Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

GUIDELINES FOR

MARK-RELEASE-RECAPTURE

PROCEDURES OF *AEDES* MOSQUITOES

Version 1.0

Food and Agriculture Organization of the United Nations
International Atomic Energy Agency
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GUIDELINES FOR

MARK-RELEASE-RECAPTURE PROCEDURES OF *AEDES* MOSQUITOES

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FOREWORD

The success of any Area-Wide Integrated Pest Management (AW-IPM) programme including a Sterile Insect Technique (SIT) component relies on the ability of the irradiated sterile males to survive, disperse and compete sexually with their wild counterparts to induce sterility in wild females.

In a phased conditional approach for such a programme, it is considered essential to estimate these quality control parameters (survival, dispersal and competitiveness) in field conditions using mark-release-recapture (MRR) trials during the baseline data collection phase before shifting to a pilot trial. In some cases, the MRR results may even prevent from going further: as an example, a field competitiveness below 0.2 should be considered as a no-go criterium. It will then be necessary to determinate which step of the production of the sterile males must be corrected, reaching from the colonized strain to the mass-rearing, irradiation and handling procedures. Another important result from MRR will be the estimation of the real density of the target population of mosquitoes. The ultimate goals of MRR results will be to set-up the release density, swath (distance between release lines) and frequency.

The protocols presented in this guideline are the results of lessons from collaborations with Member States preparing SIT pilot trials against *Aedes* species, which are still in a preliminary stage at the time of writing. They were developed to expose the problems encountered in order to provide useful insights to develop MRR protocols that should be adapted to each situation and research questions and should not be considered as final recommendations. With the extension of the number of ongoing projects, more information on field trials will become available and this guideline will be updated regularly.

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1. Objective

The objective of these guidelines is to provide guidance to organize Mark-Release-Recapture (MRR) protocols to evaluate sterile male dispersal capacity, survival rate and their field competitiveness in an open field setting, as well as the density of the wild population. The protocols presented below have been tested in the field. and derive from these tests, in Tirana, Albania for *Aedes albopictus* and Juazeiro, Brazil for *Aedes aegypti*. However, each ecological questioning and each set of particular environmental conditions require an adapted protocol. The major originality of the presented protocols is that they allow measuring competitiveness, by estimating the ratio of sterile to wild males and the induced sterility of females in the same sites.

Estimating these entomological parameters is crucial for any SIT programme and should be conducted during the feasibility phase since they are mandatory to define projects needs in terms of sterile males production, release frequency and density, and swath between release lines or distance between release points (Vreysen et al. 2013b).

2. Marking of adult mosquitoes with fluorescent pigments

2.1 Marking of sterile males at room temperature (not chilled)

This protocol was validated for *Aedes albopictus* only.

About 2.000 male pupae in a plastic Petri dish are introduced in a cardboard box (length x width x height: 12 x 12 x 18 cm; volume 2.6 litres) for emergence. The top side of the box is covered with white net. One feeder with 10% sucrose solution is placed on the top of each box as energy source for adult males.

The dust fluorescent pigment (RADGLO® JST, Radiant NV, Houthalen, Belgium or DayGlo® Color Corp., Cleveland, USA) is applied at the dose of about 300 mg/ 1000 adults to 2-3 days old males by manual insufflators (Hygienic vaginal douche, Farlin® Industrial Co., Taiwan) through the net (*Figure 1*). In order to accelerate male movement through the created fluorescent powder mist, the box is gently shaken during the next two minutes.

Dusted males are released immediately or after transport in the field. Males not taking flight in 30 minutes are considered as dead and counted. A sample of about 300 males is randomly withdrawn from several boxes and examined under a stereomicroscope to check that they are all coloured.

The time to mark one box is one minute for one person, considering that two operators are working together, and that several boxes are shaken in the same time.

Figure 1. From the left to the right, weighting of the dust and introduction into a manual insuflator, spraying of the mosquitoes within a plastic bag and a box of marked mosquito in a transport coolbox.

The survival of male *Ae. albopictus* marked with this protocol was compared with that of a control group in laboratory conditions and no difference was observed (Table 1).

Table 1. Mean Aedes albopictus male age at death (survival) in the treatment and control groups (Source: Petric, pers. Com).

Replicate*	Survival (days) Treatment*		Survival (days) Control	
	mean	SD	mean	SD
1	37.9	±18.69	40.78	±16.98
2	41.06	±18.89	39.4	±20.54
3	39.64	±16.72	34.74	±19.58
total	39.53	±18.05	38.46	±19.10

2.2 Marking procedure for chilled sterile males

This protocol was validated for *Aedes aegypti* only.

