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Modeling population dynamics based on experimental trials with genetically modified mosquitoes

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Abstract

Recently, the RIDL-SIT technology has been field-tested for control of Aedes aegypti. The technique consists of releasing genetically modified mosquitoes carrying a “lethal gene”. In 2016 the World Health Organisation (WHO) and the Pan-American Health Organization (PAHO) recommend to their constituent countries to test the new technologies proposed to control Aedes aegypti populations. However, issues concerning effectiveness and ecological impact have not been thoroughly studied so far. In order to study these issues, we develop an ecological model compatible with the information available. It presents an interdependent dynamics of mosquito populations and food in an homogeneous setting. Mosquito populations are described in an stochastic compartmental setup in terms of reaction norms depending on the available food in the environment. The development of the model allow us to indicate some critical biological knowledge that is missing and could (should) be produced. Considering the model results we show how the releases proposed could contribute to the increase of epidemic risk after they have concluded. Hybridisation levels and release numbers of mosquitoes as a function of intervention duration and target are calculated.
1. Introduction

The use of the Sterile Insect Technique (SIT) has been proposed to control populations of the mosquito *Aedes aegypti* (*Ae* ae.) because of public health reasons (Alphey et al., 2010). *Ae ae.* is the main vector of several viruses such as the *Flavivirus* producing Yellow Fever, Dengue, and Zika, as well as the *Alphavirus* producing Chikungunya. The idea is not new and follows the success obtained with the fruit fly (*Drosophila sp.*) in agricultural settings.

The early attempts to use the method with *Ae ae.* sterilised by radiation ended in failure (Morlan et al., 1962). The evolution of SIT by the introduction of genetically modified insects, GMI, incorporating an autocidal gene (Gong et al., 2005) (termed RIDL-SIT, Release of Insects carrying a Dominant Lethal) was later considered a more promising technique for mosquito control than irradiated mosquitoes (Benedict and Robinson, 2003). The RIDL-SIT technique for *Ae ae.* was developed recently and communicated in a series of papers (Phuc et al., 2007; Harris et al., 2011, 2012; Lacroix et al., 2012; Carvalho et al., 2015; Winskill et al., 2015) (all references except the first one refer to actual field-tests).

The World Health Organization (WHO) recently promoted the realisation of essays of this technique (World Health Organization, 2016) in the wake of the 2015 Zika pandemic in the Americas. More recently, the Food and Drug Administration (FDA-USA) authorised the realisation of such essays in the US (Center for Veterinary Medicine, 2016). In correspondence with technological and political developments, the Pan-American Health Organization (PAHO) endorsed WHO’s recommendations for its member states. It is apparent that there exists a growing will of performing tests of the RIDL-SIT technique *in the field*, despite the limited amount of available information and the uncertainty of impact assessments. Nevertheless, warnings were raised by scientists (The Entomological Society of America (ESA) and Sociedade Entomológica do Brasil (SEB), 2016) that foresee the ecological and social dimension of the problem, in correspondence with the considerations of social scientists regarding technological fixes (Reis-Castro and Hendrickx, 2013).

In April 20-21, 2017 a scientific meeting was held at the Ministry of Public Health of Argentina with the support of PAHO addressing the reasonability of conducting such experiments in the country. The participants were the National health authorities, a PAHO representative, a group of scientists proposing integrated control technologies (including SIT), a group of scientists not involved in technological development and two representatives of the foremost RIDL-SIT company. Despite the presence of a scientist representing the team developing the RIDL-SIT technique, some questions concerning impact assessment remained unanswered, being nevertheless relevant for the scientific community and the society as a whole, for example:

• Which lasting modifications of the environment will be produced by these essays?
• How fast will the sanitary situation previous to the environmental intervention be reestablished?

The goal of the present work is to address these and related issues using the mathematical methods of stochastic population dynamics, which, being environmentally friendly, should in general be used before any intervention. We investigate the time-evolution of a mosquito population as a result of the proposed interventions, focusing in:

• The hybridisation of the released mosquitoes with the local populations as a result of genetic diffusion

• The expected ratio between released males and local males necessary to achieve different levels of control

• The recovery time of the new, hybrid, populations when the intervention ceases

• The reasons for the difficulties found in establishing such control systems

To achieve these goals we implement a stochastic model, able to deal with small population numbers, based upon the available information. Because of the scarcity of biological information concerning the released mosquitoes, we have kept the model as general as possible, avoiding to replace missing information with conjectures. We indicate the most noticeable missing information in our critical review of the data (see Section 2.3.1). It is also worth mentioning that construction of conclusions concerning some of the data available in the reports is occasionally inconsistent between different reports and/or existing biological knowledge.

This manuscript is structured as follows: In Section 2 we describe the biological background of the technique, including a critical review of the available data, as well as the mathematical methods supporting the simulation. Section 3 displays the outcome of the simulations and their relation to relevant biological questions, while Section 4 contains a general discussion and concluding remarks.

2. Biological background and mathematical methods

2.1. The RIDL-SIT technique

The Sterile Insect Technique (SIT) was introduced in the past century for insect pest control. The goal is to release sterile males in sufficient number to reduce (control) the population by reducing the number of offsprings. Sterility was originally achieved by radiation and it has been successful as part of the measures to control the Mediterranean fruit fly (Enkerlin et al. 2015) (see below), the screwworm fly (Hendrichs et al. 2005) and other insects considered pests (for a history see Klassen and Curtis 2005). Hence, the idea of controlling populations of mosquitoes vectors of viral diseases by sterilisation emerged clearly. By 1962 Aedes aegypti was considered a mosquito that could
be controlled with the technique. Field experiments were performed by Morlan et al. (1962) but despite overwhelming releases of sterilized males in relation to the local mosquito population, no control was made evident. In consideration of this failure the technique had to be revised. Benedict and Robinson (2003) discussed some conditions that a sterile insect technique should satisfy to be successful; we quote:

Apart from the obvious requisite of mating competitiveness, two criteria must be satisfied by any sterilization method: (1) sterility must not be suppressed by complementation with any genotype present in field populations. Any variation in the expression of the sterility factor(s) following interaction with the large and heterogeneous genome of the field population will quickly lead to selection for non-sensitive females and fertile matings. (2) Absolute assurance of effectiveness must result from the sterilization method in the factory setting.

These authors also recommended that

a first step for the future deployment of transgenic technology is to demonstrate the safety of release material in the absence of confounding concerns about the spread of alleles and drive elements. This is possible only if the released material is genetically sterile.

Several authors (Weidhaas et al., 1974; Rogers and Randolph, 1984; Dobson et al., 2002; Alphey et al., 2010) have indicated that density-dependent-effects can/will reduce the efficiency of SIT programs to the point of making it non-viable.

To overcome in part these population (ecological) effects the RIDL-SIT technique was introduced. Transgenic mosquitoes were designed to have a late acting dominant lethal gene (Phuc et al., 2007) following a technique developed for the fruit fly (Gong et al., 2005). The original strains of modified mosquitoes were constructed using the Rockefeller strain of Ae ae, a strain that has been kept in captivity for a large number of years and presents adaptation of its life-cycle to the laboratory (Tejerina et al., 2009; Grech et al., 2010), as well as manifestly larger sizes and fertility than wild strains.

