BioSystems

Follow up estimation of *Aedes aegypti* entomological parameters and mathematical modellings☆

Hyun Mo Yang [a,*,1], Maria de Lourdes da Graça Macoris [b], Karen Cristina Galvani [b], Maria Teresa Macoris Andrighetti [b]

[a] *UNICAMP – IMECC, Departamento de Matemática Aplicada, Caixa Postal 6065, CEP: 13081-970, Campinas, SP, Brazil*
[b] *SUCEN, Avenida Santo Antonio, 1627, Bairro Somenzari, CEP: 17506-040, Marília, SP, Brazil*

ARTICLE INFO

ABSTRACT

The dengue virus is a vector-borne disease transmitted by mosquito *Aedes aegypti* and the incidence is strongly influenced by temperature and humidity which vary seasonally. To assess the effects of temperature on dengue transmission, mathematical models are developed based on the population dynamics theory. However, depending on the hypotheses of the modelling, different outcomes regarding to the risk of epidemics are obtained. We address this question comparing two simple models supplied with model's parameters estimated from temperature-controlled experiments, especially the entomological parameters regarded to the mosquito's life cycle in different temperatures. Once obtained the mortality and transition rates of different stages comprising the life cycle of mosquito and the oviposition rate, we compare the capacity of vector reproduction (the basic offspring number) and the risk of infection (basic reproduction number) provided by two models. The extended model, which is more realistic, showed that both mosquito population and dengue risk are situated at higher values than the simplified model, even that the basic offspring number is lower.

© 2010 Elsevier Ireland Ltd. Open access under the Elsevier OA license.

1. Introduction

Dengue virus is a *flavivirus* transmitted by arthropod of the genus *Aedes* and the disease resulting from this infection constitutes major public health problems in many tropical and subtropical regions of the world where *Aedes aegypti* is present. When an infectious mosquito injects the dengue virus in a susceptible host during the blood meal, after a period of time, which varies in average from 4 to 6 days, the dengue disease can evolve from asymptomatic or classical dengue fever to the hemorrhagic dengue, which can lead to the death of the host. The manifestation of the symptoms varies between 3 and 7 days, which corresponds roughly to the infectious period. Thereafter, the recovered individual develops a life long specific immunity against the infecting serotype of virus. Female mosquitoes are infected during the blood meal from infectious individuals in the viremic period and they transmit the infection after a period of time which situates between 7 and 10 days, probably throughout the life-span (Mandell et al., 2005).

The extrinsic incubation period of dengue virus among female mosquitoes is large compared to their survival time, which, as other entomological parameters regarding to mosquito's life cycle (Rueda et al., 1990), is sensitively depending on the temperature (Nelson, 1986; Focks et al., 1993a,b). However, the risk of dengue transmission is highly potentiated due to the *A. aegypti* bitings occurring during the day. In order to prevent dengue outbreak, periodic surveys designed to detect changes in key adult indices are important since they allow the detection of adult population fluctuations, which may prompt changes to vector control strategy. However, ecological, behavioral and control information on population size, distribution, survivorship, seasonal abundance and insecticide susceptibility are required for an understanding of epidemic potential and for the formulation of control strategies (Monath, 1989). The size of the mosquito population can be assessed if we are able to estimate the rates of mortality and transition in different stages of the life cycle: egg, larva and pupa comprising the aquatic stage, and adult mosquito (winged stage). By the means of population dynamics theory, we take into account the flows between successive compartments of the mosquito's life cycle in order to determine the size of the dengue vector population. Mathematical model when supplied with reasonable estimation of the entomological parameters provides the trends of the mosquito population over time.

Yang et al. (2009a,b) presented mathematical models dealing with mosquito population and dengue transmission. Also, labo-

ratory experiments regarding to the estimation of entomological parameters were described. For instance, Yang et al. (2009a) estimated the entomological parameters concerned to the whole aquatic phase based on the follow up of hatched eggs up to the emerging of adult mosquitoes. Then, those parameters were supplied to a mathematical model structured in two compartments, *e.g.*, aquatic phase and adult mosquitoes, to assess the effects of temperature on the transmission of dengue (Yang et al., 2009b). Based on those previous papers, in this paper we present a mathematical model including more details about the life cycle of mosquito population, that is, we consider three compartments: larva, pupa and adult mosquitoes. Since the aquatic phase is split into two stages, larval and pupal, we must estimate the entomological parameters regarding larval and pupal stages. In order to provide larval and pupal entomological parameters to the extended model, we present an estimation procedure using data from the follow up of whole aquatic phase, by considering the parametrized survival function presented in Yang et al. (2003) and used in Yang et al. (2009a). Supplying the model with these estimated entomological parameters, we can assess the consequences of including more details in the mathematical modelling.

Laboratory experiments consisted in following up of the entire aquatic forms of mosquito and collecting data every 24 h. However, larval and pupal stages are very short, arising questions about the appropriateness of one day observations. In order to address this feature, we compare the estimations obtained from the followed up of larval stage every 24 h (one day) with those obtained from every 8 h.

The paper is structured as follows. In Section 2 we present mathematical model and estimation methods. In Section 3 we present results regarding to the estimated entomological parameters and we assess the size of mosquito population and the risk of dengue epidemics. In Section 4 we present discussions and conclusion.

2. Mathematical Model and Estimation Method

To assess the incidence of dengue varying with temperature, we first present a compartmental model encompassing three stages in the mosquito's life cycle: larva, pupa and adult. With respect to adult mosquitoes, we are taking into account only female mosquitoes, assuming that there are sufficient males to copulate with them. After this, we deal with the estimation of the entomological parameters regarding to larval and pupal stages: we describe the procedure adopted to treat appropriately the data from following up of whole aquatic phase.

2.1. Mathematical Model

In order to determine the risk of dengue due to the number of mosquitoes, we developed a deterministic model to describe the dynamics of mosquito population. The dynamics of vector population is based on the life cycle of the mosquito *A. aegypti*, which comprises three successive aquatic phases (egg, larva and pupa) and one adult form, each one allocated in a compartment. The number of eggs was determined by the oviposition rate $\varphi(M) = \phi M$, where ϕ is the per-capita oviposition rate ϕ and M is the number of female mosquitoes M. The effective larvae production rate was given by $\phi M(1 - L/C)$, where $(1 - L/C)$ was the available capacity of the recipients to receive larvae from hatched eggs, with C being the total (carrying) capacity of the recipients. The number of larvae at time t, L, increased when eggs were hatched (ϕ) and decreased according to change of larvae to pupae and death, described, respectively, by the changing π_l and the mortality μ_l rates. The number of pupae in time t, P, increased with change of larvae to pupae (π_l) and decreased according to transformation of

pupae to adult mosquitoes and death, described, respectively, by the emerging π_p and the mortality μ_p rates. Finally, the number of female mosquitoes at time t, M, increased according to the emerging of pupae (π_p) and decreased according to the mortality rate μ_f.

By joining the egg and larva phases, the model assumes that a fraction k of viable eggs is hatched to larvae without constraint (Regis et al., 2008), however larvae are constrained to the carrying capacity due to, for instance, limited amount of resources and competition among them. Another oversimplification is that a fraction f of hatching eggs will generate female mosquitoes, which comes from the assumption that there are sufficient male mosquitoes so that the probability of mating is one. Hence, the dynamics of the mosquito population can be described by the following ordinary differential equations

$$\begin{cases} \dfrac{d}{dt}L = kf\phi M \left(1 - \dfrac{L}{C}\right) - (\pi_l + \mu_l)L \\[2mm] \dfrac{d}{dt}P = \pi_l L - (\pi_p + \mu_p)P \\[2mm] \dfrac{d}{dt}M = \pi_p P - \mu_f M. \end{cases} \quad (1)$$

All the previously defined parameters of the model are temperature-depending, which in turn depends on time. We analyze the model assigning to the model's parameters averaged values with respect to calendar year. Considering constant values of the parameters, the system of Eqs. (1) has two equilibrium points. The trivial equilibrium point is given by $L^* = 0$, $P^* = 0$ and $M^* = 0$; and the non-trivial is

$$\begin{cases} L^* = C \left(1 - \dfrac{1}{Q_0}\right) \\[2mm] P^* = \dfrac{\pi_l}{\pi_p + \mu_p} C \left(1 - \dfrac{1}{Q_0}\right) \\[2mm] M^* = \dfrac{\pi_l}{\mu_f} \dfrac{\pi_p}{\pi_p + \mu_p} C \left(1 - \dfrac{1}{Q_0}\right) \end{cases} \quad (2)$$

where the basic offspring number Q_0 is given by

$$Q_0 = \frac{\pi_l}{\pi_l + \mu_l} \times \frac{\pi_p}{\pi_p + \mu_p} \times \frac{kf\phi}{\mu_f}, \quad (3)$$

with $0 \le f$, $f \le 1$. Following the procedure presented in Yang et al. (2009a), we can show that the absence of mosquito population is stable if $Q_0 \le 1$; otherwise, the presence of mosquito population in a community is stable.