The dust for each container must be weighed on an analytical balance and then transferred to the container and closed. Sterile male mosquitoes are dusted with the equivalent 5 mg/ 1,000 adult males in a 100mL cylindrical container (5.5cm of diameter and 7cm of height, see Figure 2) with fluorescent pigments (RADGLO® JST, Radiant NV, Houthalen, Belgium or DayGlo® Color Corp., Cleveland, USA). Batches of 2,400 sterile males can be marked altogether by increasing the size of the container and the quantity of dust (1L container, 11.5cm of diameter and 13.5cm of height, for 12mg of dust). To ensure that dust adheres to the walls of the dusting container, the inside surfaces must be rubbed with sandpaper to create a rough as opposed to smooth surface. The container must then be shaken vigorously to coat the inner surfaces evenly. Containers must be taken to a cold room (4°C) and left to acclimatize for at least 30 min.

Sterile male mosquitoes must then be transferred to the cold room for immobilization for 10 minutes. Mosquitoes are then introduced into a pre-dusted container and the lid closed. The container must be rotated for 10 seconds (equating to approximately 20 rotations) to coat the sterile males uniformly. It must be turned around and shacked after the ten first rotations.

It took 23 minutes for two persons to dust 70,600 sterile males with this method in Brazil (24th March 2018).

Figure 2. 100 mL cylindrical containers containing 100 mosquitoes and increasing amounts of dust (source: Maria Kaiser 2019).

For aerial release, it is recommended to operate dusting right before release, while chilling for collection, and to reduce the chilling time for this operation as much as possible (and keep it below 30 min in any case by adjusting the number of mosquitoes to me marked at once). The dusted mosquitoes can be transferred to release containers according to their dust colour and packed into a cool box for transportation to the field site.

The dose of dust applied with this technique is 60 times lower than in the one above because covering the mosquitoes with dust when they are chilled increases its toxicity. Actually, chilling is opening the respiratory spiracles (Parker A., personal communication), allowing the dust to enter the trachea. Any amount upon 5mg/ 1,000 adults (Figure 3) was found to decrease their survival significantly.