The proposed strategy is to release solely male mosquitoes outnumbering the wild males and thus strongly influencing the fecundation of wild females. The offspring is expected to express the lethal gene and die before reaching fertile age. Thus, the introduced mosquitoes would, after one generation, disappear from the natural environment along with their offspring. The expected outcome in a closed environment (no adult immigration or emigration) is to control the original wild mosquito population.

The genetic engineering of the mosquitoes proceeds by inoculating eggs with molecular constructs to “induce tetracycline-repressible dominant lethally in both males and females”, producing four transgenic lines of modified mosquitoes, which appear to be obtained by modifications at a single site in the genome.
Three of these lines gave the desired mortality. One of these three presented increased mortality near pupation and was selected for mass production because of this desired attribute. According to the report (Phuc et al., 2007), a penetrance of 93 − 97% was achieved, meaning that at the level of phenotype a 3 − 7% of the individuals carrying the gene do not manifest the early mortality. The inheritance of this trait was not studied. For the case of the Mediterranean fruit fly Gong et al. (2005) followed one generation (n = 4590) concluding that survival to adulthood despite the lethal gene is not an inheritable trait. Such conclusion is inaccurate, a proper statement is that the probability of survival being inheritable lies in the interval [0, 5.9 \times 10^{-4}] with 95% confidence. The difference matters when this probability is multiplied by millions of released individuals. Nevertheless, the authors acknowledge that “the possibility of biochemical resistance to the lethal effector molecule remains a potential drawback to RIDL that is not a significant issue for radiation-based sterilization”.

As part of the engineering process, a fluorescent maker was incorporated to the same modified gene. This marker facilitates identification of larvae of genetically modified mosquitoes.

The requisite of mating competitiveness Benedict and Robinson (2003) of the modified mosquitoes, is monitored by the quantity C, defined as the ratio between offsprings (lower case) to adult males (upper case) of modified mosquitoes, \( \frac{m}{M} \), normalized to the same ratio for the wild mosquitoes, \( \frac{w}{W} \):

\[
C = \left( \frac{m}{M} \right) \left( \frac{w}{W} \right)^{-1}.
\]

Lower estimates of C will need to be compensated by performing larger releases. The estimation of C performed in cage and field experiments produced values of 0.059 (Cayman Islands (Harris et al., 2012)) and 0.031 (Brazil, (Carvalho et al., 2015)). We leave aside a previous report for the Cayman Islands of C = 0.56 (Harris et al., 2011) obtained in the context of trying to establish that mating of wild females with modified males was actually possible.

An additional source of uncertainty is the lifespan of modified adults in the wild. Mark, release and capture experiments were performed twice. In general, for Ae ae, the ability of such experiments to produce reliable data has been questioned because of the large dispersion of results obtained when trying to estimate dispersal distances, which turn to be greatly influenced by the procedure (Otero et al., 2008; Bergero et al., 2013). The reported results appear not to escape to this rule. Survivals of 2 days are reported in (Lacroix et al., 2012) and of 5 days in (Winskill et al., 2015). In the first experiment the unmodified laboratory strain was released as well showing a 2.1 day survival in average with no significantly differences with respect to the modified strain. The release of a control strain was not repeated in the second experiment, probably because of the urban setting in which it was performed. In contrast, the survival of modified mosquitoes tested against a strain collected in Chiapas (Mexico) termed Latin Wild Type, LWT, used as the basis of the FDA report (Center for Veterinary Medicine, 2016, Appendix F, p. 6-7) contains only experiments in the laboratory, but none in the field. The experiment confirms the existence
of significant differences in phenotype between LWT and the GMI in terms of fecundity and hatch rate measured at the laboratory, the environment in which the the GMI was developed. We have not found data testing survival rates of modified mosquitoes released in the field against the local wild population under similar conditions among the reported literature ((Lacroix et al., 2012; Winskill et al. 2015; Center for Veterinary Medicine 2016)).

2.2. Mosquito, genetics and environment

The populations of the tree hole mosquito *Aedes aegypti* are mainly limited by the development during the larval stage. The two main environmental factors that have been so far identified are temperature (Sharpe and DeMichele 1977; Rueda et al. 1990) and food. It is expected that genetics will play a role but comparative studies of quantitative traits are not abundant. Studies comparing developmental rates of *Ae ae.* collected in different locations in Argentina and those of the Rockefeller strain (a laboratory strain), indicate (Grech et al., 2010) that there are significant differences, under the same temperature and laboratory feeding conditions, between local strains and the Rockefeller strain, as well as statistically significant differences between local (country wide) strains, despite being all of them identified in the same branch of the phylogenetic tree as hybrids between *Aedes aegypti aegypti* and *Aedes aegypti formosus* (Gloria-Soria et al. 2016). For example, the Rockefeller strain spends less time as larvae. Yet, the most substantial difference corresponds to the total number of eggs and the daily fecundity which is five times larger for the Rockefeller strain than for the local strains. A recent study performed in Trinidad and Tobago (Chadee and Martinez 2016) reports a fertility of local females raised in the laboratory, with local genetics, which is in line with the experiments reported in (Grech et al. 2010).

Any model (be it mathematically explicit or not) aimed at exploring the possible outcome of an environmental intervention, must include the reaction norms (Hamilton 2011) of the local, the released and the resulting hybrid mosquitoes. The traits that have been identified so far are: hatching inhibition as a response to low levels of food (the Gillett effect) (Gillett 1955b,a; 1959; Gillett et al. 1977; Livdahl and Edgerly 1987; Edgerly and Marvier 1992; Edgerly et al. 1993), duration of the larvae stadium (Southwood et al. 1972; Rueda et al. 1990; Focks et al. 1993; Macià 2006, 2009; Romeo Aznar et al. 2015), mortality of preimaginal forms (Macià 2006, 2009; Romeo Aznar et al. 2015) and fertility (Arrivillaga and Barrera 2004), directly associated to the body size of females. Furthermore, it has been observed that temperature and food are not independent factors (Padmanabha et al. 2011).

Needless to say, the feeding behavior of the larvae will alter the food resource in the container. Hence, food and phenotype must be considered dynamical variables.
2.3. Simple population dynamic model to evaluate effectiveness and gene diffusion

2.3.1. Lack of information and modeling decisions.

In the light of the limited or uncertain information concerning the RIDL-SIT technique, some modeling decisions were made, namely:

- The lack of information regarding the reaction norms to environmental conditions such as the availability of food have been decisive in the modelling decision of not considering high-level models such as (Romeo Aznar et al., 2015) which incorporate phenotype variation in terms of environmental conditions. High-level models are sensitive to differences found in the phenotype for eggs collected at different places (Romeo Aznar et al., 2013).