The basic offspring number Q_0 is key parameter in population dynamics theory, and the mathematical expression depends on the details considered in the development of the model. The parameter Q_0 deserves biological interpretation. Note π_l^{-1} is the average period of time elapsed since the hatching of eggs (the probability of an egg hatching is k) until reaching the next pupal stage, and $(\pi_l + \mu_l)^{-1}$ is the average surviving period of time in the larval stage until entering in the next pupal stage, where the removals include the passage to the next pupal stage and mortality of larvae. Hence, $\pi_l/(\pi_l + \mu_l)$ is the probability that a larva survives during the larval stage, and enters to the pupal stage. Similarly, $\pi_p/(\pi_p + \mu_p)$ is the probability that a pupa survives during the pupal stage, and transforms to adult mosquito. With respect to the last term of the product, μ_f^{-1} is the average survival time of female mosquito and $f\phi$ is the fraction of eggs originated from an average per-capita oviposition rate ϕ that will become female mosquitoes; then $f\phi/\mu_f$ is the mean number of viable 'female' eggs produced by a female mosquito during entire life-span. Therefore, Q_0 is the number of eggs that will become female mosquitoes generated by a female mosquito that hatched from an egg and survived larval and pupal stages. Q_0 can also be interpreted roughly as the mean number of

viable female offsprings produced by one female mosquito during the entire survival time.

If the mean number of viable female offsprings produced by one female mosquito is lower than one ($Q_0 < 1$), the trivial equilibrium point is an attractor disregarding the initial conditions. To prove that absence of mosquitoes is globally stable for $Q_0 \leq 1$, let us define Lyapunov function $V : R_+^3 \to R$ as

$$V = \frac{\pi_l}{\pi_l + \mu_l} L + P + \frac{\pi_p + \mu_p}{\pi_p} M. \qquad (4)$$

The orbital derivative is

$$\dot{V} = -\frac{\pi_p + \mu_p}{\pi_p} \mu_f \left[\frac{Q_0 L}{C} + (1 - Q_0) \right] M, \qquad (5)$$

which is lower than or equal to zero for $Q_0 \leq 1$. By inspecting the system of Eqs. (1), it can be seen that the maximal invariant set contained in $\dot{V} = 0$ is the trivial equilibrium point. Then, from La-Salle Lyapunov Theorem (Hale, 1969), the mosquito free equilibrium point is globally asymptotically stable for $Q_0 \leq 1$.

The dengue transmission model takes into account the interaction between mosquitoes and humans. The density of mosquito population is strongly depending on temperature and humidity and changes in a short time interval, while human population is practically unchanged in the time scale of several generations of mosquitoes. Hence, the mosquito population is allowed to vary over time t, while the human population is assumed to be constant, with N being the size of the human population. The human population divided into four compartments according to the natural history of the disease: s, e, i and r, which are the fractions at time t of, respectively, susceptible, exposed, infectious and recovered individuals. Similarly, the adult female mosquito population is divided into three compartments: M_1, M_2 and M_3, which are the number at time t of, respectively, susceptible, exposed and infectious mosquitoes. However, we can introduce a simple way to allow variation in the human population changing the constant newborns rate μN by the total input Ω, which encompasses also the migration.

Dengue transmission is sustained as follows. The susceptible humans are infected during the blood meal by infectious

incubation period, and remain infective until death. The total size of the mosquito, which varies, is assigned by $M = M_1 + M_2 + M_3$.

Based on the above considerations, the dynamics of dengue transmission can be described by

$$\begin{cases} \frac{d}{dt} L \\ \frac{d}{dt} P = \pi_l L - (\pi_p + \mu_p) P \\ \frac{d}{dt} M_1 = \pi_p P - (\beta_m i + \mu_f) M_1 \\ \frac{d}{dt} M_2 = \beta_m i M_1 - (\gamma + \mu_f) M_2 \\ \frac{d}{dt} M_3 = \gamma M_2 - \mu_f M_3, \end{cases} \qquad (6)$$

in the mosquito population, which does not take into account the transovarian transmission, and by

$$\begin{cases} \frac{d}{dt} s = \mu - (\beta_h' M_3 + \mu) s \\ \frac{d}{dt} e = \beta_h' M_3 s - (\alpha + \mu) e \\ \frac{d}{dt} i = \alpha e - (\eta + \mu) i \\ \frac{d}{dt} r = \eta i - \mu r, \end{cases} \qquad (7)$$

in the human population, where $s + e + i + r = 1$. Notice that, due to a constant population N, we have $\beta_m = N \beta_m'$.

The system of Eqs. (6) and (7) has three equilibrium points: mosquito free community given by $L^* = P^* = M_1^* = M_2^* = M_3^* = 0$, $s^* = 1$ and $e^* = i^* = r^* = 0$; disease free community given by L^*, P^*, $M_1^* = M^*$, $M_2^* = M_3^* = 0$, $s^* = 1$ and $e^* = i^* = r^* = 0$, where the non-zero coordinates of the mosquito population at equilibrium are given by the Eq. (2); and the dengue settle at an endemic level given by L^*, P^*, M_1^*, M_2^*, M_3^*, s^*, e^*, i^* and r^*, with all coordinates assuming positive values: for mosquitoes population,

$$\begin{cases} L^* = C \left(1 - \frac{1}{Q_0} \right) \\ P^* = \frac{(\gamma + \mu_f) \mu_f^2 (\alpha + \mu)(\eta + \mu)(1 + (\beta_m / \mu_f) i^*)}{\beta_h' \beta_m \alpha \gamma \pi_p [1 - ((\alpha + \mu)(\eta + \mu)/\alpha\mu) i^*]} \\ M_1^* \frac{(\gamma + \mu_f) \mu_f (\alpha + \mu)(\eta + \mu)(1 + (\beta_m / \mu_f) i^*)}{\beta_h' \beta_m \alpha \gamma [1 - ((\alpha + \mu)(\eta + \mu)/\alpha\mu) i^*]} - \frac{(\gamma + \mu_f)(\alpha + \mu)(\eta + \mu) i^*}{\beta_h' \alpha \gamma [1 - ((\alpha + \mu)(\eta + \mu)/\alpha\mu) i^*]} \\ M_2^* \frac{\mu_f (\alpha + \mu)(\eta + \mu) i^*}{\beta_h' \alpha \gamma [1 - ((\alpha + \mu)(\eta + \mu)/\alpha\mu) i^*]} \\ M_3^* \frac{(\alpha + \mu)(\eta + \mu) i^*}{\beta_h' \alpha [1 - ((\alpha + \mu)(\eta + \mu)/\alpha\mu) i^*]}, \end{cases}$$

mosquitoes, with the per-capita transmission coefficient (or rate) being designed by β_h', which depends on the frequency of bites on humans by mosquitoes. The exposed individuals are, then, transferred to infectious class by the rate α, where α^{-1} is the average intrinsic incubation period. These infectious individuals are removed to recovered (immune) class after an average recovery period η^{-1}, where η is the recovery rate. Neither loss of immunity (we are restricted to only one serotype infection) nor induced mortality due to the disease is considered, and we assume a constant mortality rate μ among humans. With respect to the adult mosquito population, the susceptible mosquitoes are infected at a total rate β_m, which is the product between human population size N and per-capita rate β_m'. These exposed mosquitoes are transferred to infectious class at a rate γ, where γ^{-1} is the average extrinsic

where Q_0 is given by Eq. (3), and for human population,

$$\begin{cases} s^* = 1 - \frac{(\alpha + \mu)(\eta + \mu)}{\alpha\mu} i^* \\ e^* = \frac{\eta + \mu}{\alpha} i^* \\ r^* = \frac{\eta}{\mu} i^*, \end{cases}$$

with the infectious humans i^* being given by

$$i^* = \frac{R_0 - 1}{\beta_m / \mu_f + ((\alpha + \mu)(\eta + \mu)/\alpha\mu) R_0},$$

which is positive if $R_0 > 1$, where the basic reproduction number R_0, using $\beta_m = N\beta'_m$, is defined by

$$R_0 = \frac{\beta'_h \beta'_m}{\beta^{th}}, \tag{8}$$

with β^{th}, which is the threshold transmission coefficient regarded to the product of the per-capita dengue transmission coefficients β'_h and β'_m, being given by

$$\beta^{th} = \frac{(\pi_p + \mu_p)(\gamma + \mu_f)\mu_f^2(\alpha + \mu)(\eta + \mu)}{\gamma \alpha \pi_l \pi_p C(1 - (1/Q_0))N}. \tag{9}$$

By the fact that R_0 depends on the number Q_0, this can be seen as a risk factor of dengue outbreak. Following the procedure presented in Yang et al. (2009b), we can show that the equilibrium point regarding to mosquito free community is stable if $Q_0 < 1$; the disease free community is stable if $Q_0 > 1$ and $R_0 < 1$; and the dengue at an endemic level is stable if $Q_0 > 1$ and $R_0 > 1$.