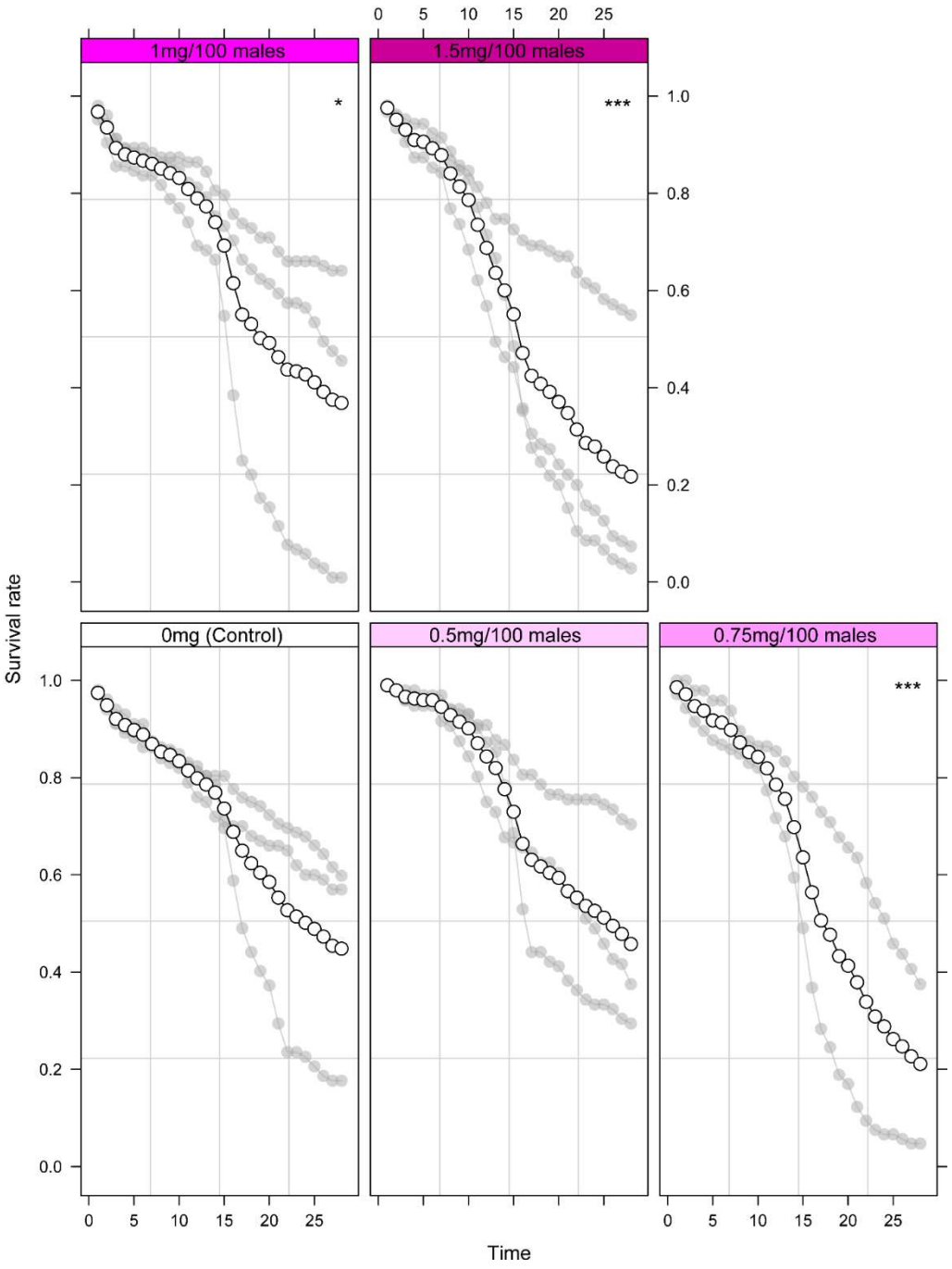


Figure 3. Survival rate of adult male Aedes aegypti depending on the amount of fluorescent dust (pink DayGlo) in mg per 100 adults (Culbert 2019).

3. Mark-Release-Recapture protocol for ground releases

3.1 Study area

The study area should be representative of the area where a pilot SIT trial is planned and the target mosquito species should be present and well established. The area should be as much isolated as possible and with only one *Aedes* species present. This should have been proven by monitoring activities. The MRR area should be at least 20ha in large cities and can be smaller in isolated village.

Field data collection on population dynamics and eggs fertility should be started with ovitraps, BG traps and/or Human Landing at least two weeks before the release of sterile males in the study area (yellow sites in Figure 4) and in a separated area (control area) at more than 2km from the release area with comparable land use and cover that will be regularly checked to estimate the natural fertility during the MRR. Fertility of wild population eggs will be assessed by standardised hatching procedures (ANNEX 1: PROTOCOL FOR EGG EMBRYONATION & HATCHING) in both sites during the MRR trials.

Figure 4. Example of an MRR study area in Tirana. The central point corresponds to the sterile males' release site. The external circle is 200m from the release point.

3.2 Mosquito release

Mosquitoes can be marked at ambient temperature or under chilled conditions according to the protocols presented upon.

Right after the marking procedures, the male mosquitoes are transported to the study area for release in an icebox to maintain controlled temperature and hygrometry (21°c and 70% rH). The dusted males are released as young adults aged 2-3 days by placing and opening the cardboard boxes in a position exposed to the sun. Cages are gently shaken for about 30 min, to induce the males to exit (Figure 5). The males that remain in the cage after 30 min are considered in the dead counting.

Different colours should be use to differentiate release sites and release times.

It is difficult to discriminate between yellow and green, between orange and red and between red and violet, and even more for combinations of colours. We thus recommend reducing as much as

possible the number of colours used simultaneously and to prefer combinations of single colours like yellow, pink and blue which can be easily discriminated.
It is suggested to release at least 10,000 males for each release site / time. Males should be released at least once a week for at least four consecutive weeks.

Figure 5. Point release of marked sterile male Aedes albopictus from the ground.

3.3 Monitoring protocol

The trapping intensity should be as much as possible homogeneous and with a density of 2 to 4 trapping stations per ha (Figure 6). The sampling stations should be arranged the day before the first males release. At least 5 ovitraps should be set in a neighbouring area serving as control (2 to 5km from the release site).
In each sampling site, a set of traps should be placed, consisting of a BG Sentinel 2™ (Biogents, Regensburg, Germany) baited with dry ice (1kg) in the case of *Ae. albopictus* and BG Lure™ (Biogents, Regensburg, Germany) and an ovitrap (500 mL black polypropylene cups; hydroculture pot; 11cm diameter x 10cm height; Luwasa Interhydro AG, Allmendingen, Switzerland) lined with heavy-weight seed germination paper (#76, Extra Heavy Weight, Anchor Paper Co., St Paul, MN, USA; 40 x 9cm) spaced 10-20m apart. Human Landing Collection (HLC) sessions should be conducted during the maximum period of activity (such as late afternoon in temperate climates and early morning in tropical environments). Ovitraps, BG-traps and HLC samples obtained in the same sampling station should be coded with the same station number.

Adult traps should be collected every day at the same time starting from the day after release and ovitraps at least once a week. The study area should be divided in sectors that will be monitored simultaneously by different teams.

The exact position of each trap should be decided in the field according to the characteristics of the local environment. Each team will be in charge of the deployment and the daily inspection of 5 to 10 stations.

During each visit, the team collects adult male and female mosquitoes flying around the team members (Human Landing Catch, HLC) with a manual battery aspirator for 15 min in each sampling station. Only one single operator per team should perform the HLC and the collected adults are recorded with an identification code referring to the sampling station and the collection date.