- The effect of the lethal gene seems to be apparent not before $L_4$ stage and it is strongest at pupae level (see Table 1 in (Phuc et al., 2007)). Hence, the relation between mortality to emergence related to unmodified pupae is adjusted so that for individuals carrying the lethal gene the probability of death as pupae is $\frac{19}{20} = 0.95$ and the probability of adult emergence is $\frac{1}{20} = 0.05$, since these are the only competing events in an exponential race (Durrett, 2001) at pupa stage.

- Another substantial missing information concerns the contributions of dead larvae and pupae as a secondary source of food. Such contribution was incorporated in earlier models (Focks et al., 1993) based upon results in (Gilpin and McClelland, 1979) and amount to a 12% recovery (the product of 40% nutritive value and 30% conversion of food in weight) according to these sources. Recycling of dead larvae was assumed to influence food availability during the developmental stages (see Section 2.3.5 for a description of the food cycle).

- Heritability of the survival trait despite the presence of the lethal gene has been set to zero.

- Mating effectiveness as compared with the wild strain has been rounded up to $C = 0.06$.

- We have made no difference in the survival of adults of different strains (other than the food deficit effect for individuals raised in the wild). None of the reported experiments checks lifespan of modified males vs lifespan of wild males in the same wild environment under similar conditions. In our simulations, that target for a pre-established reduction level of the wild population, the released males mortality will affect the release rate since a comparatively high proportion of males must be maintained throughout the process. Eventually, larger release rates may enter in conflict with the factory production capabilities. The assumption made here underestimates the strain put on the factory.
Since there is no information regarding reaction norms of hybrids between local strains of \textit{Ae ae.} and the Rockefeller strain, we have assigned to all wild and hybrid mosquitoes the same reaction to food deficit stress using the set of reaction norms available for mosquitoes collected at Buenos Aires (Argentina) \cite{RomeoAznaretal.2015}, corresponding, in the present model, to: pupation rate, fertility, mortality at the preimaginal stadiums, as well as the Gillett effect as quantitatively described in \cite{EdgerlyandMarvier1992} and the mortality of adults. However, released adult mosquitoes (overwhelmingly males) are not raised under food deficit stress and their mortality rate is not corrected by food deficit but fixed as indicated in the previous point.

A model is not just a mere transcription of experimental findings but a logical construction as well. In our schematic construction we have considered the two food regimes described in \cite{RomeoAznaretal.2015} and the dependence of phenomenological traits with the logarithm of the food concentration. A region where the life form is impossible was considered for food concentrations below $2^{-8}$ of the optimal concentration values obtained in the laboratory (which corresponds to 1 in these units). The region of high mortality was considered in the range $[2^{-8}, 2^{-6})$ while the favourable region spans the interval $[2^{-6}, 1]$. In the most unfavourable region the only life form possible are dormant eggs inhibited from hatching by the Gillett effect (see Section 2.2), all other forms have probability one of dying. In the transition region, the Gillett effect diminishes from total inhibition to no inhibition and the mortality moves from probability one to the values measured in laboratory experiments \cite{RomeoAznaretal.2015}. The fertility is considered to be proportional to the mass excess to the minimal form of the adult found at the $2^{-8}$ food concentration where it takes a zero value and grows to reach the measured value for \textit{Ae ae.} in the region of optimal laboratory conditions \cite{Grechetal.2010}. Laboratory results indicate that the increase in size as measured by the wing length is linear with the logarithm of the food concentration \cite{RomeoAznar2015} all along the considered region. Using the raw data of the experiment reported in \cite{RomeoAznaretal.2015,RomeoAznar2015} we determined that the event rate as larvae (considering both death and pupation) increased linearly (with the logarithm of food concentration) in the favorable region and was approximately constant in the transition region.

2.3.2. Stochastic Population Dynamics

This approach to population studies assumes that the dynamics is adequately described by classifying the population into \textit{compartments} $X_j$, $j = 1, \ldots, N$ (described by nonnegative integer numbers), each compartment counting the number of individuals of a given type as a function of time. For example, mosquito subpopulations in the present study are represented by the class $D$ with elements \{egg, larvae, pupae, adult female, adult male\}. Each element in $D$ will be further subdivided with respect to its genetic type according to $G$, with elements \{wild, hybrid, modified heterozygous, modified homozygous\}. Thus, the index $j$ can be thought of as listing the elements of the external product
Changes in time occur stochastically and are identified by \textit{events}, labeled by the index \( \alpha \), where \( \alpha = 1, \cdots, E \). The event \( \alpha \) produces an instantaneous change in population compartment \( j \) given by the integer \( \delta^\alpha_j \). Note that events where an individual undergoes a transition from one compartment to another (e.g., pupation where a larva becomes pupa) have exactly two nonzero indices \( \delta^\alpha_j \): For \( j = l \) (larva) \( \delta^l_l = -1 \), while for \( j = p \) (pupa) \( \delta^p_p = +1 \). Also, death events have \( \delta = -1 \) for the corresponding compartment. Birth events (oviposition) may have \( \delta > 1 \) (see Subsection 2.3.5). The time-evolution of the population, given an initial condition (here labeled as \( t = 0 \)) can be summarised as:

\[
X_j(t) = X_j(0) + \sum_{\alpha=1}^E \delta^\alpha_j n_\alpha(t)
\]

where \( n_\alpha(t) \) is the number of events of type \( \alpha \) that occurred up to time \( t \) (a stochastic variable). This approach is called a Markov Jump Process (Markov since the only necessary information to compute the next change is the knowledge of the present state; Jump since the changes that take place are described by the integer \( \delta^\alpha_j \) indicating a change in the number of individuals in compartment \( j \)). In the mathematical literature attention to these processes starts with Kolmogorov’s foundational work [Kolmogoroff 1931] and its further elaboration by Feller [Feller 1940]. A substantial effort to relate the stochastic description to deterministic equations was performed by Kurtz [Ethier and Kurtz 1986; Kurtz 1970, 1978].

Traditionally, attention was focused on the dynamics in population space, rather than event space. In the case of probability rates that are linear in the subpopulations both approaches are quite interchangeable [Solari and Natiello 2014]. However, event space is more appealing for a general understanding. For example, when the dynamics is affected by introducing a treatment or strategy regarded as an event (think e.g., of vaccination in an epidemic disease) it will be important for policy reasons (logistics, costs, etc.) to count the number of occurrences of that event, rather than attempting to sort it out by reversing the information contained in the population set \( X = \{X_j(t), j = 1, \cdots, N\} \).

The Markov assumption leads to defining probabilities per unit time \( W_\alpha \) (in the sequel called \textit{rates} or \textit{probability rates}) for each event. In this work it will suffice to consider that these probability rates depend only on the population \( X \) at each given time (other dependencies will be handled along the way). In a small time-interval \( h \) the probability of occurrence/non occurrence of event \( \alpha \) is the pair \( (W_\alpha(X)h + o(h), 1 - W_\alpha(X)h + o(h)) \), where \( o(h) \) is a quantity that goes to zero with \( h \) faster than linearly.