Let us interpret R_0 rewriting it as

$$R_0 = \frac{\gamma}{\gamma + \mu_f} \times \frac{\beta'_h M^*}{\mu_f} \times \frac{\alpha}{\alpha + \mu} \times \frac{\beta'_m N}{\eta + \mu},$$

where $M^* = M_1^* + M_2^* + M_3^*$ is given by Eq. (2). This expression results from a model where an entire aquatic phase was split into two classes, resulting in a more reliable estimation than the simplified model (Leite et al., 2000). Let us suppose that one infectious mosquito is introduced in a completely susceptible populations of mosquitoes and humans, which have M^* and N, respectively, as the numbers that can potentially be infected. This unique infectious mosquito bites, in average, $\beta'_h M^*/\mu_f$ number of susceptible humans (β'_h/μ_f is the per-capita number of bites) during the infectious period. Afterwards, these exposed humans must survive the intrinsic incubation period, with probability $\alpha/(\alpha + \mu)$, and, then, are bitten, in average, by $\beta'_m N/(\eta + \mu)$ number of susceptible mosquitoes during the infectious period. Finally, the probability that these exposed mosquitoes survive during the extrinsic incubation period and become infectious mosquitoes is given by $\gamma/(\gamma + \mu_f)$. Therefore, R_0 is the average number of secondary infectious mosquitoes produced by one infectious mosquito introduced in completely homogeneous and susceptible populations of mosquitoes and humans. This number also measures both the severity of the infection in a community and the necessary efforts to eradicate the epidemics. The introduction of one infectious human follows similar interpretation.

Both Q_0 and R_0 differ from those presented in Yang et al. (2009a,b), but result in them by letting $\pi_l \to \infty$ or $\pi_p \to \infty$, and calling the remaining compartment as aquatic phase. Yang et al. (2009a,b) considered whole aquatic phase, and for this reason mortality and transition rates regarding to the aquatic phase were estimated. Now, we split aquatic phase in larval and pupal stages. From the same set of data considering whole aquatic phase (follow up from larva until emerging of adult mosquito), we can determine mortality and transition rates regarding to separated larval and pupal stages.

2.2. Estimation of the Entomological Parameters

First, we reproduce the description of the temperature-controlled experiments given in Yang et al. (2009a), and then we show how we must proceed to determine entomological parameters of larval and pupal stages.

Temperature-controlled experiments aim to assess the effects of temperature in the development and in the survival of immature (aquatic) forms and the survival and oviposition of adult A. aegypti mosquitoes. In each experiment, a fixed number of larvae or mosquitoes was set in a germination chamber where the temperature was regulated (the device did not allow the control of humidity). The strain of the A. aegypti used in the tests was that captured from the City of Marlia ($-22°12'50''$ latitude and $49°56'45''$ longitude, 350 km far from the City of São Paulo), situated at northwest of the São Paulo State, Brazil.

The condition with respect to the light inside the germination chamber was programmed to simulate the seasonal photoperiod that occurs in the nature. The period of one day was divided in 'day' (light turned on) and 'night' (light turned off), according to the photoperiod that occurs in the City of Marlia. Inside the germination chamber two temperatures were fixed, corresponding to light and dark periods. The weighted (with respect to periods of time when the light was turned on or turned off) mean temperature calculated from records on a week-period paper in a thermohigrograph settle inside the chamber was taken to be the temperature of the experiment.

With respect to the adult mosquitoes, the experiment consisted in following up mosquitoes, since they emerged from pupae, to estimate the mean survival time, from which we derive the mortality rate, as well as the number of eggs laid in each temperature. In a cage we put together newly emerged 100 female and 30 male mosquitoes, and also an amber glass containing a filter paper for egg-laying. Inside the cage, necessary food (water with honey) was supplied abundantly, and once a day they received blood meal from immobilized mouse in order to allow the development of the fertilized eggs. These eggs that were laid on the filter paper were counted everyday, and the filter paper was replaced. The numbers of surviving male and female mosquitoes were also recorded every day. This temperature-controlled experiment was aimed to evaluate the influence of temperature on longevity and on one aspect of fertility, through egg production. The experiment was carried on from 16 August 2002 to 12 December 2004.

With respect to the aquatic phase, we dealt with the survival time and the length of time they spent in the stages of larva and pupa on different temperatures. We selected 100 newly hatched eggs, in the first instar larvae, and they were placed in a basin with the availability of food (diet of fish) inside the germination chamber. When fourth instar larvae turned into pupae, they were transferred to individual hearing vials with caps and remained inside the chamber. The observation persisted until the emergence of adult mosquito. As the foregoing procedure, the numbers of dead immatures as well as the instar change to the next stage (and also to pupal stage) were recorded every day. However, to the experiment at the temperature of 10 °C, we proceeded as follows: we allowed the emerging of larvae at the stages L2, L3 and L4 and pupae in a favorable temperature (250 °C), and then we exposed them at 10 °C. This temperature-controlled experiment was carried on from 21 January 2004 to 25 July 2005.

The experiments in the germination chamber aimed to measure the effects of temperature in the development and survival in the aquatic phase of mosquito's life cycle. This is the reason why we started the follow up with first larval stage and not with eggs because, beyond the temperature, the hatchability of eggs are influenced by many factors as bad embryonal development and desiccation. In a naive experiment (eggs were allowed to hatch to larvae in constant temperature), we obtained the following average eclosion rates (in percentage) 0, 94.1, 84.7 and 69.4, respectively for 15, 20, 25 and 30 °C. In a more refined experiment, Farnesi et al. (2009) obtained higher eclosion rates.

The data collected from temperature-controlled germination chamber were fitted taking into account the probability of maintaining the original state and the probability density to occur the change of state. These functions were parametrized in terms of the parameters τ and β, which are, respectively, the half time and heterogeneity degree. The half time is the observation time at which

the probability of the original state being maintained is 1/2, and the heterogeneity degree measures how the change of state occurs broadly scattered in the observation times. The probability distributions are described in Yang et al. (2003). Briefly, the probability of maintaining the original state at the observation time a is given by

$$S(a) = \frac{1}{1 + ((e^{\beta a} - 1)/(e^{\beta \tau} - 1))}, \tag{10}$$

where at $a = 0$ the follow up is initiated. The probability density to occur the change of state at the observation time a is

$$s(a) = \frac{\beta e^{\beta a}}{e^{\beta \tau} - 1(1 + ((e^{\beta a} - 1)/(e^{\beta \tau} - 1)))^2}, \tag{11}$$

from $s(a) = -dS(a)/da$. The parameters τ and β are fitted by the nonlinear likelihood estimation method based on the set of n recorded data $\Omega = \{(a_i, \omega_i)\}$, with $i = 1, 2, \cdots, n$, where the index i stands for the observation of ith individual and ω_i is the outcome at the observation time a_i (see Yang et al., 2009a).

With respect to the adult mosquitoes, censored individuals are those lost at each day's counting. Also the remaining mosquitoes at the last day of observation (ending of experiment) are considered censored. The figure is completely different with respect to the aquatic forms, because larvae and pupae do not escape as well as they ultimately changes to next stage. The censoring is, then, defined according to the phenomenon: in the survival study, the number of larvae emerging as adult mosquitoes is considered censored; while in the transition study, the number of died larvae and pupae is taken as censored. The observation time can be understood as the age of the mosquito or, in the case of the aquatic phase, the age of the immature form.

From the estimated parameters τ and β, we are able to calculate the average period of time in keeping the original state η or average period of time elapsed before changing to next stage ξ (both are generically represented by ψ) according to

$$\psi = \frac{\int_0^\infty as(a)da}{\int_0^\infty s(a)da} = \int_0^\infty as(a)da, \tag{12}$$

because $\int_0^\infty s(a)da = 1$. The parameters η and ξ are called as, respectively, the survival time and the transition time (time spent in a specified stage). The corresponding error (Frank, 1978) is calculated from

$$\sigma_\psi \approx \left(\frac{\partial}{\partial \tau}\psi\right)\sigma_\tau + \left(\frac{\partial}{\partial \beta}\psi\right)\sigma_\beta = \left(\int_0^\infty a\frac{\partial}{\partial \tau}s(a)da\right)\sigma_\tau$$

$$+ \left(\int_0^\infty a\frac{\partial}{\partial \beta}s(a)da\right)\sigma_\beta. \tag{13}$$

The mortality rate μ or the transition rate π (both are generically represented by ρ), regarded to, respectively, survival time and transition time, can be roughly approximated by

$$\rho = \frac{1}{\psi}, \tag{14}$$

and the corresponding error as

$$\sigma_\rho = \frac{1}{\psi^2}\sigma_\psi = \rho^2\sigma_\psi. \tag{15}$$

Now we introduce a simple method to determine the survival and transition rates regarding larval and pupal stages. As we have pointed out above, in the follow up of entire aquatic phase, the mortality of larvae and pupae were registered, together with the transformation of larvae to pupae. Hence, we can estimate, collecting data regarded only to the larval stage, the survival and transition

rates of larvae. From the estimated values of whole aquatic phase and larval stage, we can determine the survival and transition times of pupal stage as

$$\begin{cases} \eta_p = \eta_q - \xi_l \\ \xi_p = \xi_q - \xi_l, \end{cases} \tag{16}$$

where the subscripts q, p and l stand for, respectively, whole aquatic phase, pupal stage and larval stage. The survival and transition times in pupal stage comprise the mean time they spent in larval stage, which is the reason to subtract this period of time. The corresponding mortality and transition rates are calculated by Eq. (14).

