Joint FAO/IAEA Programme
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SPREADSHEET FOR DESIGNING AEDES MOSQUITO MASS-REARING AND RELEASE FACILITIES

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SPREADSHEET FOR DESIGNING AEDES MOSQUITO MASS-REARING AND RELEASE FACILITIES

Edited by:

Rafael Argilés Herrero, Carlos Cáceres and Jeremy Bouyer
Insect Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

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FOREWORD

Sterile insects have been defined as beneficial organisms by the International Plant Protection Convention. The sterile insect technique (SIT) has been applied in more than 30 countries worldwide for pest suppression, eradication, containment and prevention. Current production of sterile fruit flies supporting pest control programmes is over 3 billion insects per week. Conversely to fruit flies and other plant pests, less than ten million sterile mosquitoes are currently produced per week in a small number of insectaries at the global scale with a low production capacity.

The number and production capacity of mass-rearing insectaries for mosquitos is expected to increase in the coming years and this interactive FAO/IAEA Spreadsheet for Designing *Aede*s Mosquito Mass-Rearing and Release Facilities has been developed to assists in technical and economic decision making associated with facility design, cost, construction, equipment, and facility operation. The spreadsheet is user friendly and thus largely self-explanatory. Nevertheless, it includes this basic instruction manual that has been prepared to guide the user, and thus should be used together with the software. Ideally the model should be used as a support tool by consortia assessing facility design, investment and possible economic returns of different facility size and production scenarios. A consortium should include an entomologist with experience in mass-rearing management and facility design, an architect, a civil engineer and an A/C expert.

If users need to consult more details of this or other processes do not hesitate in contacting the editors of this manual.

The Officers responsible for the publication were Rafael Argilés Herrero, Carlos Cáceres and Jeremy Bouyer of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture.

CONTENTS

0. Introduction: How to use this spreadsheet

The FAO/IAEA Interactive Spreadsheet is intended to be used for the design of insect mass-rearing facilities for *Aedes* mosquito. It can assist in technical and economic decision-making processes associated with the design construction, costing and operation procedures of mass-rearing facilities. This spreadsheet is not intended to be used for small scale rearing or laboratory colonies.

The model is user-friendly and thus largely self-explanatory. Nevertheless, the current basic instruction manual has been prepared to guide the user and should be used together with the software. Ideally the model should be used as a support tool by multidisciplinary working groups aiming at assessing facility design, investment and possible economic returns from facilities of different sizes and production capacities. The working group should include an entomologist with experience in mass-rearing management and mass-rearing facility design, an architect, a civil engineer and an air-conditioning expert.

Start using the spreadsheet by defining the weekly pupal production and number of weeks with releases per year in the sheet “Start-up & Summary”.

As in similar spreadsheets for other insects’ mass-rearing facilities, the cells with yellow background contain basic parameters whose values can be changed by the user to adapt it to his local conditions. Most of these parameters are in sheet 3 “Pro Par” but there are many other parameters whose value needs to be changed in other sheets (equipment, diet formulation, construction costs, labour costs…). The original values in these cells can be used by default if specific information for the user’s project is unknown.

The cells with white background are calculated automatically by formulas derived from the basic values. They should not be modified unless an error in the formula is detected. To avoid unintended changes, these cells are protected and can’t be edited.

<u>Software</u>: The spreadsheet was created using Microsoft® Office Excel. It uses the workbook/page - calculation Microsoft® Office Excel structure - to present a series of inputs/outputs related to the different aspects of the insect mass-rearing process. The spreadsheet model is set out in a series of ‘sheets’ that contain individual components of the model. These are kept in separate pages to make its use easier and more understandable. The user will be able to input information at different levels of complexity depending on his/her own needs. Outputs are calculated based on simple formulas constructed by the combination of the critical parameters and inputs involved in the rearing process.

