014. For the OPTIS project, the mosquitoes were mass-reared in a climate-controlled insectary at $31 \pm 0.5^\circ\text{C}$, $85 \pm 5\%$ RH, and a 12:12 h (light: dark) photoperiod at the IRD (Institut de Recherche pour le Développement) insectary located at CYROI Saint-Denis, La Reunion. Eggs corresponding to 14,600 first instar larvae, were hatched in cylindrical, 125-mL plastic containers with screw caps using a hatching solution prepared the previous night. The eggs were allowed to hatch for two and a half hours after which the larvae were rinsed into larval mass-rearing trays stacked in a rack unit, as described in Experiment 1 above. The larvae were fed with a 4% (w/v) diet consisting of 57.5% tuna meal and 42.5% black soldier fly powder, following the four-day feeding regime described by Mamai et al. [24]. Pupae were sex-separated using the Wolbaki automated pupae sex sorter. Male pupae were aliquoted into groups of 2,000 and transferred into $30 \times 30 \times 30 \text{ cm}^3$ cages (BugDorm, DP1000, Taichung, Taiwan), which were supplied with a 10% sucrose solution until irradiation. Cages containing only virgin males and virgin females were also maintained separately.

The wild-type (F0) male and female mosquitoes used in the experiment were collected as eggs in Sainte Marie just before each experiment. Oviposition papers were collected and dried for fourteen days before egg hatching induction. The larvae reared in plastic containers (7 cm diameter, 11 cm height) at a density of 100 larvae per container (density of 3 larvae/ml) and fed the same 4% (w/v) liquid diet as described above. The male and female pupae were sexed based on external genital morphology using a stereomicroscope. They were then placed in small bowls containing water, and transferred into $45 \times 45 \times 45 \text{ cm}^3$ emergence cages (BugDorm, Taichung, Taiwan).

Irradiation

Four- to five-day-old adult laboratory-reared male mosquitoes were knock-down in a cold room (4°C) for approximately 10 minutes. The mosquitoes were compacted into square plastic boxes ($2.4 \times 2.1 \times 2.3 \text{ cm}^3$) at a compaction density of 86 mosquitoes/ cm^3 , and transported in a portable refrigerator (Dometic CoolFreeze CFX-50), maintained at 8°C , and 80% relative humidity (RH) to the irradiation facility, located approximately 80 km from the insectary. Irradiation was performed in a chilled environmental box with 50 Gy using an X-ray irradiator (X-RAD 320 Cabinet Style Irradiator, Precision X-Ray, Inc., Madison, CT, USA) [47]. After irradiation, the sterile males were returned to the laboratory. They were then divided into two groups. One group was marked with pink fluorescent dust (DayGLO Color Corp., Cleveland, OH, USA) at a dose of 10 g per 1,000 males; the other group remained unmarked. The total transport time was about six hours. Both the marked and unmarked sterile males were subsequently used for quality assessments, including survival, flight ability, and mating competitiveness.

Survival

The survival of four cohorts of males was evaluated over a 10-day period, representing the maximum interval between field releases, and the following experimental groups were evaluated (i) the laboratory mass-reared controls, (ii) the wild males, (iii) the irradiated and transported males, and (iv) the irradiated, transported and marked males. For each cohort, survival was monitored under three feeding regimes: (a) a 10% sucrose solution, (b) water only, and (c) under stress or complete starvation (no water and no sugar). For each cohort-feeding regime combination, twenty-five males were placed in a $15 \times 15 \times 15$ cm³ cage (BugDorm, Taichung, Taiwan) and mortality was recorded daily for a period of ten consecutive days.

Escape rate

The male escape rate was evaluated as described above across the four groups of males: (i) the laboratory mass-reared controls, (ii) the wild males, (iii) the irradiated and transported males, and (iv) the irradiated, transported and marked males. Three technical replicates were performed for each group and the experiment was repeated three times.

Mating competitiveness of sterile males against wild males

Mating competitiveness was evaluated in a large enclosure consisting of a metal frame with the roof that was partially covered with polyethylene plastic. The structure was covered with a shade netting that served as a double roof and three side panels, providing a semi-natural environment that maintained ambient temperature and relative humidity. Climatic conditions were recorded during experiments using a TempU03 data logger (Tzone Digital Technology Co., Ltd.), and averaged $29.86 \pm 0.28^\circ\text{C}$ (range $21.2\text{--}49.7^\circ\text{C}$), and $70.13 \pm 0.55\%$ RH (range $35.4\text{--}87.3\%$). Irradiated and transported males as well as marked, irradiated, and transported males, competed with wild males to mate with wild females inside $47.5 \times 47.5 \times 47.5$ cm³ mesh cages (BugDorm-4H4545, Taichung, Taiwan). Six cages ($47.5 \times 47.5 \times 47.5$ cm³) were mounted on chairs, each placed on plastic cups containing talc powder to prevent ant intrusion.

For each technical replicate, 25 sterile males and 25 fertile wild males (4–5 day-old) from each treatment group were introduced into the mating cages for one hour first to allow them to recover from handling stress and become fully active. Then, 25 fertile wild females were added. Three technical replicates were conducted per treatment and the experiment was repeated three times. For the third experiment only, laboratory-reared females were used due to an insufficient availability of wild females. A 10% sucrose solution on filter paper was provided as a food source. Mating was allowed to occur for 24 hours after the females were introduced. Thereafter, the males were removed, and the cages were transported to the laboratory. Females were offered blood meals on two consecutive days, using fresh bovine blood obtained from the regional slaughterhouse in Saint-Pierre, La Réunion. The blood was supplemented with 0.1% EDTA. Oviposition substrates (germination paper) were provided, and the eggs were collected three days post-feeding. The eggs were subsequently air-dried and hatched using the procedures described by Marquereau et al. [48].