The process is further modeled implementing the Feller-Kendall algorithm [Kendall 1949, 1950] that can be summarised in the following steps [Solari and Natiello 2014]:

\begin{enumerate}
  \item Starting at \( t = 0 \) or immediately after an event has occurred, the waiting time to the next event is exponentially distributed, with rate \( R = \sum_\alpha W_\alpha(X) \) equal to the sum of all event probability rates, where \( X \) refers
to the population values after the most recent event has occurred (or to $X(0)$). The time $\tau$ to the next event is simulated by picking an exponential random deviate with rate $R$.

ii. Given that an event has occurred at time $\tau$, the probability of it being event $\alpha$ is

$$P(\alpha / \text{event at } \tau) = \frac{W_\alpha(X)}{\sum_\alpha W_\alpha(X)} = \frac{W_\alpha(X)}{R}.$$ 

Picking an arbitrary ordering of the events, the event that took place at $\tau$ is simulated by picking an uniformly distributed random number $Y$ in $[0, R]$ and checking to which event in the order it corresponds. For example, if $\sum_{\alpha=1}^{K} W_\alpha(X) < Y < \sum_{\alpha=1}^{K+1} W_\alpha(X)$, we assign the occurrence to event $K + 1$.

iii. Populations are updated according to $\delta_\alpha^j$ for the assigned event and a new cycle 1-3 is computed until the final simulation time is reached.

2.3.3. Schematic description of the model

The setup in consideration is that within a limited natural population of $\text{Ae. aef}$. male adult mosquitoes are introduced (with an error of 1 female every 4300 releases (Carvalho et al., 2015)). These mosquitoes differ in two ways from the wild population. Firstly, they have two copies of a dominant “lethal gene” (not present in the wild population) that induces massive death of the offspring before adult stage. The released mosquitoes have been previously raised in the laboratory, from the Rockefeller strain of $\text{Ae. aef}$, a laboratory-adapted strain. These mosquitoes are genetically different from the original wild population. If their offspring does not completely die before reproduction, there will be some degree of population mixing and genetically modified individuals (relative to the original wild population) could arise.

Hence, to describe this problem a model has to be designed covering three goals:

i. Describe the wild population with sufficient accuracy taking care of environmental constraints.

ii. Take care of the evolution of the lethal gene in the offspring along generations. A released homozygous mosquito fecundating a wild female (where the corresponding gene is not lethal) will yield heterozygous offspring and any eventual subsequent offspring will propagate this special gene according to the mendelian rules.

iii. Take care of the mixing between the natural population and the released, genetically different, mosquitoes (mixing of other genes apart from the lethal one).

Within this approach, the specification of the model requires (a) to identify the (sub)populations (compartments) participating in the dynamics and (b) to specify the events that define the dynamics, their reach, biological content and environmental conditions.
As mentioned in the previous Subsection, the subpopulations involved are chosen according to the developmental stages (egg, larva, pupa, male adult and female adult). In the absence of released mosquitoes the events governing the population dynamics are egg hatching and mortality, larval pupation and mortality, pupa emergence and mortality, fecundation and oviposition and finally adult mortality. These events take care of modeling goal i.

All compartments are further classified according to their genetical content. There is a compartment for the wild lineage and three for the mixed lineage where 0, 1 or 2 copies of the lethal gene may be present. This population space allows for dealing with modeling goal ii.

Finally, each subpopulation has attached a number in $[0, 1]$ representing the average percentage of extraneous genetic material. Released mosquitoes contribute with 1, wild mosquitoes with 0 and the mixing propagates according to the law of independent assortment of alleles (Hamilton, 2011). This takes care of modeling goal iii.

A detailed scheme of compartments and relating events is shown in Figure 1.

Figure 1: Compartments and events. The four copies of each compartmental stage correspond to the cases (wild, 0, 1, or 2 copies of the lethal gene, H: hatching, P: pupation, E: emergence, F: fecundation, O: oviposition, Death: Death rate (specific for each stage), Death LG: modified death rate at the pupa stage due to the presence of the lethal gene.

### 2.3.4. Food dynamics

Most rates, especially larval pupation and mortality rates, depend on the availability of food. Following the model in (Romeo Aznar et al. 2013) we assume that the totality of oviposition sites can optimally host $l_{opt}$ larvae (grown to their potential size), that may feed unrestrictedly. At the same time, food in the sites is produced by (environmental) bacterial activity, it degrades at a certain rate $u$ (also depending on the environment, in this work $u = \frac{1}{days}$) and it is consumed at a pace depending on the amount of larvae.
Dead larvae and pupae are to some extent recycled as available food, as mentioned in Section 2.3.1. Let \( \frac{P_f}{C_f} \) denote the ratio of produced to consumed food. Initially, we set its value to the deterministic equilibrium value, an environment-dependent estimate. We denote the leftover food (relative to consumed food) as \( L_f \). In conditions of food scarcity, \( L_f = 0 \). The dead larvae or pupae recycled as food is denoted as \( X_l \). This last parameter is initially zero and it is updated at every larval or pupal mortality event (see below). The ratio \( \frac{P_f}{C_f} \) indicates the environmental scarcity conditions: if lesser than one, there is no food available for the larvae to reach the potential weight at pupation (this ratio enters in several steps of the modeling procedure). The food-cycle for each simulation time-step \( \tau \), where the initial total larva population is \( L \) and the ideal estimated capacity of the oviposition sites is \( l_{opt} \), proceeds as follows:

\[
L_f = \max \left( \frac{P_f}{C_f} - 1, 0 \right)
\]

\[
\frac{P_f}{C_f} = L_f \exp(-u\tau) + \frac{l_{opt} + X_l}{L}
\]

\[
X_l = X_l \exp(-u\tau)
\]

At each time-step, the values of \( L \) and \( X_l \) are updated and all above quantities recalculated. The influence of the food in mosquito dynamics is described by larval event rate \( LE(x) \), mortality probability as larvae and pupae \( ML(x) \), hatching inhibition \( GL(x) \) and fertility rate \( FT(x) \):

\[
LE(x) = \max(0.025, 0.25269 + 0.031974 x)
\]

\[
ML(x) = \begin{cases} 
1 & x < -8 \\
\max(0.033, 0.033 - 0.4835 (x + 6.)) & x \geq -8
\end{cases}
\]

\[
GL(x) = \begin{cases} 
1 & x \geq -6 \\
(1 + 0.5 (x + 6.)) & -8 \leq x < -6 \\
0 & x < -8
\end{cases}
\]

\[
FT(x) = \max(0, 0.127742 (x + 8.))
\]

where \( x = \log 2(\frac{C_f}{P_f}) \). The fertility rate changes the fertility of the emerging females, and then the average fertility of females (see below). The values for the reaction norms correspond to the values reported in (Romeo Aznar et al., 2015). In addition, the average lifetime as adult is corrected as \( m_a = \frac{m_{a0}}{1 - ML(x)} \) obtaining in this form a reasonable correspondence between times measured at the laboratory (see for example (Chadee and Martinez, 2016)) and in field experiments (for example (Southwood et al., 1972)). Released male mosquitoes, that had no food deficit while raised, were set to have average lifetime \( m_{a0} \) (see values below).