<u>Opening the file</u>: When first opening the original file, it is very important that it is saved under a new name so that the empty original document is kept in an unaltered form so that it can be used for future projects. It is also important to make back-ups of the original file and also files that are being worked on so that in the event of accidents there is always an up to date copy of the work available.

1. Start-up parameters

The target production level per week of the mass-rearing facility must be defined here. The units of the production level are millions of sterile male pupae per week.

The number of weeks of operation in the year shall also be defined here. Some programmes in temperate climates may not need to release sterile *Aedes* males during the overwintering season if field data indicate that there is no mating activity of the wild population. This will not affect the sizing of the rooms or equipment but will determine the period for operations and the yearly requirements of hand labour and supplies of larval ingredients.

2. Mass-rearing process

This is an informative tab that summarizes the mass-rearing process workflow and quantifies the number of operations to be conducted every day, which have been calculated using the basic data provided by the user in the Production Parameters tab.

No data shall be entered in this tab.

It is assumed that the workload and production level remain constant throughout the week, as is currently the case in operational programmes. This implies that all the mass-rearing tasks are conducted evenly throughout the week. For small production levels such as laboratory colonies, this may not apply.

The production system has one bisexual colony that produces the eggs for the male-only for field release and for its own feedback. After hatching, the L1 are seeded in common larval trays for both the bisexual and the male-only release. Pupae obtained after the first tray tilting -most of which will be male due to the protandry- are sex-sorted to form the male-only release. Remaining larvae from the sorting operation can be used to load the adult cages of the bisexual colony -the sex ratio is close to the optimal 3:1 female:male – or can be put back in larval trays to increase the male production. The user of the spreadsheet shall define the number of trays tilting and pupae sorting operations in the corresponding cell in the tab Pro Par according to their standard rearing procedures.

The tilting of larval trays for the recovery of pupae is usually done only once per tray, since most of the males (approximately 75-80%) pupate in the first day and it is uneconomical to reuse the remaining larvae with a low percentage of males to fill new trays. Larval trays are not earmarked for the bi-sexual colony or for male-only release.

This production system differs in essence from the colony amplification system in that no filtering is required as in the case of fruit fly Genetic Sexing Strains (GSS) with a recombination rate that requires a filtering system to avoid the establishment of recombinants in the colony.

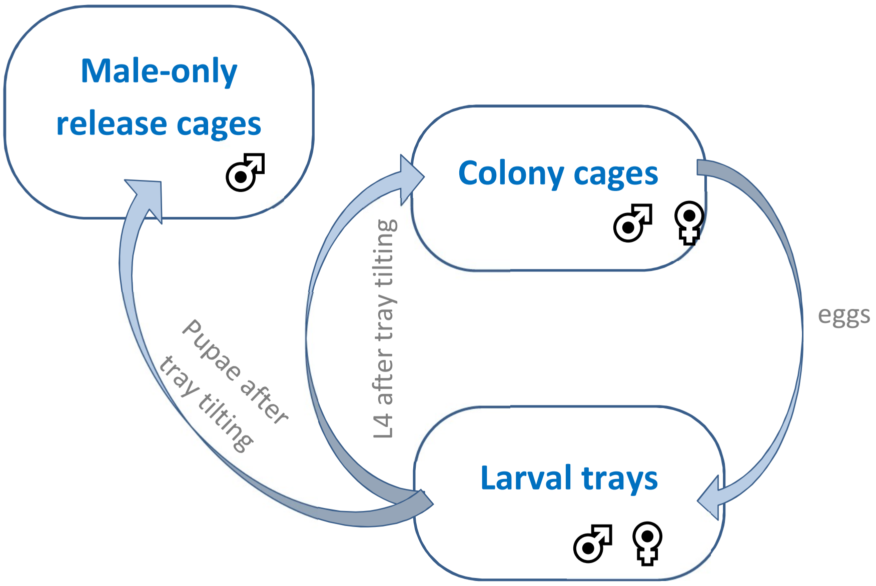

Figure 1. Aedes *mosquito production system.*

3. Production parameters

Most of the values of the basic production parameters that are needed for the calculations must be entered by the user in this tab. These parameters are grouped in biological parameters (duration of life cycle, egg production, larvae and pupae yields, efficiency in terms of egg-larvae-pupae transformation), equipment capacity and tasks schedule.