Teams should be rotated between sectors.

Weather parameters (air temperature and relative humidity) should be recorded throughout the course of the study by a weather data logger placed in the study area and collected from a station situated in the neighbourhood (rainfall, wind speed and direction).

Figure 6. BG-trap, ovitrap and HLC session as used in a trial conducted in Albania on Ae. albopictus

3.4 Entomological analyses

Each team should be provided with an insulated thermal container to transport dry ice and to store the adult samples caught during the daily collection. If dry ice is not available, ice packs can be used. Collected adult mosquitoes are stored overnight and screened for identification of species and coloration the following day under a stereomicroscope and using a 12 volts UV lamp or using a USB fluorescent microscope (Dinolite®) and a laptop.

The number of eggs in each paper should be counted the following day under a stereomicroscope and the portions of paper with eggs cut out in stripes, stored, maturated and hatched at least seven days after collection according to the procedures described in Annex 1.

Each day after collection, the field data samples are analysed, classified and stored into a database to calculate the distances between release and recapture sites.

The surveillance activity should be continued on this schedule for two weeks after the last release.

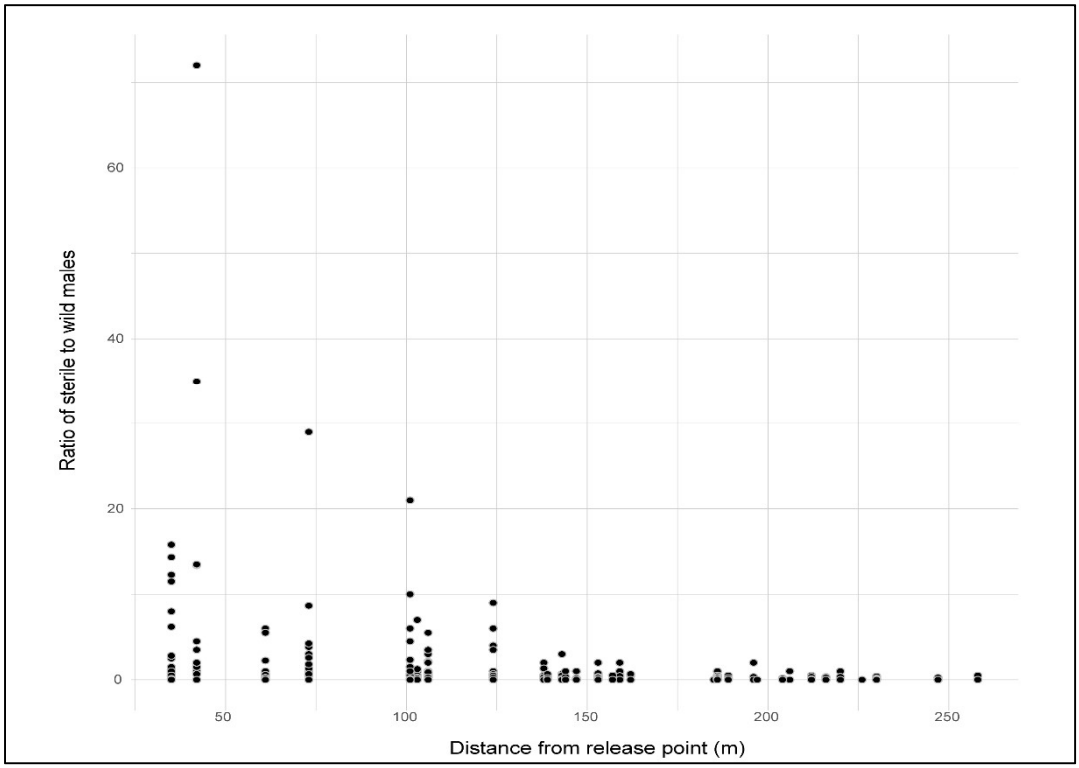
3.5 General comments

In Albania, BG-traps and HLC lead to similar estimations of the mortality and dispersal of the sterile males which show that they can both be used to make these estimations. However, whereas BG traps are set for 24H periods and thus cover a full activity period for mosquitoes, HLC is only 15 mins and should be performed only during the maximal activity periods of *Aedes*, to reduce the noise for statistical analyses. Whenever possible, it is advised to use BG-traps baited with CO_2 in the case of *Ae. albopictus* or any other trap model offering a standard attractiveness and covering a full period of activity. HLC will however be useful in areas where electrical supply is missing or where traps can be stolen or damaged.

In order to assess the competitiveness of sterile males, the induced sterility and the ratio of sterile to wild males must be estimated in the same area. The sterile to wild ratio generally drops quickly within 200m from the release point, whereas the spatial trend in the induced sterility is much smoother because fecundated females disperse more (Figure 7). This higher dispersal for females than males was demonstrated before by other authors in *Aedes aegypti* in Singapore (Liew and Curtis 2004).