2.3.5. Specific details

The theoretical basis of the model when it comes to the population dynamics of \( Ae \) \( ae. \) refers to Romeo Aznar et al. (2013; Otero et al.) 2006 2008. The
problem is quasi-linear \cite{Solari2014} in the populations, meaning that (a) all events that decrease a subpopulation produce a decrease in one unit in only one population compartment and (b) all non constant rates can be written in the form $W_{a} = m_{\alpha} X_{i}$, for the relevant population $X_{i}$. However, the coefficients $m_{\alpha}$ need not be all constant.

The events hatching and pupation are assumed to have the same coefficient for all four compartments of the participating subpopulation. All these events pick one individual from the original subpopulation and passes it to the corresponding subpopulation of the next stage. Pupa emergence is less likely for the compartments carrying 1 or 2 copies of the lethal gene (see below for the explicit values). Emergence events are randomly assigned to yield 50% males and females. Each mortality event simply decreases the corresponding subpopulation in one unit. Except in the case of pupa mortality, where the effect of the lethal gene also distinguishes two cases, the mortality coefficient is taken to be the same along each stage. The pupa mortality is much higher for the compartments having 1 or 2 copies of the lethal gene (see Appendix I for details of the estimates). As indicated in Section 2.3.1 adult mortality was assumed to be equal for all strains. This modeling decision may overestimate the size of non wild subpopulations, an effect that may be mitigated by larger releases.

The release was acted weekly during the treatment period, following the implementations of this technique discussed above, that report only weekly releases. The release goals of \cite{Harris2012} were a relation of 10 : 1 in adult males (later increased to 25 : 1) and 50% fluorescence in the larvae from ovitrap-collected eggs. The initial size of the release is therefore set to be 11 times larger than the adult male population in our computations. Later release events are increased or reduced adaptively every week, in order to adjust the weekly proportion of eggs with lethal gene against a target value. There is however a maximum release size corresponding to the maximal capacity of the production plant. In this work it is set to 100000 individuals per release. Release is intended to concern only modified males, but a few females are released as well, since the accuracy of the separation technique is limited (one female every 4300 releases \cite{Carvalho2015}).

The model simulates a closed environment, i.e., no emigration/immigration of adult individuals to and from adjacent areas is taken into account. Clearly, such effects could only delay or reduce any effects of the release since immigrants, at least initially, would be wild individuals while emigrants could be any among released, wild and mixed individuals.

In this implementation, fecundation is not treated as a separate event. Instead, we assume that given that the adult male population is nonzero, all females are fecundated immediately after emergence. Thus, each emerged female has two associated indices, corresponding to the adult female compartment (inherited from the pupa) and the fecundating male compartment. The latter is chosen randomly among the four male subpopulations, weighing the size of the subpopulation with two copies of the lethal gene with a factor 0.06, as described in the previous Sections. We assume hence that this reduced effectiveness is coupled to a high proportion of non-wild genes and to the homozygous property.
The index pair given by fecundation is used to propagate to the offspring both the lethal gene and the percentage of introduced genes from the released population (according to the independent assortment of alleles (Hamilton, 2011), i.e., with the average of parental percentages). The propagation takes place in the oviposition event that adds individuals to the egg population (distributed among the four egg compartments according to mendelian rules) without decreasing any other subpopulation. The amount of eggs laid by each female at each oviposition depends on temperature and on the size of the female, which in turn depends on the environmental food availability. In average, wild mosquitoes collected in Argentina and reared in an environment with optimal food availability ($P_f = 1$) at $T = 26$ °C, lay 2 eggs per day (Grech et al., 2010). Hence, the number of eggs per oviposition is $\delta_{egg} = \frac{2}{m_{ov}(26)FT(1)}FT(P_fC_f) \equiv c_{lay}FT(P_fC_f)$. Once a female has emerged, its size is fixed as well as its egg-laying capacity, both by the value of $P_f$ at emergence (which varies among events according to food dynamics). Thus, at each emergence event, $\delta_{egg}$ is updated as follows:

$$\delta_{egg} := \frac{\delta_{egg}F + c_{lay}FT(P_f)}{F + 1},$$

where $F$ is the total number of adult females existing previous to the actual emergence. This is a sort of “moving average”.

Dead larvae and pupae are partially recycled by the environment within the hatching sites. For each death event we add to $X_l$ the amount $x_l = \beta FT(P_f)$. The quotient estimates the reduced size of each dead larva or pupae relative to ideal food conditions. The values $\beta = 0$, $0.12$ (as suggested by results in (Focks et al., 1993) and (Gilpin and McClelland, 1979), see Section 2.3.1) and $1$ were tested.

Most rates are known to be temperature dependent (Rueda et al., 1990; Otero et al., 2006; Padmanabha et al., 2011). In this work we fixed temperature at the value $T = 26$ °C, corresponding to a year-round stable tropical environment. The coefficients for the different rates are (in units of $(days)^{-1}$) are displayed in Table 1, along with the references where the model structure and rates have been discussed:

2.3.6. Hybridisation degree

The inheritance of genotype is acted at fecundation. On one hand, the different subpopulations are classified in four compartments, depending on they being “purely wild” or mixed (e.g., a wild female mating a released male), with 0, 1 or 2 copies of the lethal gene (here labeled $W$, $M_0$, $M$, $M_2$). When the treatment starts, the first generation offspring of the released males with wild females will invariably land on compartment $M_1$ but later generations may mix further, since pre-adult mortality of the mixed offspring is not total. In any case, the offspring falls always in one of the four compartments.
### Table 1: Events and event rates. (LG stands for Lethal Gene)

| Event                                      | Rate                                                                 |
|--------------------------------------------|----------------------------------------------------------------------|
| Hatching\(^{a,b}\)                         | \( m_{e\rightarrow t} = (-0.0167105 + 0.03866 \exp\left(\frac{T + 7.26}{18.37906}\right)) GL\left(\frac{T_f}{C_f}\right) \) |
| Egg mortality\(^{a,c}\)                    | \( m_e = 0.01 \)                                                     |
| Pupation\(^{b}\)                           | \( m_{t\rightarrow p} = LE\left(\frac{T_f}{C_f}\right) \left(1 - ML\left(\frac{T_f}{C_f}\right)\right) \) |
| Larval mortality\(^{b}\)                   | \( m_l = LE\left(\frac{T_f}{C_f}\right) ML\left(\frac{T_f}{C_f}\right) \) |
| Pupa emergence no LG\(^{b}\)               | \( m_{p\rightarrow a} = 0.5787 \left(1 - ML\left(\frac{T_f}{C_f}\right)\right) \) |
| Pupa mortality no LG\(^{b}\)               | \( m_p = 0.5787 ML\left(\frac{T_f}{C_f}\right) \) |
| Pupa emergence with LG                     | \( m_{pl\rightarrow a} = \frac{1}{20} \left( m_p + m_{p\rightarrow a} \right) = \frac{1}{20} \times 0.5787 \) |
| Pupa mortality with LG                     | \( m_{pl} = \frac{19}{20} \left( m_p + m_{p\rightarrow a} \right) = \frac{19}{20} \times 0.5787 \) |
| Adult mortality\(^{b}\)                    | \( m_a = \frac{0.04}{(1 - ML\left(\frac{T_f}{C_f}\right))} \) |
| Adult mortality (2 LG)\(^{d}\)             | \( m_a = 0.04 \)                                                     |
| Oviposition\(^{b}\)                        | \( m_{ovip} = 0.03154 \exp\left(\frac{T - 4.7511}{10.590}\right) \) |

\(^a\) See (Otero et al., 2006).  \(^b\) See (Romeo Aznar et al., 2013; Romeo Aznar, 2015).  \(^c\) See (Trpis, 1972).  \(^d\) See (Center for Veterinary Medicine, 2016).