To adjust temperature-depending entomological parameters we choose polynomial function of degree m,

$$P_m(T) = \sum_{i=0}^m b_i T^i, \tag{17}$$

where T is temperature in $^\circ$C and the coefficients b_i, with $i = 0, 1, 2, \ldots, m$, are fitted by the linear least square estimation method (Yang et al., 2009a).

Finally, we established the suitability of the estimation methodology presented here, especially when the transitions occur quickly. All the followed up data were recorded every 24 h. In order to show the advantage of parametrized estimation procedure to determine the transition from one stage to other, we performed a follow up experiment recording the observations every 8 h. Then, we grouped three sequential observations and treated them as observations every 24 h, and compare the transition rate with that obtained from observations every 8 h, which is taken as gold standard. To enhance the practicality (in reducing observation times and efforts) of this method to estimate followed up data, we choose elevated temperatures (the transition process occurs quickly at high temperature) to maximize the difference between estimated transition rates.

3. Results

In this section we present the adjusted entomological parameters and how they are manipulated simultaneously by mathematical modelling.

3.1. Adjusted Entomological Parameters

We present the feasibility of estimating the entomological parameters regarding larval and pupal stages from a unique experiment involving whole aquatic phase. To estimate the survival and transition times with respect to pupal stage, we must obtain large number of pupae being transformed from larval stage simultaneously. This procedure is similar to that employed to recruit the number of adult mosquitoes or number of larvae from eggs to be followed up. We deliberately did not concern with pupal stage in order to enhance the usefulness of the methodology we are proposing: to describe phenomena in which development and maturation depend on the time elapsed since the 'birth'. In the mosquito's life cycle, pupal stage is preceded by larval stage before emerging in adult form. If we estimate survival and transition times regarded to larval stage and whole aquatic phase, then they can be handled to yield survival and transition times concerning to pupal stage according to Eq. (16).

The estimated temperature depending entomological parameters as polynomial given by (17) regarding to adult mosquito, oviposition rate and whole aquatic phase were presented in Yang et al. (2009a). In Table 1 we present the estimated coefficients of the polynomials, recalling that the subscript q stands for the whole aquatic phase.

Table 1

The entomological parameters regarding female mosquito and whole aquatic phase were estimated using an nth degree polynomial $P(T) = b_0 + b_1 T + b_2 T^2 + \cdots + b_n T^n$ (from Yang et al., 2009a, except π_q, which is a new fitting using an arbitrary error 0.01 for transitions that are not occurred). Unit of b_i is days^{-1} ($^\circ$C)$^{-i}$ and standard deviations are shown between parentheses.

Parameter	b_0	b_1	b_2	b_3	b_4
ϕ	-5.400	1.800	-2.124×10^{-1}	1.015×10^{-2}	-1.515×10^{-4}
μ_f	8.692×10^{-1}	-1.590×10^{-1}	1.116×10^{-2}	-3.408×10^{-4}	3.809×10^{-6}
μ_q	2.130	-3.797×10^{-1}	2.457×10^{-2}	-6.778×10^{-4}	6.794×10^{-6}
π_q	1.310×10^{-1}	-5.723×10^{-1}	1.164×10^{-2}	-1.341×10^{-3}	8.723×10^{-5}

In this paper we supply the extended mathematical model entomological parameters regarding to larval and pupal stages. First we fit the entomological parameters regarded to larval stage, π_l and μ_l, by selecting data corresponding to larval stage from the experiment involving entire aquatic phase. With this subset of data, we fit the parameters τ and β as follows.

The survival study is determined by the number of deaths occurring during the follow up in the larval stage. The aquatic phase of mosquito comprises larval and pupal stages, by which the hatched eggs must be passed through in order to emerge in adult form. In Table 2 we present the parameters τ and β fitted against the followed up data corresponding to survival of larval stage.

At very low (10 $^\circ$C) and high (40 $^\circ$C) temperatures all larvae died reproducing the observations in Vector Topics (1980). At low temperatures larvae survived more time than at high temperature, but they behaved more heterogeneously. Let us explain the fitting where $\tau < \sigma_\tau^2$ or $\beta < \sigma_\beta^2$, which occurred at 19.04 and 19.18 $^\circ$C in Table 7 and 18.86, 19.04, 26.84, 26.85, 31.61 and 36.55 $^\circ$C in Table 8. In the survival of larval stage, at 19.04 $^\circ$C almost all of 14 larvae died in the beginning of the experiment and at 19.18 $^\circ$C the unique death of larva occurred in the last day of observation. In the survival of aquatic phase, the deaths at 18.86 (two) and 36.55 $^\circ$C (four) occurred exactly in the, respectively, 15th and 8th days of observation; at 19.04, 26.84 and 31.61 $^\circ$C all deaths occurred before the occurrence of the first transition to pupal stage and at 26.84 $^\circ$C the unique death occurred in the last day of observation. However, the two deaths occurring at 36.41 $^\circ$C illustrate the case $\tau > \sigma_\tau^2$ or

$\beta > \sigma_\beta^2$: they occurred at two different days, 10th and 14th day of observation.

The average survival time η and its error σ_η are calculated using Eqs. (12) and (13) with τ and β given in Table 2. From these values we calculate the mortality rate of larval stage μ and its error σ_π using Eqs. (14) and (15). Table 3 presents the average survival time and the mortality rate of larval stage.

In the aquatic phase, it is not enough to survive larval and pupal stages. Each aquatic form must successfully be transformed in sequence (egg–larva–pupa) to emerge as an adult form in order to perpetuate its species. In Table 4 we present the parameters τ and β fitted against the followed up data corresponding to transitions from larval stage to pupal stage.

The larvae and pupae behaved heterogeneously at low temperature, and at 14.92 $^\circ$C we observed the most heterogenous behavior. In temperatures higher than 20 $^\circ$C the behavior was more homogeneous, especially between 26 and 35 $^\circ$C. The heterogeneous behavior outside the optimal temperature seems to be an attempt to face adverse environment which results in high mortality rate.

The average transition time ξ and its error σ_ξ are calculated using Eqs. (12) and (13) with τ and β given in Table 4. From these values we calculate the transition rate π and its error σ_π using Eqs. (14) and (15). Table 5 presents the average transition time and the transition rate from larval stage to pupal stage.

Finally, we derive the entomological parameters taking into account the previously estimated values using Eqs. (16) and (14) with corresponding errors. This indirect method allows the estimation of parameters in larval and pupal stages from a unique follow up from the hatching of eggs until emerging of mosquitoes.

Table 2

The estimations of the parameters half age τ (and variance σ_τ^t) and heterogeneity β (and variance σ_β^2) with respect to survival of larva stage are shown. The estimated parameters measure the period of time from hatching of eggs until transformation to pupa. All larvae survived at temperatures 18.86, 26.84, 26.85, 31.61, 36.47 and 36.55 $^\circ$C.

Parameter	τ (days)	σ_τ^2 (days^2)	β (days^{-1})	σ_β^2 (days^{-2})
10.00	6.02407	0.46797	0.55800	0.05616
10.00	8.41771	1.54342	0.14786	0.02437
10.00	–	–	–	–
10.00	4.48730	0.12298	1.42136	0.11437
10.38	2.09152	0.10056	1.75153	0.19013
10.45	5.71771	0.15105	1.05837	0.11924
10.45	4.75667	0.33633	0.82575	0.09452
14.74	47.3410	2.96399	0.08024	0.01627
14.84	45.1516	3.67524	0.11696	0.02241
14.92	71.6009	7.73810	0.03911	0.00937
18.86	–	–	–	–
19.04	36.1113	676.743	0.03618	0.00472
19.18	37.0003	$>10^5$	1.05579	$>10^5$
26.56	10.8940	0.43309	3.03758	2.51025
26.84	–	–	–	–
26.85	–	–	–	–
30.83	7.51052	1.53369	1.41566	1.01480
31.61	–	–	–	–
34.95	8.63987	2.06229	0.92946	0.67130
36.47	–	–	–	–
36.55	–	–	–	–
39.95	3.12507	0.07151	2.74786	0.19295
40.16	3.75184	0.08723	2.08406	0.087232
40.64	3.46030	0.16423	1.14837	0.11239

Table 3

The calculations of the average survival time η (and error σ_η) and the mortality rate μ (and error σ_μ) with respect to larva stage are shown. All larvae survived at temperatures 18.86, 26.84, 26.85, 31.61, 36.47 and 36.55 $^\circ$C.