For each experiment and repetition, control assays were also performed using $30 \times 30 \times 30 \text{ cm}^3$ cages (BugDorm-4H4545, Taichung, Taiwan) each containing 25 fertile, wild-type females and one of the following groups of males: (i) 25 fertile, laboratory mass-reared males, (ii) 25 sterile, laboratory mass-reared males.

Statistical analysis

The percentage of egg-induced sterility (IS) was calculated using the Abbott-corrected formula [49]: $IS = 100 \times (1 - H_o/H_n)$, where H_o represents the hatch rate in the treatment cage and H_n the hatch rate in the fertile male control. Competitiveness values (C) were computed following Fried index [50]: $C = ((H_n - H_o)/(H_o - H_s)) \times (N/S)$, where H_n and H_s denote the hatch rates from females mated exclusively with fertile males and sterile males, respectively; H_o is the observed hatch rate in the treatment cage at a given sterile-to-wild male ratio; N and S correspond to the numbers of fertile and sterile males, respectively. Analysis were performed using R Software version 4.3.2 (R Development Core Team 2008) along with RStudio version 2024.10.31. environment (RStudio, Inc. Boston, MA, USA, 2016). Binomial generalized linear mixed models (GLMM), fit by maximum likelihood (Laplace Approximation) were used to assess differences in survival and escape rate where they served as response variables, with treatment as a fixed effect, replicate and experiment as random effects. For competitiveness index and induced sterility, linear mixed-effect models (LMM) were used after applying an angular (arcsine square-root) transformation to percentage data to stabilize variances and normalize distributions. The survival was analyzed using cox proportional hazards mixed-effects models (coxme), and the log-rank test to assess differences in survival probabilities among groups. Differences in mean survival were analyzed using a LMM.

Results

Experiment 1: Quality assessment of sterile *Aedes albopictus* males reared following FAO/IAEA procedures

Post-treatment survival rate

The survival rates (mean % $\pm$ SE) in *Ae. albopictus* (Fig. 1) were significantly affected by irradiation, long-distance transport, and recovery conditions. Survival rate remained high in irradiated males ($97.53 \pm 0.24\%$) and in the transported males ($90.73 \pm 0.81\%$) after a 24-hour recovery period with access to sugar. However, these survival values were significantly lower than those observed in the non-irradiated control group ($98.15 \pm 0.14\%$), confirming the negative effect of irradiation (GLMM, $z = -4.23$, $p = 2.35e-05$) and long-distance transportation (GLMM, $z = -26.48$, $p < 2e-16$). Regardless of recovery duration, stress (starvation) conditions markedly decreased the survival in non-transported irradiated males ($78.63 \pm 1.95\%$) and in transported sterile males ($55.41 \pm 2.84\%$), emphasizing the beneficial effect of sugar feeding during the recovery period.

Escape rate

The escape rate of *Ae. albopictus* males, expressed as the percentage of males escaping from the FAO/IAEA flight test device, is shown in Fig. 2. Non-irradiated (control group) males exhibited the highest escape rate ($68.93 \pm 3.22\%$), which was significantly higher than that of all irradiated groups (GLMM, $p < 0.05$). Although irradiated males maintained a relatively high escape rate ($58.76 \pm 0.99\%$) compared with the two experimental groups exposed to transporting conditions, irradiation significantly reduced the escape rate as compared with the control group ($z = -4.35$, $p = 1.39e-05$). Transported sterile males showed a marked decrease in escape rate, particularly when tested after only two hours of recovery ($19.19 \pm 2.33\%$, see Fig. 2). However, when transported males were given a 24-hour recovery period with access to a 10% sucrose solution, their escape rate increased significantly ($33.12 \pm 1.55\%$) compared to those without sugar ($19.19 \pm 2.33\%$), although this escape rate remained lower than that of non-transported sterile males ($68.93 \pm 3.22\%$).

Mating competitiveness

Mating competitiveness, as measured by the Fried index, is presented in Fig. 3A. Results revealed significant variation in sexual competitiveness among treatments (ANOVA, $F_{(48.075)} = 5.33$, $p < 0.05$), indicating that transport conditions, and recovery time influenced male mating performance. Sterile males subjected to long-distance transport exhibited significantly lower competitiveness index values (0.25 ± 0.18) compared with their sterile counterparts that had not been transported (0.76 ± 0.13) when tested after 4 hours of recovery (LMM, $df = 45.77$, $t = -2.68$, $p = 0.01$). Furthermore, even when they were allowed 24 hours of recovery with access to a 10% sucrose solution, transported sterile males consistently showed lower competitiveness (0.20 ± 0.11 to 0.55 ± 0.13) than non-transported sterile males (0.76 ± 0.13 to 1.15 ± 0.23), regardless of the sterile-to-fertile male ratio. Increasing the ratio of sterile to fertile males did not result in a higher competitiveness index for either non-transported or transported sterile males (Fig. 3A, $p > 0.05$). Although extending the recovery period to 24 hours with access to a 10% sucrose solution slightly increased the competitiveness index of irradiated and transported males, the increase was not statistically significant ($p > 0.05$). These results underscore the detrimental impact of long-distance transport on male mating competitiveness.

The levels of induced sterility obtained from the different groups of irradiated males are shown in Fig. 3B. Induced sterility ranged from $9.17 \pm 4.08\%$ to $80.35 \pm 8.07\%$. A linear mixed-effects model revealed a significant effect of treatments on induced sterility (ANOVA, $F_{(7, 42.06)} = 20.47$, $p < 0.001$). A 24-hour recovery period with access to a 10% sucrose solution resulted in slight but significant increase in induced sterility among irradiated non-transported males compared with males tested 4 hours after irradiation (LMM, $df = 43.77$, $t = 1.94$, $p = 0.05$). The effect of transport was also evident, as the transported males consistently induced lower sterility than non-transported males, particularly when tested after only a 4-hour recovery period (LMM, $df = 42.54$, $t = -4.53$, $p = 4.69e-05$). Increasing the sterile-to-fertile male ratio from 1:1 to 5:1 or to 10:1 in both transported and non-transported groups significantly increased induced sterility (LMMs, $p < 0.05$), reaching values as high as 80%. This pattern reflects the compensatory effect of increasing sterile male densities in overcoming moderate reductions

in individual male competitiveness. However, no significant difference in induced sterility was detected between the 5:1 and 10:1 sterile-to-fertile male ratios for either non-transported (LMM, $df = 40.48$, $t = 0.49$, $p = 0.63$) or transported males (LMM, $df = 48.16$, $t = -0.51$, $p = 0.62$).