Regarding the rest of the genetic material, the average degree of mixing \( R \) of each compartment is computed in several steps. This value changes for each individual at fecundation/oviposition. Further, when new individuals enter a compartment, the compartmental average for \( R \) changes accordingly and this modification propagates along the life cycle. Wild population has initial mixing value 0, while released mosquitoes have value 1, corresponding to 100% Rockefeller-strain genetic material. Following the law of independent assortment of alleles, offspring has a mixing equal to the average of the parental values, \( R_o = \frac{1}{2} (R_m + R_f) \). Laid eggs (eq. 1), however, distribute among compartments according to mendelian laws. Finally, the average mixing of the affected compartments is consequently moved as,

\[
R_n := R_n X(n) + R_e E \times \frac{X(n) + E}{}
\]

where \( E \) is the amount of eggs (with inherited mixing \( R_0 \)) belonging to compartment \( n \) and \( X(n) \) is the preexistent number of eggs in that compartment (with previous average mixing \( R_n \)).

### 3. Results

Model simulations were performed using the following scheme:

- The initial wild populations and environmental food conditions were set to the deterministic equilibrium value (see Appendix II for a description).
- A few years transient was run to produce a stable population, and this data was saved to start the simulation runs.
• For a transient time \( TT = 156\) weeks (about 3 years), the wild population was allowed to proceed without interferences. The population data was saved to start the next simulation.

• After the previous transient, intervention was started, for a duration of \( TI = 52,260\) weeks.

• After finishing the intervention, the system was simulated for another 156 weeks.

• The exhibited results are the average of 10 runs of the previous scheme.

• The short treatment was targeted to a weekly fluorescence ratio of 0.5 and the larger to ratios of 0.5 and 0.75 between eggs hatching to fluorescent vs total number of hatched eggs (per week).

• The recycle coefficient \( x_l \) at equilibrium was set to 0.12 for the short treatment while for the long treatment we illustrate the dynamics with \( x_l = 0, 0.12 \) and 0.5.

3.1. Summary of results

We illustrate the simulations results with a few graphs indicating the time-evolution of the displayed properties. The abscissa of all graphs has units of days, counted from the beginning of the simulation. The transient, treatment and final portions are evident.

In Figure 2 we display the time-evolution of the number of adult females carrying no lethal gene for the short treatment (1 year), where the ratio of eggs with lethal gene to total number of eggs each week is targeted to 50%. The recycling coefficient of dead larvae and pupae is set to 0.12. The drop in population size during the intervention is evident. Initially, the drop is more intense since the adjusting mechanism to target fluorescent eggs is slow (see below for a discussion).

![Figure 2: Females with no lethal gene, 1-year treatment, 50% eggs with lethal gene. Vertical lines indicate beginning and end of treatment.](image-url)
Figure 3 displays the time-evolution of the number of adult females carrying no lethal gene for the long treatment (5 years), where the ratio of eggs with lethal gene to total number of eggs each week is targeted to 50% or 75%. Same considerations as in the previous picture hold. The three curves correspond to different values of the recycling coefficient of dead larvae and pupae (see caption).

Figure 3: Females with no lethal gene, 5-year treatment, 50% (left) or 75% (right) eggs with lethal gene. Curves correspond to values 0 (red), 0.12 (green) or 0.5 (blue) for the recycling coefficient of dead larvae and pupae. Vertical lines indicate beginning and end of treatment.

The target goal we attempt to simulate follows (Harris et al., 2012) and aims to obtain a 50% or 75% proportion of laid eggs every week carrying one or two copies of the lethal gene. The idea of monitoring eggs in order to regulate the treatment may be simple, but the control procedure is quite involved. On one hand, eggs have to be hatched in order to observe the larval fluorescence, meaning that the information about the system in actual implementations is obtained with certain delay (of the order of days). On the other hand, the control is acted by modifying the release, i.e., mainly adult males. In the model, it takes two (target 50%) or three (target 75%) weeks before the release of adults propagates to a modified proportion of eggs of about half the desired target.

The simulations display a reduction of adult females (and all other sub-populations except adult males) during the treatment period. This reduction could be said to be environment dependent, since it varies with the ability of the oviposition/breeding sites to recycle dead larvae and pupae into more food. There is an initial drop passing the target since the control procedure is indirect (eggs are monitored but the only modification to the system acts through the introduction of adult males). The other target in (Harris et al., 2012) is satisfied in excess, as seen in Figure 4 left. The adult male ratio is permanently above 10 : 1 during the treatment, while in the most demanding treatments it can be as high as 40 : 1.

After finishing the treatment, the system returns to a situation that is comparable with the initial equilibrium condition, the main difference being displayed in Figure 4 right. This figure displays the time-evolution of the pro-
portion of alien genes in the population. Only the recycling coefficient 0.12 is plotted, since the differences with the other situations are minor. Three of the four female compartments die out, remaining only the hybrid compartment with no lethal gene. The new equilibrium condition, although being quantitatively similar to the one before treatment, bears now different mosquitoes, the difference being larger for the longer and more demanding treatments.

The fact that the system returns to its previous equilibrium value can be understood in different levels. From the simulation viewpoint, the system was assumed to be in a stable equilibrium before treatment. Despite the more or less drastic influence of the treatment, it eventually returns to this equilibrium when left alone. However, simulations with large weekly releases (about eight times larger than the maintenance release for the 0.5 target) under a sufficiently long time (more than one year) shift the final equilibrium to a smaller permanent population with 100% Rockefeller hybridization homozygous in the lethal gene. From the modeling viewpoint, we pointed out before that there exists no knowledge about the adaptability of the modified hybrid mosquito populations to the actual environment. This possibility is consequently not considered in the model. Similarly, it is not known whether mosquitoes with 65% of the genetic content of the Rockefeller strain differ from the wild strain in their behaviour and their efficiency as vectors of viral diseases.

3.1.1. Cleaning of breeding sites

Release procedures described in the literature are frequently initiated by cleaning the environment removing breeding sites, in order to reduce preexistent preimaginal populations and the carrying capacity of the environment. This additional treatment is in fact independent of the RIDL-SIT technique. For the sake of the model, “cleaning” amounts to modifying the initial conditions (in relation to the actual conditions of a given environment), in such a way that the environment has less oviposition sites, smaller initial preimaginal populations and consequently a different initial condition also for the food dynamics.
3.2. Report of simulated scenarios: Questions and answers.