Rearing efficiency:

Among the rearing efficiency, egg hatching rates, larval survival and adult emergence rates need to be defined. Low values in the above parameters will lead to over-sizing the larval rearing.

By default, the survival of L1 to pupa does not need to be defined by the user since it is automatically derived from the value of the tray's depth based on a graph built with the information from Briegel 2003 (Physiological bases of mosquito ecology). The larval survival depends mainly on the tray depth, so the calculation considers the depth of the tray selected in the 'Equipment Parameters'. However, the user can change manually the value of this parameter if needed.

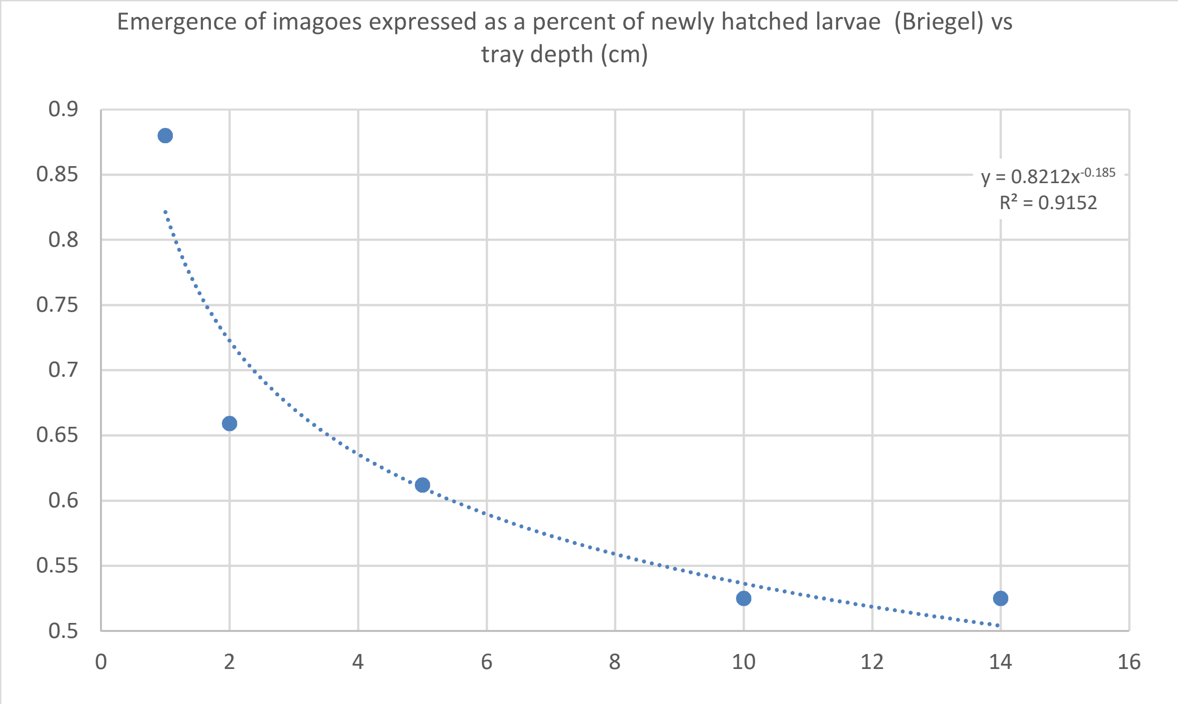

Figure 2. Emergence of imagoes expressed as a percent of newly hatched larvae (Briegel) vs tray depth (cm).