Experiment 2: Quality assessment of sterile *Aedes albopictus* males in an SIT program in La Reunion

Escape rate

As shown in Fig. 4, the escape rate of *Ae. albopictus* males differed significantly among treatments. Compared to the wild males as the reference group, the escape rate was significantly lower in irradiated and transported males (GLMM, estimate = -0.466, $z = -3.258$, $p = 0.001$) and in irradiated, transported and marked males (GLMM, estimate = -0.615, $z = -4.196$, $p = 2.71 \times 10^{-5}$). However, there was no significant difference between irradiated-transported and irradiated-transported-marked males (GLMM, estimate = -0.149, $z = -1.014$, $p = 0.311$). Furthermore, the odds of laboratory mass-reared males escaping the flight device did not differ significantly from those of wild males (reference group) (GLMM, estimate = -0.084, $z = -0.589$, $p = 0.556$), indicating that colonization and mass-rearing procedures did not adversely affect the males' escape rate, but that irradiation, combined with transport and additional marking, was associated with a significant reduction in this rate.

Survival over ten-day observation period

Additional file 1 and Table 1 present the 10-day survival probabilities of the different male groups under the various nutritional regimes. Male survival varied according to both treatment group (Log-rank (Mantel-Cox) test, $\chi^2 = 12.1$, $df = 3$, $p = 0.007$) and nutritional regime (Log-rank (Mantel-Cox) test, $\chi^2 = 886$, $df = 2$, $p < 2e-16$).

Table 1

Ten-day survival and mean longevity of adult *Aedes albopictus* males maintained under three nutritional conditions: 10% sucrose, water only, and stress, conducted in the context of an SIT program on Réunion Island

Nutritional conditions	10% sucrose	Water	Under stress
Treatments	Survival rate after 10 days $\pm$ SE (%)	Mean survival $\pm$ SE (days)	Mean survival $\pm$ SE (days)
Wild	93.00 $\pm$ 5.79 ^a	3.98 $\pm$ 0.17 ^a	3.15 $\pm$ 0.11 ^a
Control Lab-reared	89.33 $\pm$ 5.88 ^{ab}	3.46 $\pm$ 0.17 ^{ab}	2.22 $\pm$ 0.09 ^b
Irradiated and transported	78.67 $\pm$ 5.89 ^b	3.38 $\pm$ 0.12 ^{ab}	2.18 $\pm$ 0.07 ^b
I Irradiated, transported and marked	91.88 $\pm$ 2.71 ^{ab}	2.91 $\pm$ 0.13 ^b	1.46 $\pm$ 0.07 ^b
. Within the column, means followed by different superscript letters are significantly different (LMM). Means sharing the same letter are not significantly different at $p < 0.05$.			

Under the 10% sucrose treatment, survival after a period of 10 days was high in all groups, ranging from 78.67 $\pm$ 5.89% to 93.00 $\pm$ 5.79% (see Table 1). The irradiated and transported males showed a significantly higher risk of mortality than the wild males (hazard ratio [HR] = 3.30, $z = 2.33$, $p = 0.02$) under this nutrition condition, whereas no significant differences were detected for control lab-reared males (HR = 1.55, $z = 0.77$, $p = 0.441$) or irradiated, transported and marked males (HR = 1.19, $z = 0.29$, $p = 0.769$) compared to wild males.

Under water-only conditions, survival decreased markedly in all groups, and all males died during the observation period, with none surviving beyond eight days. Compared with wild males, all other groups showed a significantly higher risk of mortality ($p < 0.05$). The irradiated, transported and marked males showed the significantly lowest mean longevity (2.91 $\pm$ 0.13 days) compared to wild males (3.46 $\pm$ 0.17 days) (Table 1, LMM, $df = 6.0$, $t = -3.957$, $p = 0.007$).

Under stress conditions, 100% mortality was reached by day 6 in wild males, by day 4 in laboratory-reared and irradiated and transported males, and by day 3 in irradiated, transported and marked males. The mean longevity of wild males was the longest (3.15 $\pm$ 0.11 days), while that of irradiated, transported and marked males was the shortest (1.46 $\pm$ 0.07 days).

Mating competitiveness

In semi-natural environmental conditions mating competitiveness assays, irradiated and transported males exhibited a competitiveness index of 0.95 $\pm$ 0.19. Interestingly, irradiated, transported and marked males exhibited a higher index of 1.23 $\pm$ 0.27. However, this difference was not statistically significant (LMM, $df = 14.00$, $t = 0.827$, $p = 0.42$). These results suggest that sterile males maintained high mating

competitiveness compared to wild males under the experimental conditions. Irradiated and transported males achieved approximately 95% of wild mating performance, while irradiated, transported and marked males performed at least comparably.

Induced sterility level was $45.88 \pm 6.13\%$ in the irradiated and transported males and $48.33 \pm 5.31\%$ in the irradiated, transported and marked males. This difference was not statistically significant (LMM, $df = 14.00$, $t = 0.45$, $p = 0.66$). Taken together, these results suggest that irradiation and the additional handling associated with transport and marking did not impair the ability of *Ae. albopictus* males to effectively compete with wild males for mates under semi-field conditions.