T ($^\circ$C)	η (days)	σ_η (days)	μ (days^{-1})	σ_μ (days^{-1})
10.00	6.18297	0.49208	0.16173	0.1617
10.00	10.2783	1.83554	0.09729	0.27831.8
10.00	–	–	–	–
10.00	4.49375	0.12529	0.22253	0.00620
10.38	2.13283	0.11131	0.46886	0.02447
10.45	5.72900	0.15708	0.17455	0.00479
10.45	4.82959	0.35350	0.20706	0.01516
14.74	48.1622	3.43440	0.02076	0.00148
14.84	45.3399	3.79649	0.02206	0.00185
14.92	74.8001	9.14403	0.01337	0.00163
18.86	–	–	–	–
19.04	43.5404	43.8765	0.02297	0.02314
19.18	37.0100	$>10^5$	0.02702	$>10^5$
26.56	10.8940	0.43309	0.09179	0.00365
26.84	–	–	–	–
26.85	–	–	–	–
30.83	7.51068	1.53461	0.13314	0.02720
31.61	–	–	–	–
34.95	8.64234	2.07228	0.11571	0.02775
36.47	–	–	–	–
36.55	–	–	–	–
39.95	3.12558	0.07173	0.31994	0.00734
40.16	3.75316	0.08768	0.26644	0.00623
40.64	3.51105	0.17638	0.28482	0.01431

Table 4
The estimations of the parameters half age τ (and variance σ_τ^2) and heterogeneity β (and variance σ_β^2) with respect to transition of larva stage are shown. The estimated parameters measure the period of time from hatching of eggs until transformation to pupae. All followed up larvae died for $T < 10.45\,^\circ$C and $T > 39.95\,^\circ$C.

$T\,(^\circ$C)	τ (days)	σ_τ^2 (days^2)	β (days^{-1})	σ_β^2 (days^{-2})
10.00	–	–	–	–
10.00	–	–	–	–
10.00	–	–	–	–
10.00	–	–	–	–
10.38	–	–	–	–
10.45	–	–	–	–
10.45	–	–	–	–
14.74	56.0539	2.24179	0.10629	0.02431
14.84	32.4369	0.96884	0.23552	0.02835
14.92	55.1385	3.52788	0.06520	0.00890
18.86	10.3280	0.17538	1.25465	0.06932
19.04	11.9910	0.10279	1.75584	0.18720
19.18	12.4410	0.25012	0.80121	0.03862
26.56	7.83359	0.09920	1.83344	0.18097
26.84	6.85701	0.06939	2.67580	0.19205
26.85	6.08382	0.06355	3.91212	0.24670
30.83	4.86750	0.06065	3.24865	0.25738
31.61	5.85002	0.08186	2.09756	0.21559
34.95	3.91510	0.06783	3.16557	0.12797
36.47	6.66252	0.20089	1.29339	0.13883
36.55	4.80303	0.22171	1.28927	0.18830
39.95	–	–	–	–
40.16	–	–	–	–
40.64	–	–	–	–

Table 6
The derivations of the average survival time η (and error σ_η) and the mortality rate μ (and error σ_μ) with respect to pupa stage are shown. All larvae survived at temperatures 18.86, 26.84, 26.85, 31.61, 36.47 and 36.55 $^\circ$C.

$T\,(^\circ$C)	η (days)	σ_η (days)	μ (days^{-1})	σ_μ (days^{-1})
10.00	–	–	–	–
10.00	–	–	–	–
10.00	–	–	–	–
10.00	–	–	–	–
10.38	–	–	–	–
10.45	–	–	–	–
10.45	–	–	–	–
14.74	−8.2529	5.30102	−0.1212	0.07783
14.84	10.2122	3.07363	0.09792	0.02947
14.92	−1.0085	7.44476	−0.9916	7.31979
18.86	16.9053	11.2444	0.05915	0.03935
19.04	36.4790	33.4569	0.02741	0.02514
19.18	17.7674	4.28964	0.05628	0.01359
26.56	6.91721	3.11579	0.14457	0.06512
26.84	8.98919	38.4055	0.11124	0.47528
26.85	9.57738	11.9611	0.10441	0.13040
30.83	4.65792	1.33522	0.21469	0.06154
31.61	10.9952	162.716	0.09095	1.34594
34.95	4.73055	1.81534	0.21139	0.08112
36.47	7.49772	1.86159	0.13337	0.03312
36.55	5.19600	1.62414	0.19246	0.06016
39.95	–	–	–	–
40.16	–	–	–	–
40.64	–	–	–	–

Table 6 presents the average survival time and the mortality rate of pupal stage using Eqs. (16) and (14) with corresponding errors. The values of η_q are those given in Table 8 from Yang et al. (2009a).

The negative values at 14.74 and 14.92 $^\circ$C are due to smaller values of σ_β^2. In both temperatures several larvae died near the last observation day. The reason is that at low temperatures, larvae spend large period of time in the larval stage and die without being transformed to pupae in the ending of experiment. Contrarily, the other smaller value of σ_β^2 at 19.04 $^\circ$C did not present larvae dying near last observation days. The negative mortality rates are changed to zero. Hence, the indirect yielding of survival time in the pupal stage does not furnish good estimation at low temperatures.

Table 7 presents the average transition time and the transition rate from pupal stage to emerging of mosquitoes using Eqs. (16) and (14) with corresponding errors. The values of ξ_q are those given in Table 8 from Yang et al. (2009a).

In contrast to the survival time, transition time of pupal stage is estimated with good accuracy in all temperatures. The reason is that the natural flow in the life cycle of mosquito is the emerging from pupae, which in turn come out from larvae. Due to the fact that we are dealing with non-ideal environment, deaths among larvae and pupae occur during the progression along the aquatic phase.

Let us compare the mortality and transition rates associated to larval and pupal stages. We observe that pupae are under slightly

Table 5
The calculations of the average transition time ξ (and error σ_ξ) and the transition rate π (and error σ_π) with respect to larva stage are shown. All followed up larvae died for $T < 10.45\,^\circ$C and $T > 39.95\,^\circ$C.

$T\,(^\circ$C)	ξ (days)	σ_ξ (days)	π (days^{-1})	σ_π (days^{-1})
10.00	–	–	–	–
10.00	–	–	–	–
10.00	–	–	–	–
10.00	–	–	–	–
10.38	–	–	–	–
10.45	–	–	–	–
10.45	–	–	–	–
14.74	56.1754	2.37911	0.01780	0.00075
14.84	32.4505	0.97845	0.03082	0.00093
14.92	56.3015	3.89307	0.01776	0.00123
18.86	10.3281	0.17539	0.09682	0.00164
19.04	11.9910	0.10279	0.08340	0.00072
19.18	12.4415	0.25025	0.08038	0.00162
26.56	7.83359	0.09920	0.12766	0.00162
26.84	6.85701	0.06939	0.14584	0.1458
26.85	6.08382	0.06355	0.16437	0.00172
30.83	4.86750	0.06065	0.20544	0.00256
31.61	5.85004	0.08189	0.17094	0.00239
34.95	3.91511	0.06784	0.25542	0.00443
36.47	6.66358	0.20162	0.15007	0.00454
36.55	4.81130	0.22707	0.20784	0.00981
39.95	–	–	–	–
40.16	–	–	–	–
40.64	–	–	–	–

Table 7
The derivations of the average transition time ξ (and error σ_ξ) and the transition rate π (and error σ_π) with respect to pupa stage are shown. All followed up larvae died for $T < 10.45\,^\circ$C and $T > 39.95\,^\circ$C.

$T\,(^\circ$C)	ξ (days)	σ_ξ (days)	π (days^{-1})	σ_π (days^{-2})
10.00	–	–	–	–
10.00	–	–	–	–
10.00	–	–	–	–
10.00	–	–	–	–
10.38	–	–	–	–
10.45	–	–	–	–
10.45	–	–	–	–
14.74	5.56510	4.38033	0.17969	0.14144
14.84	7.25580	1.80948	0.13782	0.03437
14.92	28.0859	10.3487	0.03561	0.01312
18.86	4.71540	0.32926	0.21207	0.01481
19.04	4.05790	0.21701	0.24643	0.01318
19.18	4.04970	0.50020	0.24693	0.03050
26.56	2.17841	0.20630	0.45905	0.04347
26.84	2.36875	0.15266	0.42216	0.02721
26.85	2.26668	0.15182	0.44117	0.02955
30.83	1.44307	0.17660	0.69297	0.08481
31.61	1.54566	0.18016	0.64697	0.07541
34.95	1.20538	0.14777	0.82961	0.10170
36.47	1.45127	0.41769	0.68905	0.19831
36.55	1.45948	0.43351	0.68518	0.20352
39.95	–	–	–	–
40.16	–	–	–	–
40.64	–	–	–	–

Table 8
The estimated and calculated parameters for the transition from larva to pupa and from larva to adult mosquito, using followed up data recorded every 8 and 24 h. The units are: days (for τ, σ_τ, ξ and σ_ξ) and days^{-1} (for β, σ_β, π and σ_π).