We summarize our findings with a set of questions and answers. The first two questions are those present in the Introduction and originally triggering this study.

A. Which lasting modifications of the environment will be produced by these essays?
The main modification is the hybridization of the released mosquitoes with the local populations as a result of genetic diffusion. The degree of hybridization depends both on the duration of the treatment and its intensity (target), as well as on the (environmental) differences in food dynamics.

B. How fast will the sanitary situation previous to the environmental intervention be reestablished?
The recovery time of populations can be counted in months. There is no difference in population sizes before and after the treatment for the targeted programmes.

C. What is the expected ratio between released males and local males necessary to achieve different levels of control?
The ratio ranges from 12 : 1 to 40 : 1 to sustain a target of 0.5 to 0.75 of the eggs having the modified genetics. Ratios larger than 100 : 1 are needed to detect sizeable effects at the beginning of the intervention. This can be compared with the reports in [Harris et al., 2012] where the essay started setting a ratio of 10 : 1 in a comparatively large area but it soon became clear that a smaller (or much smaller) area and a higher ratio (25 : 1) were necessary in order to observe some effects.

D. How are the results affected by the limitations of an hypothetical mosquito factory?
In the simulations we have arbitrarily chosen to limit the production to 100000 modified mosquitoes per week. Some essays have managed to pass this limit. The present simulations remain always below the limit. In practice, a smaller area requires less mosquitoes. Therefore, the production limit is not an issue at this level. Repeatedly hitting the limit would slow down or reduce the possibility of attaining the targets of the treatment.

E. How does recycling of dead larvae and pupae affect the dynamics?
Dead individuals could be reprocessed in different ways depending on e.g., temperature, bacterial contents, etc. This issue is not intrinsic to the RIDL-SIT technique apart from the fact that this or any other technique must act on a given natural environment. The model allows for sensing this issue to some extent. The effects appear to be less important regarding the genetic diffusion but sensibly larger.
regarding the temporary reduction in subpopulation sizes during the treatments.

F. Is it possible to eliminate the mosquito?
The model does not consider immigration (or emigration) to (from) the intervention region. Under this condition, if the fertility of the released females carrying the modified gene is close to the Rockefeller strain from which they were produced, the targeted treatment will not eliminate the mosquito but rather replace it with some hybrid strain. Simulations releasing weekly all the production of the factory suggest that a 100% Rockefeller strain with two copies of the lethal gene will replace the wild population (remaining stable) if the treatment is long enough. Analytical results confirm this observation. Population levels are expected to be somewhat smaller and mosquitoes larger than in the untreated situation.

G. How does the technique compare to other infrastructural measures when it comes to costs, permanent benefits, risks and mosquito populations?
The model does not contemplate social and economic costs (in part because of lack of information). The simulations suggest that the technique will become a permanent patch, this is, there will be no end to the release programme and as such it represents a structural cost that should be compared with other long-term policies (such as running water, a sewage system, proper handling of water reservoirs, etc.) that set the possibility of a healthier environment.

4. Final discussion and conclusions

The processes of development of models and of introduction of new technologies present some parallels that are worth discussing. In the first place, both processes respond to the different attitudes towards knowledge and learning, or more generally to the epistemic frame(s) in action.

>From pre-modeling (intuition-based) conclusions we enter the realm of theoretical conclusions based on mathematical models. A first stage in model development can be called the chimeric level where the modeled mosquito only roughly resembles the real one (take this expression as self criticism (Otero et al., 2006, 2008)), but at least the time-scales and life-cycle characteristics are present. Models mature into ecologically oriented models (Romeo Aznar et al., 2013, 2015; Romeo Aznar, 2015) fundamentally including phenotypic plasticity (Reed et al., 2010) and focusing on one strain at a time (subpopulations of mosquitoes in different cities may be strikingly different (Tejerina et al., 2009; Grech et al., 2010) and even within the same city (Paupy et al., 2004), different genetics is associated to different breeding sites) and may further progress towards evolutionary models (Scheiner, 1993) accounting for the evolution of phenotypic plasticity. In this higher level we still have everything to learn.
In parallel, technologies start as laboratory tests, essentially ignorant of the hazards and singularities of life outside the laboratory. At this level, technologies are short-sighted; they cannot look ahead the time and space scales of the controlled laboratory tests. In order to mature into reliable tools, they too have to develop, including insight about the mutual influences between technologies and environment. This triggers the need of a research program and an action policy which eventually may outcome a strategy to handle the challenges of the environment and of (co)evolution. Business may interfere with this process, attempting to obtain profits now and ignoring losses until later.

The perspectives of the RIDL-SIT technique considered before this work are clearly at the first stage. We have pointed out in the previous sections issues about missing information on both the life-cycle of the modified mosquitoes and on their interaction with the environment and with the wild strain. Moreover, already at the level of bibliographical background research we note that important requirements mentioned e.g., in Benedict and Robinson (2003) are not fulfilled.

The information so far produced corresponds to the development of the technology and extrapolations of laboratory results based upon intuitions. We have termed this epistemic frame the technological frame (Solari, 2016, 2017). It implies the fundamental decision of ignoring the differences between a wild environment and the laboratory using the simple resource of not exploring them. In this work we implemented an environment-aware model of the life-cycle of a strain of Aedes aegypti compatible with field observations (Romeo Aznar et al., 2013, 2015; Romeo Aznar, 2015) also introducing some genetics since the issue at stake with the RIDL-SIT technique is to take advantage of genetical differences between mosquito strains. We have demonstrated that, with the help of mathematical models, it is possible to detect some of the information needed to make less risky decisions than just “trial and error” (which has been the method of technology in all civilizations but it is not the method of science), and that there is more to monitor than the success of the method considered only in the restricted terms of the developers of the technology. The model suggests that not even in a minimal area protected from immigration of wild specimens the technique is able to eliminate the mosquito population. Even worse, the output of the treatment is to produce a new, hybrid, mosquito strain, whose characteristics are unknown. The RIDL-SIT strategy can be viewed as a technological patch for an unsustainable control program, a conclusion that has been reached previously from social considerations (Reis-Castro and Hendrickx, 2013). Indeed, the results of this work indicate that there is a long path that needs to be travelled before moving into field tests.