The dynamics of the pupation is a very important information that must be investigated to assess the efficiency of the rearing. The level of protandry in the larval development must be confirmed. If a large percentage of the males pupates in the first day, it is recommended to sort the pupae only once per tray and use of the pupae collected for the male-only production, while the remaining larvae will be used for feeding back the colonies. Conversely, if the pupation of the males extends in several days, the tilting of the larval trays for pupae sorting will need to be conducted more than once per tray, which will increase the rearing costs.

The egg production rate per female in the different gonotrophic cycles will define the relative size of the bisexual adult colony. Large variation in the egg-laying rates has been observed in different insectaries, probably due to the level of colonization of the strains.

Life cycle information:

The duration of the different development stages will be affected by the environmental conditions and other rearing conditions.

The user must define the duration of the larval development and the duration of the gonotrophic cycles. Similarly, the number of gonotrophic cycles that the colony cages will be kept before been discarded, shall be defined by the user. To make an informed decision, the user shall consider the oviposition rate in the different gonotrophic cycles and the workload to maintain the cages. In many mosquito insectaries, 2 gonotrophic cycles seem a sound balance.

Equipment capacity:

The capacity of the different type of equipment (larval trays, colony cages, etc.) must be defined by the user of the spreadsheet. As a general rule, larger larval trays and cages will reduce the daily workload. However, large equipment that cannot be handled by a single staff should be avoided (regulations on prevention of occupational risk due to heavy loads handling must be observed).

The capacity of the adult cage is defined by the dimension of the cage and by the number of adult mosquitoes per square centimetre of vertical resting surface in a cage (so-called Density-Resting Surface, or DRS). A DRS value of 0.8-1 is generally reported to promote suitable adult mosquito-rearing conditions (Gerberg 1970, Balestrino 2014). The spreadsheet can accept different cage dimensions and DRS values and will update the cage capacity accordingly.

Different cages for the male-only mosquitos for field releases and egg production colony with different design, dimensions and DRS must be defined.

The default value for the sex ratio in the colony cages is defined by the pupation dynamics information already entered by the user. However, the user can change this value manually if needed.

The capacity of the larval trays is defined by the volume of water and by the density of L1 that are seeded per mL of water. A value of 3 L1/mL is considered appropriate for *Ae. albopictus* and *Ae. aegypti* (Guidelines for routine Colony Maintenance of *Aedes* Mosquito Species, FAO/IAEA, 2017). Other species like *Ae. polynesiensis* require a much lower larval density. The larval density is also related to the availability of food and accumulation of faeces. The spreadsheet can accept different tray dimensions and water depth values, and will update the L1 capacity of the trays and larval survival accordingly.

Rearing tasks schedule

The parameters under 'Rearing Tasks Schedule' tab, such as the frequency of blood feeding will depend on the operation procedures of the insectary. They will be used to calculate the workload of the different daily tasks.

4. Release facility

This tab serves to assess the dimension of the male-only release colony including number of cages required to be handled per day and sterilization operations. No input values are needed in this tab.

5. Mass-rearing facility

This tab serves to assess the dimension of the bisexual colony (both adult cages and larval trays) and the daily number of larval trays for the production of male pupae for field releases.

There is only one parameter whose value must be defined: the increase factor of amplification colonies needed to maintain the mother colony. This parameter refers to the possibility of having a filter colony to preserve a desired genetic background. This mother colony should be maintained in relaxed rearing conditions. In some cases of fruit flies, like for the GSS Vienna 8 for *Ceratitis capitata*, a filter system with different amplification steps is needed to preserve the purity of the strain and avoid recombinants without the temperature sensitive lethal mutations taking over the colony. The number and size of these amplification colonies will depend on the stability of the strain (recombination rate) and competitive advantages of the mutants. If no filter colony will be used, this value must be set to 1.