From larva	(30 °C) to pupa		(30 °C) to adult		(35 °C) to pupa		(35 °C) to adult	
	8 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h
β	4.2055	3.2487	4.0156	2.10	2.0197	2.1017	1.8883	1.7472
σ_β	0.3957	0.2574	0.3498	0.212	0.2137	0.2154	0.2129	0.1921
τ	4.4353	4.8675	6.0805	6.3106	5.4886	5.8610	7.1085	7.4117
σ_τ	0.0461	0.0607	0.0482	0.1160	0.0851	0.0823	0.0908	0.0982
ξ	4.4353	4.8675	6.0805	6.3106	5.4887	57.4117	7.1085	7.4117
σ_ξ	0.0461	0.0607	0.0482	0.1160	09080	0.0822	0.0908	0.0981
π	0.2255	0.2054	0.1645	0.1585	0.1822	0.1706	0.1407	0.1349
σ_π	0.0023	0.0026	0.0013	0.0029	0.0018	0.0024	0.0018	0.0018
$\frac{\pi_8 - \pi_{24}}{\pi_8}$	0.0891		0.0365		0.0637		0.0412	

high mortality than larvae. This finding is not expected, because larva is more fragile than pupa. The simple explanation is that the survival of aquatic phase comprised in counting died larvae and pupae. However, we observe that larval stage is at least 3-fold longer than pupal stage, an expected result.

We have estimated all parameters from data collected every 1 day. To address the question about the accuracy of these estimations, we compare the transition rate with that resulted from observations every 8 h, which is taken as the gold standard. In Table 8 we show the estimated entomological parameters regarding to the transitions from larva to pupa and from larva to adult mosquito for 30 °C and 35 °C, because at higher temperatures, the transitions between successive stages occur quickly. Being, therefore, the period of time spending at each stage short, the comparison between the estimated transition rates obtained from 8 and 24 h of observations will enhance different outcomes. We observe that the difference of transition rates between successive observations elapsing 8 h and 24 h are at most 9%, which decreases when the transition time increases. Even sparse observations do not deviate so much from 'gold standard value' when the parametrized survival function (10) is considered.

Let us now adjust the rates of mortality and transition using the polynomial (17). Based on Tables 3 and 6, we fit the mortality rate as a function of temperature of larval (Fig. 1a) and pupal (Fig. 1b) stages. For $T > 40.38$ °C, the negative values in the larvae mortality rate must be changed to zero, and for $T > 39.95$ °C, the negative values in the pupae mortality rate must be changed to zero.

Based on Tables 5 and 7, we fit the transition rates as a function of temperature of larval (Fig. 2a) and pupal (Fig. 2b) stages. We let $\pi = 0$ and $\sigma_\pi = 10^{-2}$ in the temperatures (10 and 40 °C) at which larvae really do not develop to pupae.

Hence, we have temperature depending entomological parameters ϕ and μ_f (Table 1), and π_l, μ_l, π_p and μ_l. Thu et al. (1998) estimated development of mosquito stages at different temperatures, and their findings are comprised in the estimated values given in Tables 5 and 7. The survival of adult mosquitoes found by Thu et al. (1998) corroborates those estimated in Yang et al. (2009a): the adult mosquitoes survived long period of time at intermediate temperatures, and at very high temperature, the mortality rate is very high. Next we assess how these parameters influence the mosquitoes population and dengue risk.

3.2. Epidemiology Based on Mathematical Model

In foregoing section, we applied population dynamics theory to evaluate the number of mosquito population based on the entomological parameters. The key parameters to determine the size of mosquito population and the dengue risk are the basic offspring number Q_0 and the basic reproduction number R_0.

In Fig. 3 we present curves of the basic offspring number Q_0 for two models, using Eq. (3). The estimation of Q_0 differs according to
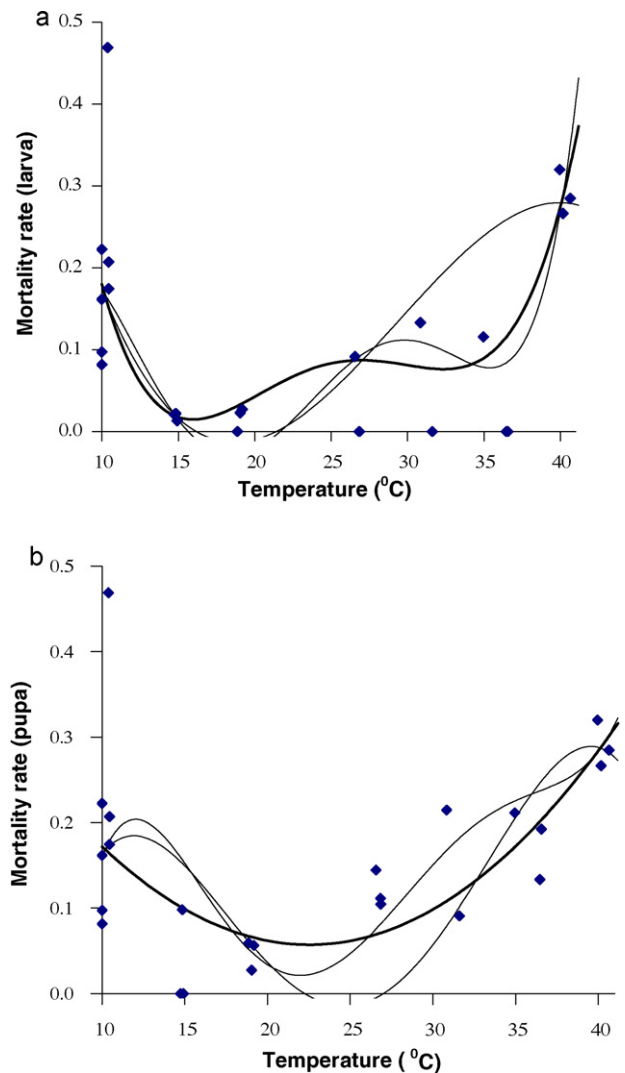

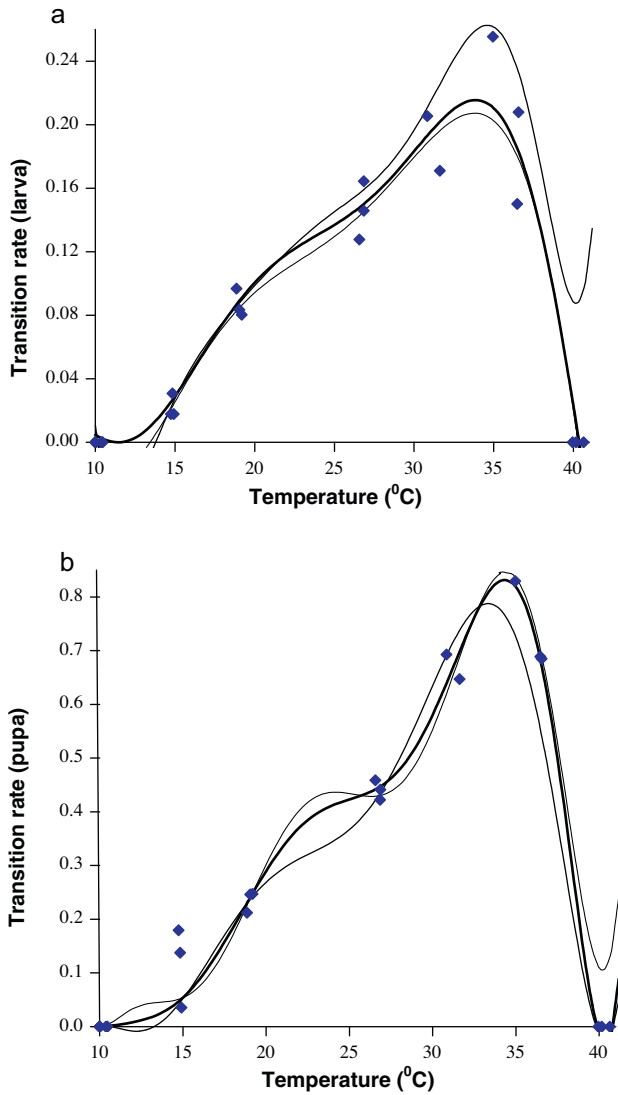**Fig. 1.** The fitting of larvae and pupae mortality rates as a function of temperature. We show the estimated coefficients b_i (days$^{-1} \times [°C]^{-i}$) and errors (between parentheses). Larvae fittings are (a): $b_0 = 2.315$ (1.143×10^{-1}), $b_1 = -4.191 \times 10^{-1}$ (2.547×10^{-2}), $b_2 = 2.735 \times 10^{-2}$ (1.970×10^{-3}), $b_3 = -7.538 \times 10^{-4}$ (6.194×10^{-5}) and $b_4 = 7.503 \times 10^{-6}$ (6.704×10^{-7}) (thick curve). Fitting for third (thinner) and fifth (thin) degree polynomials and calculated values ($\blacklozenge$) are also shown. Pupae fittings are (b): $b_0 = 4.256 \times 10^{-1}$ (1.015×10^{-1}), $b_1 = -3.248 \times 10^{-2}$ (1.720×10^{-2}), $b_2 = 7.060 \times 10^{-4}$ (8.398×10^{-4}) and $b_3 = 4.395 \times 10^{-7}$ (1.188×10^{-5}) (thick curve). Fitting for fourth (thinner) and fifth (thin) degree polynomials and calculated values ($\blacklozenge$) are also shown.