The limited nature of the model manifests the need of understanding evolutionary issues in order to proceed further: How might the mosquito change under selective pressure? How will the differences between hybrid and wild mosquitoes manifest themselves? The results of hybridisation cannot be forecast in the present context of lack of information, yet this difficulty should not be rephrased as “there are no consequences expected from hybridisation”. In all cases, the larger genetic pool available will result in a better matching of the lo-
cal forms of the mosquito to the environment. If the larger fertility provided by the Rockefeller background of the released mosquitoes results in larger fertility of the new hybrid population established in place of the original wild population, without altering the available biomass of food, a larger number of adults of smaller size than the original wild population is to be expected. A larger number of vectors facilitates the propagation of mosquito transmitted diseases, while the favorable or unfavorable incidence of the size of the vector is a matter of discussion (Juliano et al., 2014). In the same form, a co-evolution of bacteria in which \textit{Ae} \textit{ae.} feeds prompted by the increase in mortality in the breeding sites is expected to result in substantially larger populations because of the sensitivity detected to “recycling” dead pupae and larvae. Thus, an “arms race” scenario might arise (increased population retaliated by increased releases). The epidemiological situation after the intervention will then be worse than before the intervention. We must conclude that the possibility of a larger risk for epidemics after the intervention has ceased should be taken into account. This observation warns us about the risks of attributing to the species what is in fact the result of the genetics and environment. Mosquito strains are different, they interact in (so far) unexpected ways and are subject to a large, and mostly unknown today, number of influences. It is worth to recall that a few years after the intervention described in (Carvalho et al., 2015) had concluded, Itaberaba, a suburb of Juazeiro (Bahia, Brazil) was stricken again by Zika, Dengue and Chikungunya in 2016.

The standard analogy between controlling fruit flies and controlling mosquitoes such as \textit{Ae} \textit{ae.} is not a very close one from an ecological point of view. A very obvious difference is the landscape, going from agricultural to urban, yet a deeper difference consists in that \textit{Ae} \textit{ae.} as an established domestic insect is limited only by the environment, while the fruit fly, when production has not been abandoned, is not limited by the available environment, but rather the fly is just attempting to colonize it, being in short number relative to the carrying capacity. Current knowledge in SIT indicates that “releasing sterile insects routinely in certain areas may be more expedient to prevent establishment of major pests than eliminating them after they become established” (Hendrichs et al., 2005).

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\footnote{See \url{http://www.itaberabanoticias.com.br/itaberaba/quase-6-mil-pessoas-foram-infectadas-com-dengue-zika-e-chikungunya-em-itaberaba}}
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Appendix A. Lethal-gene enhanced mortality at pupae level

The assumptions behind the modified death and emergence rates for pupae are as follows. For the model, pupae can undergo only two events, namely emergence $m_{p\rightarrow a}$ and death $m_p$. We assume that the time to the next event is not altered with respect to the wild population, so in both cases this time responds to an exponential distribution with parameter $R_{pupa} = m_{p\rightarrow a} + m_p = m_{pl\rightarrow a} + m_{pl}$ (where $l$ stands for “lethal”). Further, we assume that 5% of pupae carrying the lethal gene emerge while the other 95% dies. Hence,

$$\frac{m_{pl\rightarrow a}}{m_{pl\rightarrow a} + m_{pl}} = 0.05$$

and therefore

$$\frac{m_{pl\rightarrow a} + m_{pl}}{m_{pl\rightarrow a}} = 20 \text{ or } m_{pl} = 19m_{pl\rightarrow a}$$

Hence,

$$m_{pl\rightarrow a} = \frac{1}{20} (m_{p\rightarrow a} + m_p), \text{ and } m_{pl} = \frac{19}{20} (m_{p\rightarrow a} + m_p).$$

Appendix B. Deterministic equilibrium population

Deterministic population equations as those are frequently seen in epidemiological modeling, have an older history than the stochastic approach. However, deterministic models have a more limited value, since they average away relevant issues of the dynamics. The most conspicuous limitation is that these models cannot properly describe few individuals, as would be the case in problems where there is a potential extinction risk (such as ours). In certain situations (Ethier and Kurtz [1986]; Solari and Natiello [2003]) the deterministic dynamics can be seen as a sort of large-population limit of the stochastic approach. However, since the wild population is not spontaneously facing extinction, the deterministic equilibrium values are a reasonable starting point for our simulations.

To produce deterministic equations out of the stochastic setup amounts to combine the rates $W_\alpha$ and associated modifications $\delta_\alpha^a$ populationwise.

The general expression for the deterministic dynamics of each population species in the absence of released individuals reads,

$$\frac{dX_j}{dt} = \sum_\alpha W_\alpha (X_1, \cdots, X_n) \delta_j^a = \sum_\alpha m_\alpha^i X_i \delta_j^0$$

where the sum over $\alpha$ is relevant only for those events modifying population $X_j$ and $X_i$ is the specific subpopulation associated to the event with coefficient $m_\alpha^i$. At this point recall that fecundation is not treated as an event, i.e., it is
assumed to occur in a time-scale that is negligible in front of the life-span of adult females. Letting $E, L, P, F$ denote the size of the wild populations of eggs, larvae, pupae and females, we obtain,

$$
\frac{dE}{dt} = m_{ovl} c_{ovl} FT \left( \frac{P}{C_f} \right) F - \left( m_{e\rightarrow l} + m_e \right) E
$$

$$
\frac{dL}{dt} = m_{e\rightarrow l} E - LE \left( \frac{P}{C_f} \right) L
$$

$$
\frac{dP}{dt} = LE \left( \frac{P}{C_f} \right) \left( 1 - ML \left( \frac{P}{C_f} \right) \right) L - 0.5787 P
$$

$$
\frac{dF}{dt} = \frac{1}{2} 0.5787 \left( 1 - ML \left( \frac{P}{C_f} \right) \right) P - \frac{m_a}{1 - ML \left( \frac{P}{C_f} \right)} F
$$

The food equilibrium condition influences all equations and also the larvae population $L$. Being the deterministic system quasilinear \cite{Solari and Natiello 2014} in the populations, the equilibrium is obtained when the determinant of the coefficient matrix is zero, determining in such a form the food level of the equilibrium. In terms of exponential races, the equilibrium is independent of the average time required for the race and depends only on the probability of occurrence of one or another outcome. The population number is determined by the production of food by the environment. The equilibrium values were obtained solving the equilibrium food dynamics to obtain the equilibrium $P_f C_f$ value and the equilibrium larvae population $L$. Subsequently, all other populations were obtained by solving $\frac{dE}{dt} = 0$, $\frac{dP}{dt} = 0$, $\frac{dF}{dt} = 0$ as a function of $L$ and $\frac{P_f}{C_f}$.

Appendix C. Reaction norms determined for this work

The data available from the experiment reported in \cite{Romeo Aznar et al. 2015, Romeo Aznar 2015} was used to produce the estimates for the dependence of body size (surrogated by wing length) and the rate for events (pupation or death) as larvae. The results are displayed in Figure C.5. The data displays clearly two different responses to food depletion: the starvation region where the probability of mortality increases but the average time for the next event in the larvae compartment does not change significantly; and the scarcity region where mortality is not increased but the average time between events (the reciprocal of the rate) changes monotonically. The left panel of Figure C.5 shows the wing length as a function of the logarithm of the fraction of optimal food density. Wing length, body size and fertility are monotonically related. The right panel shows the event rate for larvae as a function of the same environmental variable.
Figure C.5: Reaction norms for fertility and event rate (death plus pupation) for larvae. (No females emerged for the $2^{-10}$ treatment).