Discussion

The success of the SIT programs critically hinges on the biological quality and field performance of the released sterile insect males. Throughout the complex production-to-release chain, various biological, physical, and operational factors may impact males' performance, making rigorous quality assessment of the "end product" release-ready sterile males an indispensable asset for program effectiveness. This study evaluated key biological quality indicators of sterile *Ae. albopictus* males produced under FAO/IAEA standardized procedures at the IPCL and within an ongoing operational SIT program in the island of La Réunion, focusing on their ability to withstand cumulative stresses and remain competitive with their natural counterparts for mates. Parameters evaluated include post-treatment survival, escape rate and mating competitiveness.

The proportion of males successfully recovered after irradiation and/or transport is a critical operational indicator in SIT programs. Losses during these steps can significantly reduce the number of sterile males available for field release, which compromises the planned overflooding ratio and the overall efficiency of the program. This study revealed that *Ae. albopictus* sterile males produced under the FAO/IAEA operational workflow maintained high post-treatment survival rates, even after irradiation ($97.5 \pm 0.14\%$) and after 48 hours of simulated transport conditions ($90.7 \pm 0.81\%$) in IPCL, provided they received a 24-hour recovery period with access to a 10% sucrose solution. In contrast, a two-day transport of *Ae. albopictus* from Italy to Montenegro led to 40% mortality upon arrival [51]. The high survival rates observed in irradiated and transported males after a 24-hour recovery period with access to a 10% sucrose solution indicate remarkable physiological resilience of *Ae. albopictus* to the combined effects of optimized chilling, handling, and irradiation. However, the IPCL experiment clearly highlighted the detrimental impact of stress conditions, which significantly decreased the percentage of survival rate to $78.63 \pm 1.95\%$ and $55.41 \pm 2.84\%$ after irradiation and two days of transportation, respectively. This underscores the importance of carbohydrate replenishment to restore metabolic reserves and support flight muscle activity after stressful handling and transport.

For recovered males to perform well in the field, they must be able to fly and disperse effectively after irradiation and/or transport. Flight ability, assessed by the escape rate, serves as reliable indicator of adult vigor and dispersal potential. In the IPCL experiment, irradiation alone significantly reduced escape to $58.76 \pm 0.99\%$ compared to the control group ($68.93 \pm 3.22\%$). The simulated 48-hour long-distance

transport further exacerbated this reduction, leading to a marked decrease in escape rate, particularly when males were tested shortly after arrival ($19.19 \pm 2.33\%$ after two hours of recovery). Crucially, a 24-hour recovery period with access to sugar significantly improved escape rates to $33.12 \pm 1.55\%$, demonstrating that some negative effects of transport are reversible. These results corroborate previous findings that appropriate post-transport recovery can restore flight performance to acceptable levels for field release [18, 29].

Mating competitiveness, as quantified using the Fried index, is a more robust measure of male quality. Mass-irradiated males that were not transported and were allowed time to recover for 24 hours after irradiation were equally competitive with fertile males ($C = 1.15 \pm 0.23$). This result suggests that chilling and adult irradiation, as outlined in FAO/IAEA procedures does not inherently compromise mating performance if adequate recovery time is allotted. These results are consistent with those of Madakacherry et al. [36], who reported a C value of 1.17 for the same *Ae. albopictus* strain under smaller-scale rearing and irradiation conditions. Compared with other male-based release strategies, our findings demonstrate a level of competitiveness similar to that reported for RIDL-513A *Ae. aegypti* males in lab cages in Malaysia [52, 53]. However, after two days of transportation, male competitiveness decreased to 0.25 ± 0.18 , a value far below the 0.7 threshold considered acceptable for field releases when competitiveness is evaluated in lab conditions [54, 55]. This indicates that a 48-hour transport duration is unsuitable for *Ae. albopictus*, as observed previously [18]. Similarly, a mark-release-recapture (MRR) study involving *Ae. albopictus* transported from Italy to Albania, reported a C value of 0.28 [56], which corroborates our findings. Such transport-induced decrease in quality likely arises from the cumulative effects of handling, prolonged compaction, and energy depletion during shipment. These factors are known to affect survival, mating propensity, and competitiveness [51, 57, 58]. The results of the induced egg sterility followed these trends, with non-transported males achieving higher levels of induced sterility ($38.78 \pm 5.21\%$ to $80.35 \pm 8.07\%$) than their transported counterparts ($5.82 \pm 2.42\%$ to $47.83 \pm 14.58\%$). However, a 24-hour recovery period improved induced sterility, particularly among irradiated, non-transported males. This confirms the positive influence of recovery on overall quality. These results, obtained under semi-field conditions, are further confirmed by recent preliminary field results from a two-month mark-release-recapture trials conducted in Graz, Austria using the same *Ae. albopictus* strain maintained at the IPCL. In that trial, releasing sterile males resulted in approximately 70% eggs induced sterility, with a concurrent decline in the number of *Ae. albopictus* females captured in monitoring traps compared to an untreated control area nearby, according to IAEA [59]. Previous field studies have shown that low levels of induced sterility (e.g., below 50%) do not necessarily result in a significant reduction in the adult mosquito population [60, 61]. This occurs because a moderate reduction in egg hatching is offset by an increase in larval survival [61–63]. In this context, achieving only 47% induced sterility, as obtained with long-distance transported *Ae. albopictus* males underscores the fact that transporting these sterile males for two days or more cannot be recommended, as it could compromise the program's effectiveness. Even when released sterile males are equally competitive with wild males, Dobson [64] emphasized that overflooding number of sterile males must still be released to effectively suppress or eliminate the target mosquito population. No significant difference in mating competitiveness was

observed among sterile-to-fertile male ratios of 1:1, 5:1, and 10:1, indicating that increasing sterile male density does not enhance individual competitiveness. These findings contrast with those of Bond et al. [37] and Kittayapong et al. [35], who reported a surprising increase in competitiveness with rising sterile-to-fertile male ratios in both *Ae. albopictus* and *Ae. aegypti*. However, induced sterility increased significantly from the 1:1 to the 5:1 ratio, with no additional improvement at 10:1. This suggests a plateau effect, which is well described when the competitiveness of the sterile males is too low.[54]. Similar results were reported by Oliva et al. [17], who observed a twofold reduction in population fertility at a 5:1 ratio. The absence of significant differences between the 5:1 and 10:1 ratios, as also reported by Damiens et al. [39], suggests that beyond a ratio of 5:1, additional sterile males (as of 10:1) did not result in significantly greater induced sterility. In this study conducted at IPCL, a limitation is that competitiveness was assessed relative to laboratory-reared males for mating with laboratory-reared females. Thus, it is essential to compare sterile males directly with wild populations to obtain more realistic estimates of field performance.