Fig. 2. The fitting of larvae and pupae transition rates as a function of temperature. We show the estimated coefficients b_i (days$^{-1} \times [°C]^{-i}$) and errors (between parentheses). Larvae fittings are (a): $b_0 = -1.847$ (3.823), $b_1 = 8.291 \times 10^{-1}$ (1.300), $b_2 = -1.457 \times 10^{-1}$ (1.814×10^{-1}), $b_3 = 1.304 \times 10^{-2}$ (1.349×10^{-2}), $b_4 = -6.461 \times 10^{-4}$ (5.804×10^{-4}), $b_5 = 1.796 \times 10^{-5}$ (1.448×10^{-5}), $b_6 = -2.617 \times 10^{-7}$ (1.946×10^{-7}) and $b_7 = 1.551 \times 10^{-9}$ (1.090×10^{-9}) (thick curve). Fitting for sixth (thinner) and eighth (thin) degree polynomial and calculated values (♦) are also shown. Pupae fittings are (b): $b_0 = 21.902$ (28.146), $b_1 = -10.311$ (11.995), $b_2 = 2.051$ (2.153), $b_3 = -2.242 \times 10^{-1}$ (2.122×10^{-1}), $b_4 = 1.469 \times 10^{-2}$ (1.256×10^{-2}), $b_5 = -5.887 \times 10^{-4}$ (4.576×10^{-4}), $b_6 = 1.411 \times 10^{-5}$ (1.003×10^{-5}), $b_7 = -1.852 \times 10^{-7}$ (1.213×10^{-7}), and $b_8 = 1.022 \times 10^{-9}$ (6.206×10^{-10}) (thick curve). Fitting for seventh (thinner) and ninth (thin) degree polynomials and calculated values (♦) are also shown.

model specification. In general, mathematical model that takes into account more details regarded to biological phenomenon furnishes more reliable information and avoids catastrophic forecasting. As we have shown in foregoing section, the mosquito population cannot be maintained in a region if $Q_0 \leq 1$. From Fig. 3, we have $Q_0 \leq 1$ (we do not have dengue transmission because the region is free of mosquito) if $T \leq 14.66\,°C$ and $T \geq 36.94\,°C$ for one aquatic phase; and $T \leq 15.41\,°C$ and $T \geq 36.86$ for model considering larval and pupal stages.

The model structured with one more compartment shows that the curve of Q_0 situates always below and inside with respect to the curve provided by the simplest model. Additionally, the interval of temperature at which mosquito population can invade and colonize new areas is slightly diminished in more detailed model. In
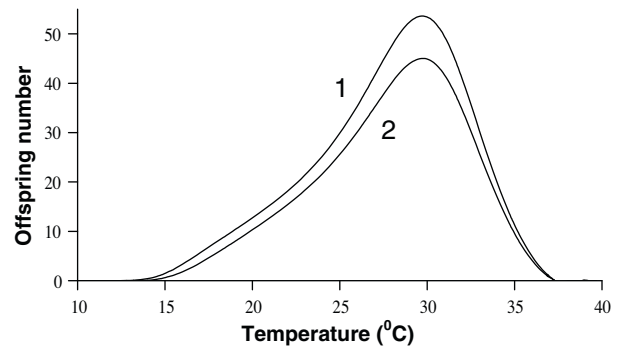

Fig. 3. The basic offspring number Q_0 as a function of temperature is shown, with $kf = 0.25$. The modelling assumes one compartment comprising larva and pupa stages (1) and larva and pupa stages composing two distinct compartments (2).

another words, more detailed model seems to predict less capacity of mosquito's invasion and infestation than the simplified version of the model.

When $Q_0 > 1$, the size of the mosquito population M^*, from the third equation of (2), increases proportionally with the carrying capacity C. In Fig. 4 we show the number of mosquito population M^*, using arbitrarily $C = 1$ (for instance, if C is increased 10 times, the vertical axis, the number of mosquitoes, must be multiplied by this factor). Note that M^* is not proportional to Q_0, because entomological parameters affecting Q_0 affects M^*. For instance, for temperatures near lower and upper bounds where we have Q_0 near one, simplified version of the model predicts slightly higher number of mosquitoes, but for intermediate temperatures, detailed model shows higher number of mosquitoes, even that the basic offspring number is low.

Q_0 measures the reproductive capacity of female mosquitoes, for this reason the number of larvae L^* increases depending only on Q_0. These larvae must survive and become pupae, which in turn must survive and emerge as adult mosquitoes. Hence M^* depends not only on Q_0, but also on the capacity of becoming pupae, on the probability of surviving pupal stage and on the surviving adult phase. These combinations resulted in lower number of female mosquitoes even when the capacity of generating descendants
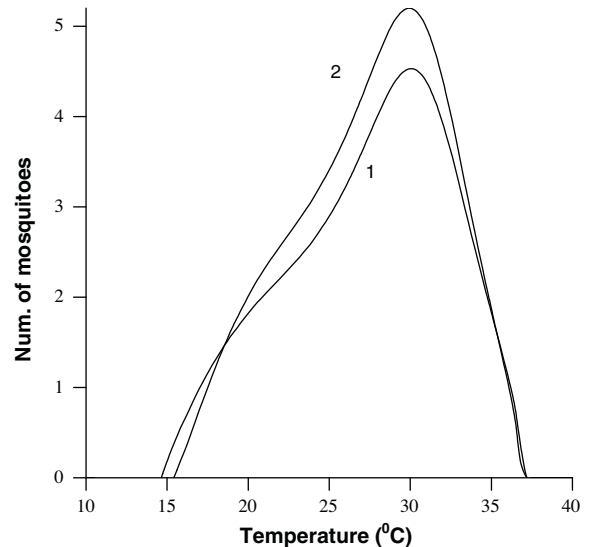

Fig. 4. The number of mosquitoes M^* as a function of temperature is shown, with $kf = 0.25$ and $C = 1$. The modelling assumes one compartment comprising larva and pupa stages (1) and larva and pupa stages composing two distinct compartments (2).
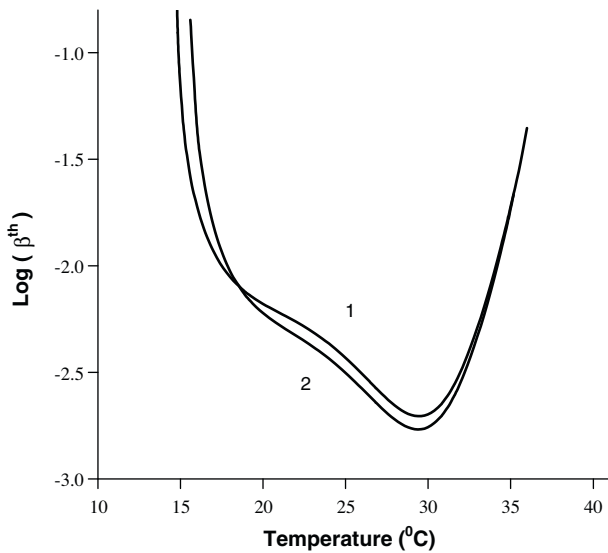
Fig. 5. The threshold transmission coefficients β^{th} as a function of temperature are shown. The modelling assumes one compartment comprising.

is higher. However, the capacity of invading and colonizing new regions is depending only on Q_0 (Maidana and Yang, 2008).