6. Diet formulation

The larval diet ingredients, composition and feeding regime must be defined in this tab. A standard diet based on beef liver powder, tuna meal and brewer yeast appears by default, following the recommendations in Guidelines for routine Colony Maintenance of *Aedes* Mosquito Species, FAO/IAEA, 2017. Other ingredients can be used by amending the percent of the ingredients listed in the recipe.

To standardise the larval diet formulation, the ratio must be expressed in mg of ingredients (not included water) per L1. The dilution in water of the ingredients must also be defined.

The volume of diluted diet that must be added to the larval trays each day is calculated based on the composition of the diet, concentration, feeding regime, dimensions of the larval tray and density of L1.

For the adults, the average blood intake per female per feed must be defined here and will affect the requirements in terms of fresh blood supply per week.

7. Diet requirements

This tab calculates the inputs of diet for larvae and adults to be used daily and weekly. This information shall be used for dimensioning the minimum stocks available in the warehouse.

8. Storage of diet ingredients

The storage capacity for the diet ingredients is calculated in this tab.

9. Rearing equipment

The needs in equipment (larval trays, racks, colony cages, male only cages, irradiator, sex sorter, diet mixers, blood feeders, L1 counters, larval diet feeders, tray washing machine, etc.) is calculated in this tab. Consumable material and small equipment common in laboratories such as balance that may be used for quality control are not listed here.

An oversize factor is applied to most of the equipment to account for unforeseen situations in the production process (e.g. the number of male-only cages at the release centre must be increased by 50% to foresee delays in releases caused by bad weather).

For the key equipment, such as the sex sorter or the irradiator, there is an option to consider a backup unit.

For some operations, such as irradiation, the user must define the throughput and capacity per operation of the equipment. Also, the maximum time available per day for the operation must be defined.

10. Environmental conditions

This tab summarizes the information of the optimal ranges of temperature and relative humidity in the different rearing rooms. The values in this tab are not used for any calculation, although they certainly have an impact in some of the parameters such as development time of the mosquito, size of the rearing rooms or number of pieces of equipment.

11. Water requirements

The water requirements for rearing (larval trays + larval diet + adults' diet), equipment washing and rearing room cleaning is estimated here. There is little information on the requirements for washing and room cleaning, which is estimated as a percentage of the rest of the items.

12. Floor area calculations

The area for the rearing rooms in the mass-rearing and release facility are estimated based on the number of equipment units and their footprints. The footprints for larval racks and adult cages are calculated based on their dimensions as entered in the 'Production Parameter' tab. An oversize factor is applied to account for the internal corridors and dead spaces to allow access to cages and racks within the rearing rooms.

Working areas (for example for tray loading, cage loading, etc.) have been estimated as a percentage of the room areas.

Common areas for labs, washing areas, warehouse, offices, corridors, toilets, etc., can vary according to the floorplan distribution, automation level, local regulations, etc., and should be revised by the user of the spreadsheet. Some of the common areas, like offices, toilettes or storage, are duplicated in both mass-rearing and release facility. The space needed for the common areas can be reduced if both facilities are merged. Merging facilities will foster economies of scale and make operations more cost-effective.

Water treatment, back-up power generator and electricity rooms have not been considered.

13. Construction costs

The present tab aims at giving a very broad estimation of the construction costs of the whole infrastructure, including mass-rearing and release facilities. The cost per unit of construction will largely vary across countries and must be entered by the user. Three different unit costs (building costs for office and labs, building costs for rearing rooms and building costs for storage and warehouse) can be defined by the user. The unit costs of the rearing rooms must include the cost of climatization. The estimated construction costs do not include the purchase of the land for building.

The expected lifespan of the infrastructure and equipment is used to estimate a depreciation value. No residual value after lifespan has been considered for the used facilities.

The cost of supporting infrastructure, such as water treatment or power station if needed, have not been considered in this construction cost estimation.

Administration costs are associated to any insect mass rearing facility. As a rule of thumb, this is estimated as a percentage of the total operational costs. Between 6 to 10% of the operational costs is considered appropriate.