The transfer and implementation of FAO/IAEA standardized procedures in a field-based SIT program in La Reunion confirms the robustness and operational reproducibility of the protocols. Overall, the results were consistent with those obtained at the IPCL. Survival is a key indicator of sterile male fitness, as it determines the likelihood of successful mating within the limited reproductive window. A 10-day observation period is operationally relevant, as it encompasses the peak mating activity of *Aedes* males and aligns with the maximum weekly release intervals in SIT programs. Survival was strongly influenced by adult nutritional conditions, with relatively high survival rate observed under 10% sucrose feeding. While mass-rearing and irradiation alone did not drastically reduce survival under optimal feeding conditions, the cumulative stress of irradiation, transport, and marking led to decreased survival, particularly under nutrient-deprived conditions. The operational transport duration of approximately 6 hours, showed that while irradiation and transport measurably reduced escape rate compared to wild males, laboratory mass-reared males initially exhibited escape rates comparable to wild-type males. Furthermore, the additional process of fluorescent marking did not significantly reduce the escape rate beyond the combined effects of irradiation and transport. The findings of this study suggest that under the shorter transport durations as implemented in the pilot trial in La Reunion, escape rate remained within acceptable operational limits. Moreover, semi-field mating assays revealed that the irradiated, transported, and marked males remained competitive. Irradiated and transported males achieved a competitiveness index of 0.87, while irradiated, transported, and marked males achieved a substantially higher index value of 1.15. These indices correspond to induced sterility levels of $42.91 \pm 6.72\%$ and $48.01 \pm 6.72\%$, respectively. This clear distinction underscores the critical role of transport duration: the 6-hour operational transport in La Reunion, between the mass-production insectary in Saint-Denis and the SIT field trial site in Saint-Joseph, allowed for significantly better-quality retention than the 48-hour simulated transport at IPCL. These values are higher compared to those previously reported by Damiens et al [39] for the same strain reared under small-scale conditions. In that study, the induced sterility reached only $33.6\% \pm 5.2\%$, and the competitiveness index was 0.51 ± 0.11 . These results suggest that

males produced under current operational conditions maintained a high level of mating performance despite undergoing mass rearing, irradiation, transportation, and marking procedures.

While the assessments of the final product's key quality parameters were robust, they were conducted under controlled or semi-field conditions that may not fully capture the environmental variability encountered during actual field releases. Therefore, future work should focus on evaluating the field performance of released males, including their dispersal capacity, survival rate, mating competitiveness, and resulting impact on population suppression.

Conclusion

This study rigorously demonstrates that key biological quality parameters, including survival, flight ability, mating competitiveness and induced sterility, were generally maintained at high levels throughout the production workflow. However, our findings critically highlight that certain downstream operational factors, particularly prolonged long-distance transport and short recovery period, can significantly reduce male quality, notably affecting flight ability and mating competitiveness. We confirmed that a 24-hour recovery period with adequate sugar feeding prior to release is crucial for restoring male vigor and mitigating the negative impacts of handling and transport stress. Furthermore, while individual male competitiveness may be affected by these stressors, our results stress that employing an optimal sterile-to-fertile male ratio of approximately 5:1 can effectively compensate for moderate competitiveness losses (as observed during shorter transport durations), thereby ensuring effective induced sterility in the target population. The successful implementation and robust quality performance observed in the operational SIT program in La Reunion provide strong evidence for the effectiveness and transferability of these FAO/IAEA standardized workflows under real-world, field-relevant conditions. This comprehensive assessment provides valuable insights for optimizing SIT components to enhance the success of future *Aedes* mosquito control programs.

Abbreviations

WHO

World Health Organization

SIT

Sterile Insect Technique

AW-IPM

area-wide integrated pest management

FAO

Food and Agriculture Organization of the United Nations

IAEA

International Atomic Energy Agency

IPCL

Insect Pest Control Laboratory

OPTIS

Opérationnalisation de la Technique de l'Insecte Stérile contre les Aedes vecteurs de la dengue à La Réunion

ORF

overflowing sterile-to-fertile male ratio

IRD

Institut de Recherche pour le Développement

EDTA

Ethylenediaminetetraacetic acid

IS

Induced sterility

GLMM

Generalized linear mixed models

LMM

Linear mixed-effect models

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors approved the final manuscript and the submission to this journal.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no competing interests.

Funding

The research conducted at the IPCL presented in this paper was funded by the United States of America under the grant to the IAEA entitled: Enhance Agency's Capacity to Provide Support to Member States to Control Aedes Mosquitoes as Vectors of Human Pathogens, Particularly Zika Virus, Using Integrated Vector Management Approaches with a Sterile Insect Technique Component. In La Reunion, this work was supported by the OPTIS project, co-financed through the French Ministry of Higher Education and Research, the Regional Health Agency of Reunion, the FEDER program co-funded by the European Union

and the Réunion Region. Europe is committed to La Réunion through the FEDER program. This article reflects only the authors' views, and the agency is not responsible for any use that may be made of the information it contains. The mention of specific companies or a certain manufacturers' products in this document does not imply that they are endorsed or recommended by the FAO/IAEA in preference to others of a similar nature that are not mentioned.