This means that traps should be set far enough from the release points to be able to assess the decrease in egg fertility with distance (up to 500m). To obtain a good estimation of the competitiveness in the release area, it is thus necessary to release sterile males homogeneously (every 50-80m depending on the dispersal distance measured) over the monitored area, but also to release additional sterile males at the same density in a buffer of at least 250m around the monitored section where ovitraps may allow measuring the dispersal of sterile females outside the release area (and reversely of wild female into the release area). Finally, the release frequency should be adapted to the estimated survival: if the mean survival is lower than 7 days, two releases per week will need to be undertaken to maintain a more homogeneous ratio of sterile to wild males. Otherwise, the competitiveness will be underestimated because part of the wild females will not be exposed to sterile males between the release events.

It is then necessary to mark males released in the center of the study area and those released over the full studied area with different colours (like in the other example presented below) to assess dispersal and competitiveness simultaneously. Actually, a single release in the center of the monitored area will lead to a heterogeneous ratio of sterile to wild males which is sub-optimal to estimate competitiveness. Finally, it must be noted that a single release point is sub-optimal to estimate dispersal as well, since directional attraction or heterogeneity of the landscape can bias the measure of dispersal. Budget allowing, it is thus better to release males in at least three release points using different colours.



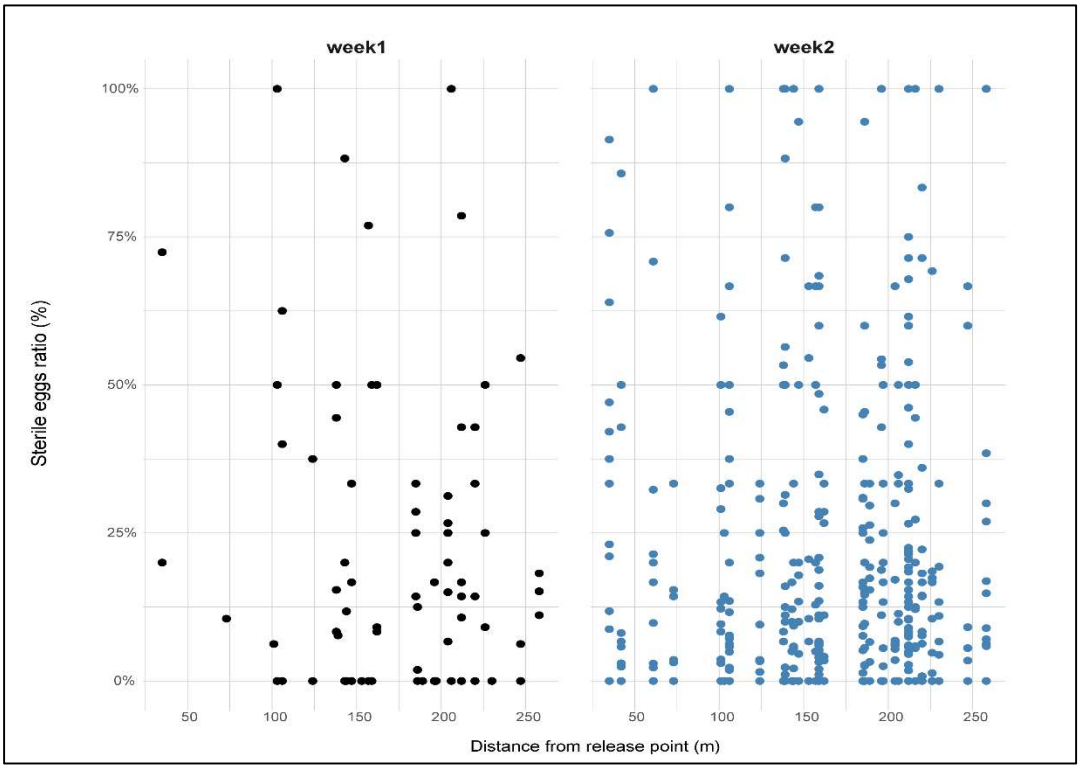


Figure 7. Ratio of sterile to wild males (top) and percentage of sterile eggs (bottom) as a function of the distance to the release point.

4. Mark-Release-Recapture protocol for aerial release

4.1 Mosquito release

Aerial releases will be conducted by drone. Point releases are used to estimate dispersal whereas line release (Figure 8 and Figure 9) are used to estimate competitiveness. Point releases should be conducted at various altitudes (50-100m) to determinate the best altitude regarding mortality and dispersal homogeneity. The swath between release lines must be parametrised as the median dispersal. Line releases can also be conducted by ground when no drone is available and, in this case, the distance between release points along the lines shall be equal to the distance between lines.