14. Workload

This tab quantifies the workload for each of the daily tasks in the mass-rearing and male handling facilities to achieve the target production level. As described in previous sections, large mass-rearing facilities are operated seven days per week and all rearing tasks are conducted on a daily basis in a balanced manner.

The workload of the following activities has been quantified:

- Hatching eggs: volume of eggs (mL) that must be hatched every day.
- Hanging trays in the racks: number of larval trays to be added to the rearing cycle every day.
- L1 dosage in larval trays: number of larval trays to be seeded with L1 every day.
- Larval feeding: number of trays and racks to be fed every day based on the feeding calendar.
- Tray tilting: number of trays and racks to be tilted daily for pupae collection
- Pupae sex sorting: the total number of pupae of both sexes to be sorted every day, which will depend on the protandry and performance of the sex sorting device.
- Tray washing: number of larval trays to be washed per day.
- Irradiation: number of irradiation operations per day.
- Packing pupae for irradiation: same as above
- Colony cages loading: number of colony cages to be loaded with pupae every day
- Colony cages blood feeding: number of colony cages to be blood fed every day.
- Egg collection: number of colony cages to be collected every day.
- Egg storage: volume (mL) and number of eggs to be stored every day.
- Colony cage washing: number of colony cages to be washed every day.
- Larval diet preparation: volume of larval diet (L) to be prepared every day.
- Adult diet preparation: volume of adult diet (L) to be prepared every day.

- Blood collection: volume of blood (L) to be collected per week. The frequency of the blood collection will depend on the availability of blood at the nearest abattoir. In most cases, blood collection will be done once per week.
- Blood doses preparation: number of doses of blood to be prepared per day to feed the colony cages. This will depend on the number of times that a single blood dose can be used before being discarded.
- Male-only cage loading: number of male only cages to be loaded every day with male pupae after sex sorting.
- Adult diet preparation: volume (L) of adults' diet to be prepared every day to feed the cages with male-only mosquitoes.
- Male-only cage chilling and adults' collection: number of male-only cages that will be sent to the chilling room every day for adults' collection.
- Adult packing for releases: number of males to be packed for releases every day.
- Male only cage washing: number of male only cages to be washed every day.

The working time for each of the rearing activities will largely depend on the automation of the different processes. Therefore, the work rates must be filled by the user of the spreadsheet according to the degree of automation of the facility. In the case of an unattended process, the time needed per unit of workload is not the time required by the machine to complete the task but the time that the staff must invest to prepare/configure/set the machine to perform the task.

15. Equipment budget

The list of equipment is summarised in this tab. The number of each type of equipment will depend of its size, capacity, yield and oversizing factor (defined by the user in the tables '03 Pro Par' and '09 Rearing Equipment').

The unit costs for each piece of equipment can vary depending of the supplier, especially if the equipment can be manufactured locally, and must be defined by the user. A unit cost indication is given by default.

16. Diet and consumables costs

The weekly and yearly cost of the different diet ingredients and consumables can be consulted in this table. As for the case of the equipment, the unit costs for the different ingredients may vary locally.

17. Final considerations

This spreadsheet is a powerful and flexible tool for project and facility managers. It provides managers with practical information regarding the list of items that should be considered when planning the establishment of rearing facilities of any insect species. Once facilities are established, they can also be used to estimate facility and production costs, as well as production projections under different rearing efficiency scenarios. During the design process it is very important that a local specialist provides the required inputs for the air conditioning (A/C), heat and electrical load assessments as the cost for these important components vary according to facility size and location. Similarly, the estimation of auxiliary areas and waste water treatment plant are also not part of this spreadsheet.

As for many industrial activities, the efficiency of the insect mass-rearing activities is largely dependent on the scale. The current spreadsheet is not intended to be used for the design of laboratory colonies but for mass-rearing facilities.

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