Authors' contributions

WM, CB and CJdB conceived the study. WM, CB, TW, HY, AH, MW, LM, SSK, TM performed the experiments. WM analyzed the data and drafted the manuscript, which was critically revised by WM, CB, TW, HY, AH, MW, LM, SSK, MG, TM, JB, FS, CJdB. CB and CJdB supervised the entire work. All authors read and approved the final version of the manuscript.

Acknowledgements

Not applicable

Authors' information (optional)

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Figures

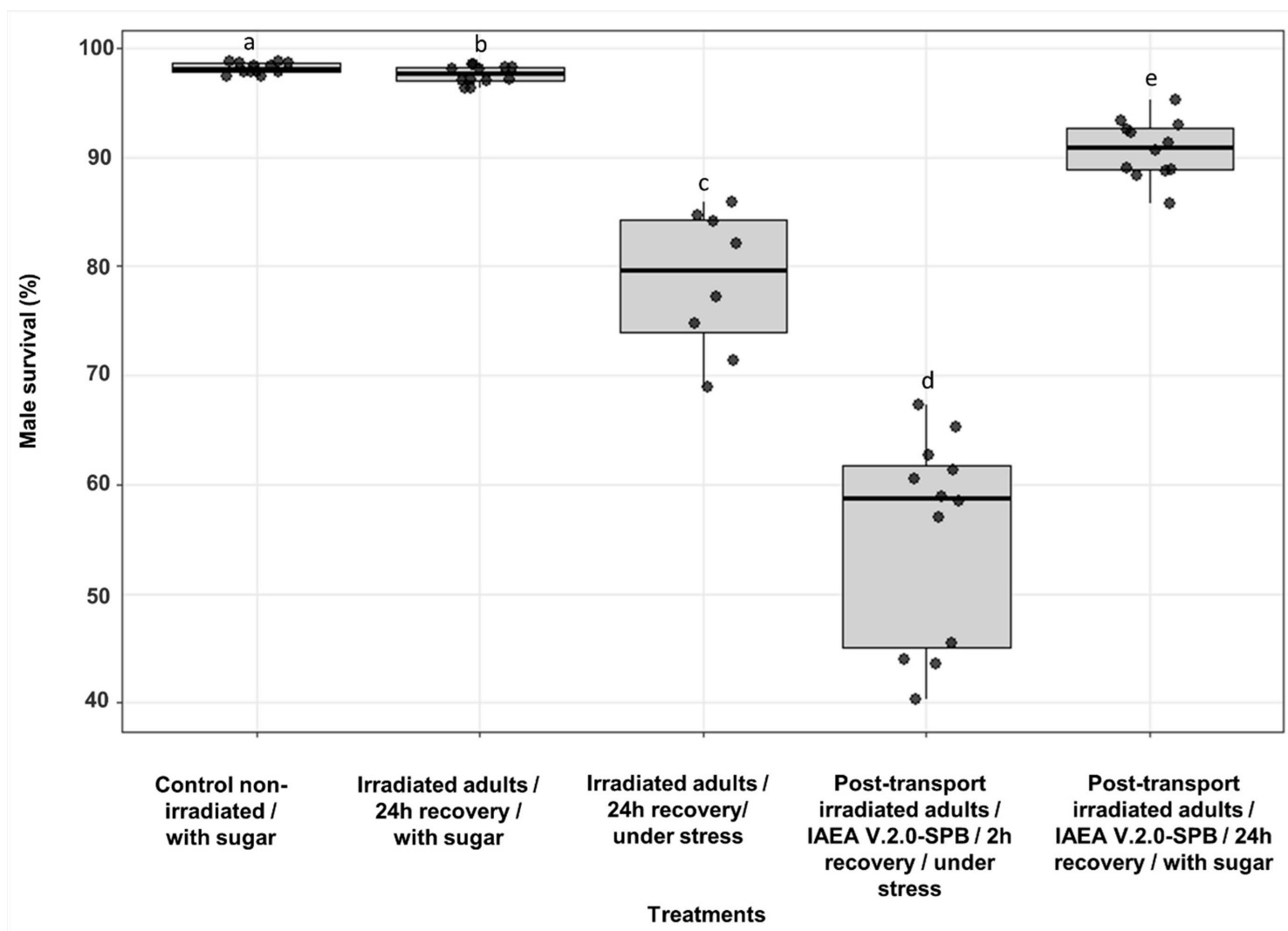


Figure 1

Post-treatment survival rates (%) in *Aedes albopictus* males, mass-reared, irradiated and transported following FAO/IAEA procedures. Results are presented as mean $\pm$ SE. Different letters between groups indicate statistically significant differences (GLMMs, $p < 0.05$).