The release should be done early in the morning, especially in tropical climates, to avoid mortality and clumping of the mosquitoes.

Figure 8. Release area of 20 ha in Carnaìba do Sertão. The red point is the central release point, the red lines are the release lines with a swath of 80m and the yellow points are the monitoring stations.

Figure 9. Air releases of sterile males were achieved in collaboration with WeRobotics using a DJi Matrice 600 pro drone (Photo WeRobotics).

4.2 Monitoring protocol

Prior to the day of the first release, battery operated BG-sentinel traps baited with BG-lure in the case of *Aedes aegypti* and BG-lure + CO2 in the case of *Aedes albopictus* should be set in the study area, referring to a rectangular area of 20ha (as shown in Figure 8), with a density of 2 to 4 traps/ha. In each of the trapping stations, one ovitrap is set in the vicinity of the BG trap 10 - 20m).

In addition, 5 ovitraps are set in a neighbouring control area (that should be located 2-5km from the release area) to measure the natural fertility of a control population during the same period.

Collection of the traps are conducted daily from the day following the release. Traps should be inspected in the late afternoon (5:00pm to 7:00pm, after the peak activity), and the samples collected brought to the laboratory. All mosquito catches should be given an identification code referring to the relevant station in order to calculate dispersal capacity. In the field, collected adults are immediately placed in an insulated storage container and placed in an ice box. They can be killed in a freezer overnight. The following day, field collected samples can be analysed, classified and data stored. Samples must be screened for colour under a UV-light stereomicroscope or using USB fluorescent microscope.

Collections should be made by 4 teams of 2 people simultaneously for a 20ha area, with each team responsible for monitoring 10 traps. Traps are monitored daily for a period of 2 weeks after the last release.

Eggs collected must be dried for 7 days and then hatched (ANNEX 1: PROTOCOL FOR EGG EMBRYONATION & HATCHING). Non-hatched eggs are bleached. Release and recapture points must be geo-referenced using a Global Positioning System device. All coordinates are entered into a Geographical Information System to calculate the distances between release and recapture sites.

4.3 Lessons and recommendations

In a trial conducted in Carnaìba do Sertão, Brazil, there was no correlation between release altitude and mortality but altitude significantly increased dispersal of the release mosquitoes: we thus recommend a release altitude of 100m in future drone release trials. Of course, the release altitude needs to be adjusted to local regulations for drone operations.

A density of 2 monitoring stations per hectare is enough to gather accurate data on the mortality, dispersal and competitiveness of sterile males.

A MRR trial as the one described above will allow estimating the Fried index (Figure 10), but several recommendations can be made to improve it:

- The total duration of the releases should be at least 4 weeks;
- In the absence of a buffer area around the study area, with release of similar densities of sterile males than in the monitored area, the competitiveness is underestimated in this area due to the immigration of fertile wild females from the surrounding areas. Therefore, a buffer around the monitored section is necessary to prevent this bias. This buffer can be 250m to 1km depending on the environmental characteristics of the release area and on the dispersal capacity of wild females. Setting ovitraps in this buffer will allow measuring the emigration rate of sterile females outside the release area and conversely the immigration of wild females into it.

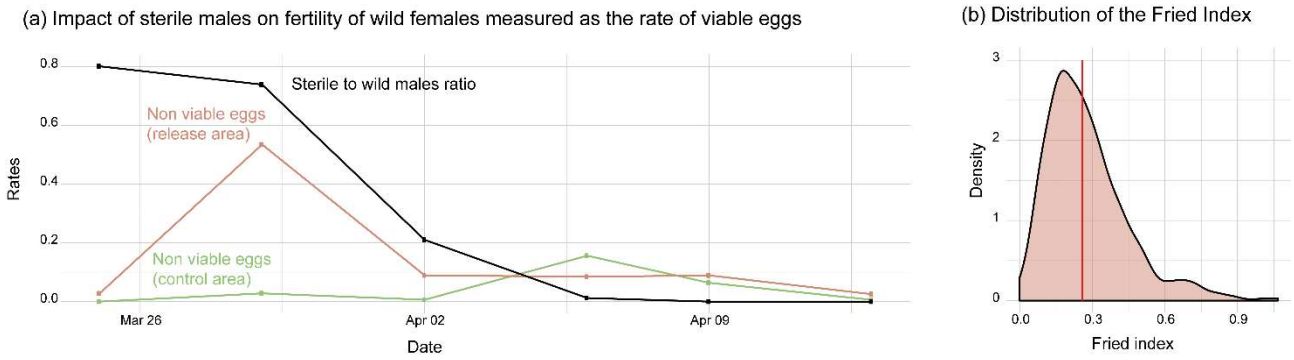


Figure 10. Induced sterility and sexual competitiveness of sterile males. (a)Temporal dynamics of the sterile to wild ratio, and rates of viable eggs in the release and reference areas. (b) Estimation of Fried index from 1,000 bootstraps in the distributions of sterile to wild males ratio in traps and viable eggs rates in ovitraps in the release and reference areas (Density indicates the number of simulations with the given x value)

5. Statistical analysis

The male dispersal can be analysed by the mean distance travelled (MDT), the maximum distance travelled (MAX), and the flight range (FR). In the first study design presented above, the estimation of the dispersal distance of *Ae. albopictus* males was facilitated by the homogeneous density of recapture stations in the study area. It should be corrected for if another design is selected.