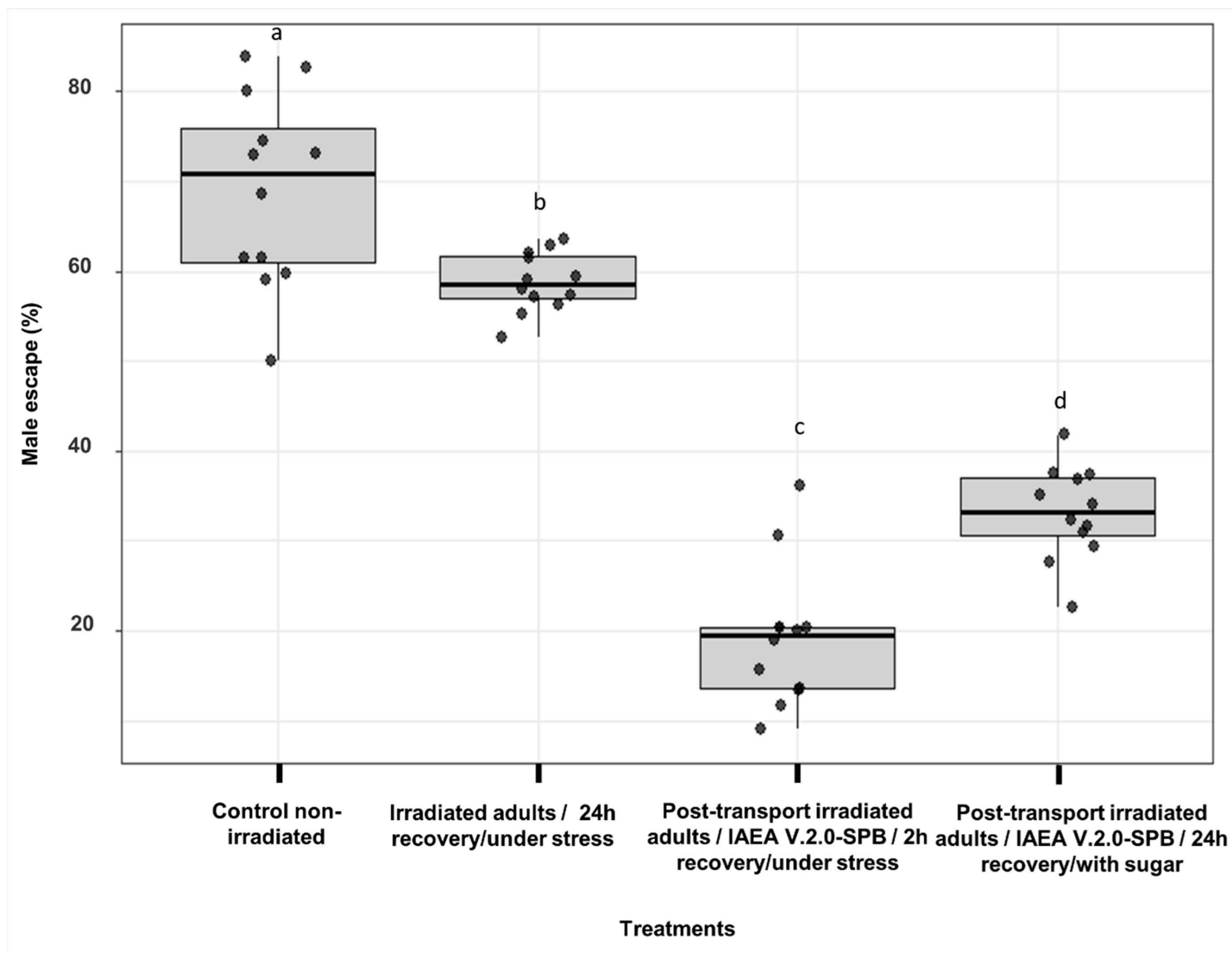


Figure 2

Escape rates (%) in *Aedes albopictus* males, mass-reared, irradiated and transported following FAO/IAEA procedures. Results are presented as mean $\pm$ SE. Different letters between groups indicate statistically significant differences (GLMMs, $p < 0.05$).

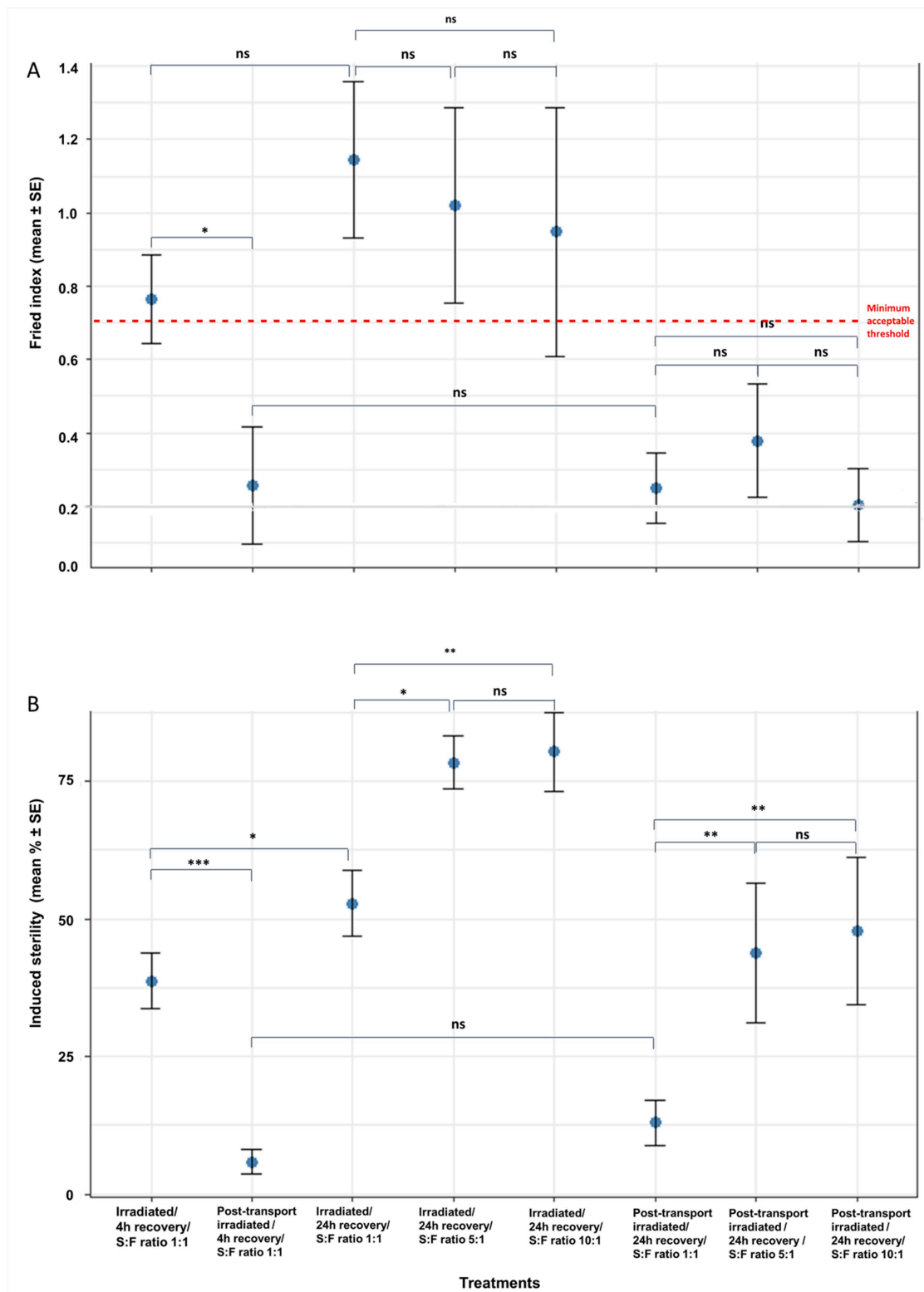


Figure 3

Fried index (A) and induced sterility (B) in radiation-sterilized *Aedes albopictus* males under various treatment conditions and sterile-to-fertile (S:F) male ratios. Data points represent treatment means, and error bars indicate the standard error of the mean (SE). The red dashed line in panel A denotes the minimum acceptable threshold ($C = 0.2$) for operational quality standards in SIT programs (WHO/IAEA

2020). Significance levels (LMMs tests) are indicated by asterisks ($p < 0.05$, $p < 0.01$, $p < 0.001$), while “ns” denotes non-significant differences.

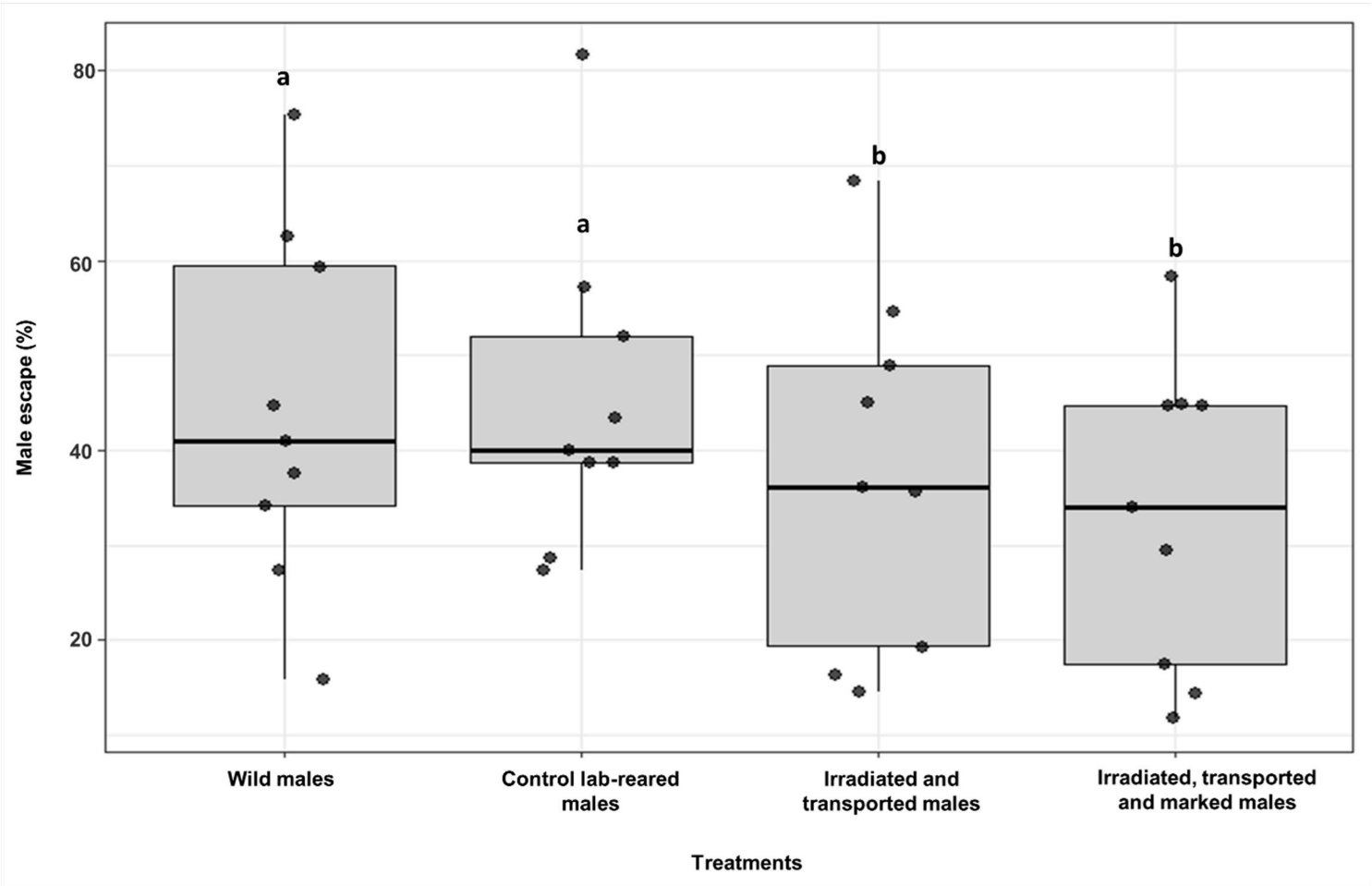


Figure 4

Escape rates (%) in *Aedes albopictus* males produced within an SIT program on La Reunion Island. Results are presented as mean $\pm$ SE. Different letters between groups indicate statistically significant differences (GLMMs, $p < 0.05$).

Supplementary Files

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