The FR can be estimated by the linear regression of the cumulative recaptures obtained in each recapture stations (x-axis) versus the decimal log of the distance between the trap and the release point. The FR50 and FR90 is the estimated distance from the release point within which 50% and 90% of the released individuals were captured. A random isotropic model in two dimensions can also be fitted to the data to calculate the diffusion coefficient (Bouyer et al. 2007, Vreysen et al. 2013a).

The direction of dispersion can be analysed by means of circular statistics (Zar 1999) and possible correlation with the Normalized Difference Vegetation Index (NDVI) or other landcover units measured through satellite imagery can be investigated. If a bias is found and the data available are sufficient, a non-isotropic model can be fitted to them.

The sterile males survival rate can be estimated by the linear corrected method (Harrington et al. 2001, Buonaccorsi et al. 2003) as follows:

$$\theta = e^a/(N + e^a)$$
$$s = e^b/(1 - \theta)^{1/d}$$

Where:

a is the ordinate at origin,

b is the regression coefficient of the linear regression of the log-transformed captures as a function of time,

N is the number of individuals released,

$\boldsymbol{\theta}$ is the recapture rate,

d is the number of days after release, and

s is the daily survival rate.

Based on the number of sterile males released and recaptured, the wild population size can also be estimated in the study area using a simple product of the wild male catches by the recapture rate.

The Fried competitiveness index must be estimated as follows (Fried 1971):

F = ((Ha - Ee)/(Ee - H0)) / R

Where:

Ha is the natural fertility for a given season/site (determined during the feasibility study)
Ee is the observed fertility rate
H0 is the residual fertility of sterile males
R is the ratio of sterile over wild males.

Most of the time, H0 can be neglected because it is often close to zero to calculate a simplified version of the competitiveness index (Sow et al. 2012), also called "Capacity to Induce Sterility" by some authors (Bellini et al. 2013, Bassène et al. 2017).

Moreover, the protocols presented upon can allow for the investigations on several issues of interest such as:

- Possible correlations between BG traps and HLC collected males (wild and marked) by stations;
- Possible correlation between ovitraps data and females collected by BG traps and HLC;
- Possible correlation between ovitraps data and males collected by BG traps and HLC;
- Possible spatial heterogeneity of F;
- Possible impact of the time of the day on HLC;
- Estimation of trap efficiency per hectare per day for each trapping system.

Finally, it must be noted that most of the analyses presented upon are based on assumptions that should be kept in mind because they are rarely fulfilled:

- Marked and unmarked animals must have equal chances of being captured;
- Marked animals intermix with the rest of the population e.g. they are not territorial, and they are not altered by marking;
- Marking does not adversely affect survival;
- Closed population (no immigration or emigration within the period of study) and a stable population (no births or deaths within the period of study);
- Marking is not lost over the period of the experiment;
- Sufficient time must be allowed between release and recapture period for all marked individuals to be randomly dispersed throughout the population;
- Recapture rates are high enough to support an accurate estimate.

To obtain better estimations of the parameters, the information generated in such MRR trials may thus be used within simulation models that can be much more flexible and include an explicit representation of space, that can be modelled using remote sensing data (Cecilia et al. 2018, Cecilia et al. 2017). This is the only method that will allow an optimized estimation of the various parameters altogether, since estimating mortality and dispersal independently for example is quite problematic and relays on hypotheses that are not necessarily fulfilled (like the isolation of the population under study). It is therefore advised to involve biostatisticians and/or modellers from the beginning of the study design to ensure a proper analysis afterwards.

ANNEX 1: PROTOCOL FOR EGG EMBRYONATION & HATCHING

Eggs collected in ovitraps are counted directly on the substrata (germination paper) under a stereomicroscope 20-30X.

Stripes of oviposition paper containing eggs are cut out without establishing the status of the eggs at collection. The stripes collected are placed inside 50 mL Falcon tubes and left with the lid unscrewed on it (air can enter, and humidity can escape) for one day. Once no water remains in the tube but paper remains humid, the tube is sealed. Plastic tube containing the eggs are maintained at room condition (23 °C) for seven days before applying the hatching procedure.

In the case of *Aedes albopictus*, the egg fertility rate can be measured by collecting wild eggs by means of ovitraps and hatching these eggs by a standardized protocol. Hatching protocol may be as follow:

- Field collected eggs will be left to embryonate on their substratum for one week.
- A given volume of hatching solution is prepared in the afternoon (18:00) according to the number of tubes. It consists of a solution of dechlorinated water with 0.36g/L of Nutrient Broth (CM0001 Oxoid Ltd., Hampshire, England) and 0.07g/L of Brewer's yeast (YBD-1KG, Sigma-Aldrich, St. Louis, MO).
- The Falcon tubes containing the egg stripes are filled with the solution, tightly sealed and maintained overnight at lab condition or climatic room (ca. 28 °C). The leftover solution should not be used for the following days.
- The following day the tubes can be opened, and eggs will be discriminated and assigned to three possible categories: 1-intact eggs; 2-hatched eggs; 3-collapsed eggs.
- The eggs normally shaped need to be double checked by squeezing them with a mounted needle under a stereomicroscope to ascertain the presence of the embryo. Embryonated or hatched eggs are considered fertile whereas empty or collapsed eggs can be counted as sterile.

ANNEX 2: PROTOCOL FOR FIELD TRAPPING

Before starting:

1. Check whether all necessary equipment is prepared (cf. checklist in ANNEX 3: EQUIPMENT LIST)
2. Prepare labels for trap and HLC catches
3. Prepare labelled germination paper for ovitrap replacement

On field station:

1. Place BG trap in a shaded area, nearby vegetation, protected from rain where possible

   To run:

   a. Mount and place the trap
   b. Add BG Lure
   c. For *Aedes albopictus*, place 1 kg of dry ice in a closed isotherm box (but with 2 small holes, diameter 5-6mm) and place the box inside the BG trap. A CO_2 dispenser based on compressed gas can also be used.
   d. Place the battery inside the BG trap and connect OR plug in to a 220V source
   e. Check whether the fan works well
   f. Close the trap
   g. Place a label (piece of paper, 2x4cm) with station ID and start date
   h. Add the nets

   To collect:

   i. Take off the net containing mosquitoes and close it, remaining at the entrance/sucking zone of the trap (without disconnecting the trap)
   j. Place the net in the dry ice box, avoiding direct contact with dry ice with a paper or plastic sheet
   k. Restart from bullet c.

2. Place an ovitrap

   To run:

   a. Place an ovitrap in a shaded area at low elevation from the soil, preferably with nearby vegetation, 10-20m apart from BG trap
   b. Write station ID and start date on the seed germination paper sheet
   c. Place the germination paper in the ovitrap, sticking on its internal side
   d. Fill the ovitrap with water up to 2/3 level (do NOT change the water during the trial, but complete with additional water when necessary)

   To collect:

   e. Collect the germination paper and place it in a plastic bag
   f. Add end date and trap code on the plastic bag

g. Restart from bullet b.

3. Perform HLC
    a. Place a black bag (ideally a 100L black plastic bag containing an empty cardboard box)
    b. Add station ID and start date on aspirator tube (catch chamber)
    c. Connect hand mechanical aspirator to its battery
    d. Catch all male mosquitoes flying around the bag and people during 15 minutes (do NOT shake bushes/vegetation in the surrounding)
    e. Perform this at proximity (in between) BG trap and ovitrap
    f. Place the catch chamber in dry ice box (to kill the mosquitoes and avoid longer flying in the tube)

Back at office/laboratory:

1. Store the samples adequately until counting and identification.
2. Clean up and store the field equipment safely
3. Recharge the batteries
4. Count and identify samples
5. Report data on forms and then in database
6. Invite collaborators to have a drink

ANNEX 3: EQUIPMENT LIST

- BG traps (x)
- Batteries for BG traps (2x)
- BG battery charger (check presence of adequate number of sockets or presence of multiple plug sockets
- 100mL urine cups (sugar feeding)
- Coffee filters (sugar feeding)
- Emergence bowls
- Larval diet
- Hand held sieves
- Counters
- Balance
- Irradiation canisters
- Irradiator
- Fluorescent dust (yellow, pink, green, orange, white, blue – DayGlo)
- USB Fluorescent microscope
- Tape (labelling) + Sharpies + forceps
- Petri dishes and forceps (QC)
- Ziplock bags
- Insulated containers (specimen collection)
- Dry ice (BG traps and for collecting samples) in the case of *Aedes albopictus*
- BG lure
- Hand held fluorescent light
- Stereomicroscopes
- Mouth aspirators (bring our own and a few spare)
- Water bottles (larval feeding)
- Weather vane and anemometer (weather conditions)
- Killing boxes plus ethyl acetate (if authorized and no cooling system available to keep the collected specimen)
- Ovitraps and paper substrate
- Place where to maturate the eggs. We used small container (see protocol hatch maturation)

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