In Figs. 3 and 4, we let kf = 0.25, based on the following reasons. Assuming that half of eggs will produce female mosquitoes, then f = 0.5, which must not change with temperature. However, the eclosion of eggs and the time of hatching of first larvae since the completion of embryogenesis depends on temperature must vary with temperature (Farnesi et al., 2009). In the calculations we assumed k = 0.5 for all range of temperatures, which corresponds to the eclosion rate at 35 °C, the lowest rate obtained by Farnesi et al. (2009). We stress the fact that the rate of embryogenic completion and the eclosion rate can be incorporated in the Eq. (3) by introducing one more compartment (eggs) in the model. The introduction of eggs compartment changes the basic offspring number Q_0 and the number of mosquitoes M^* according to

$$\begin{cases} Q_0 = \dfrac{\pi_e}{\pi_e + \mu_e} \times \dfrac{\pi_l}{\pi_l + \mu_l} \times \dfrac{\pi_p}{\pi_p + \mu_p} \times \dfrac{f\phi}{\mu_f} \\ M^* = \dfrac{\pi_e}{\mu_f} \dfrac{\pi_l}{\pi_l + \mu_l} \dfrac{\pi_p}{\pi_p + \mu_p} C \left(1 - \dfrac{1}{Q_0}\right), \end{cases}$$

where π_e is the rate of embryogenic completion and eclosion of eggs in order to become larvae, and μ_e is the mortality rate of eggs. In the interpretation of Q_0, $\pi_e/(\pi_e + \mu_e)$ is the probability that an egg survives egg phase, and hatch to larvae in aquatic environment. Notice that this probability replaces the fraction of viable eggs k in Eq. (3).

As we have pointed out in foregoing section, the intrinsic and extrinsic incubation periods, α^{-1} and γ^{-1}, are assumed to be constant with temperature (the extrinsic incubation period is strongly dependent on temperature (Lindsay and Birley, 1996; Thu et al., 1998), but we restrict the analysis taking into account only the variations provided by the estimated entomological parameters). Therefore, the risk of dengue outbreak is assessed taking into account only the variation of the entomological parameters with temperature. In Fig. 5 we present threshold transmission coefficient β^{th} as a function of temperature. The curve corresponding to the threshold transmission coefficient β^{th} was obtained using NC = 1; however, if we increase 10 times this value, the curve displaces down one unity (we are using log-scale), decreasing the threshold transmission coefficient β^{th}, and, consequently, increasing the possibility of dengue outbreak.

The model structured with one more compartment shows that the interval of temperature of the curve β^{th} situates inside with respect to the curve provided by the reduced model. However, at a large range of temperatures, β^{th} obtained from more detailed model is lower than the simplified version. This behavior is similar to that observed with respect to the number of mosquitoes (Fig. 4). Hence the detailed model shows that dengue epidemics occurs slightly more severe and in a narrow range of temperature than the simplified version of the model.

Besides the different outcomes regarding to the basic offspring number and basic reproduction number, the inclusion of one more compartment that became the model more complex allowed us to understand the curves shown in Fig. 5: the crossing in the threshold curves is essentially due to the behavior in the number of mosquitoes rather than to the basic offspring number. Hence, it should be very interesting to analyze the effects of adding egg compartment by estimating the parameters π_e and μ_e.

When we do not have precise estimation of the transmission coefficients, the corresponding threshold values can be seen as an alternative to assess the dengue outbreaks or severity of dengue epidemics. Let us consider the curve corresponding to β^{th} in Fig. 5. Let us suppose that the transmission coefficients in a certain region are nearly β_h^s and β_m^s. Then, we draw a horizontal line with value $\log(\beta_h^s \beta_m^s)$ in the $\log(\beta^{th}) \times T$ coordinates, which intercepts the curve at two temperatures, say T_1 and T_2. If this chosen region situates between T_1 and T_2, the dengue epidemics can be triggered, and the temperature at which the epidemics level is the most severe is approximately 28 °C, where β^{th} assumes the lowest value. The basic reproduction number R_0 can be thought of comparable to the vector capacity: both are structured in terms of the biting rates of mosquitoes (squared), survival rate and extrinsic incubation period. Barbazan et al. (2010) assessed the effect of temperature on the vector capacity, and they concluded that halving the length of the biting interval with 10 °C rise in temperature increases the transmission rate by at least 2.4 times.

The basic reproduction number R_0 is a quotient between the transmission coefficients and its threshold value. For this reason, the distance between them, for instance $\left|\beta_h \beta_m - \beta^{th}\right|$, in some extent measures the welfare of a community (or region) with respect to dengue disease. If $\beta_h \beta_m < \beta^{th}$ and the difference is very high, the community is practically free of dengue outbreaks; otherwise, the dengue outbreaks should be very severe. However, the levels of recurrent dengue epidemics are not depending only on transmission coefficients, but also on the fractions of susceptible populations of mosquitoes and humans: if both fractions are nearly one (exactly one when the dengue disease is introduced in a completely susceptible population), more severe will be the next dengue outbreak (Yang, 1998).

The modern life style produces as residuals recipients that are appropriate to receive eggs from female mosquitoes. This is one of the reasons to explain the reemerging of A. aegypti mosquitoes in heavily populated areas and also to the persistence of them facing great efforts of eradication. To eradicate the dengue vector, chemical and mechanical controls are applied to mosquito population, besides the remotion of breeding sites (mechanical control). These controls can be incorporated in the model: the chemical control can be incorporated in the model introducing additional mortality rates in the larval and pupal stages and in adult mosquitoes, and mechanical control decreases the carrying capacity C besides increasing the mortality rate of eggs. The introduction of these forms of control aims to reduce the offspring number to Q',

$$Q' = \dfrac{\pi_e + \mu_e}{\pi_e + \mu_e + \mu_e'} \times \dfrac{\pi_l + \mu_l}{\pi_l + \mu_l + \mu_l'} \times \dfrac{\pi_p + \mu_p}{\pi_p + \mu_p + \mu_p'} \times Q_0,$$

where prime (′) stands for the additional mortality rates due to control mechanisms. The effect is the reduction in the basic offspring number Q_0. The mechanical control affects the basic offspring number, but also reduces the total amount of breeding sites, decreasing the carrying capacity C. Maybe the unique adversity is the active adherence of the individuals to maintain the reduced breeding sites, in order to avoid the quick return to the previous infestation level (Kroeger et al., 1995). Therefore, the basic offspring number Q_0, in conjunction with the past incidences of dengue infection, should be a very useful information to help in designing and implementing the controlling mechanisms to diminish the population size of mosquito by public health authorities.

4. Discussions and Conclusion

A previously developed mathematical model taking into account whole aquatic phase as one compartment (Yang et al., 2009a) was modified to split aquatic phase in larval and pupal stages. To obtain new entomological parameters, we used data collected from temperature-controlled experiments aiming to study the influence of temperature in mortality and transition rates regarding to mosquito's life cycle. The followed up mosquitoes were those found in the City of Marlia (350 km far from the City of São Paulo), situated at northwest of the São Paulo State, Brazil.

We estimated the entomological parameters regarding to larval and pupal stages (Figs. 1 and 2) from data considering whole aquatic phase (from eggs until emerging of adult mosquitoes). The estimations were obtained by considering the methodology presented here, which presented good fittings, even that we used polynomial function and, consequently, a simple linear fitting. Non-linear fittings could be attempted, as the generalized additive model.

The parametrized probability functions used to fit followed up data showed some advantages. Even in extreme temperatures at which very low number of individuals suffered change of state (from alive to death in different stages of life cycle and transition from one stage to another stage in the aquatic phase), the adjusted probability functions provided reasonable estimations (Yang et al., 2009a). Another advantage is the possibility of deriving estimations of more parameters from a unique followed up data: we illustrate this feature obtaining mortality and transition rates regarding larval and pupal stages from a unique follow up of entire aquatic phase encompassing two successive stages. The transitions in aquatic phase of mosquito's life cycle occur quickly in higher temperatures. We showed that the estimation of entomological parameters from observations recorded every 24 h does not result in higher errors. In order to show this, we compared two sets of observations, collected every 8 and 24 h, and we observed that both estimations are closer. There are several advantages of following up every 24 h: diminishing costs, reducing perturbations in the experiments during counting, minimizing the locomotion of researchers to the laboratory which saves times, etc.

We analyzed the model assuming constant entomological parameters, from which we obtained the basic offspring number Q_0 and basic reproduction number R_0. Our main purpose was to compare the results provided by these epidemiological parameters obtained from two models, where one is simplified version of the other. We stress the fact that we did not divide the whole aquatic phase into three phases presenting very different characteristics: egg, lava (this can be split into different instars, but they have similar aspects) and pupa. The reason is that we did not estimate any parameters regarding to egg phase. The extended model is more realistic because the larval and pupal stages are physiologically and behaviorally different. Moreover, this model can evaluate better the use of larvicides to control mosquito population because larvicides induce additional mortality on larval stage, leaving pupal stage practically unaffected.

The estimated entomological parameters regarding to larval and pupal stages were used to analyze the effects of details being introduced in mathematical modellings. Figs. 3–5 illustrate how the predictive capacity of models is affected by details. The model encompassing larval and pupal stages showed high infestation of mosquitoes (M^* higher, Fig. 4) and more risk of dengue epidemics (β^{th} lower, Fig. 5) than the corresponding model considering only one aquatic phase for intermediate temperatures. The comparison between two models allowed us to show that the reproduction capacity of the vector measured by the basic offspring number alone cannot be considered to establish the absolute risk of dengue disease. However, lower and upper bounds of temperature at which there is an outbreak of dengue epidemics is lower and higher than the bounds provided by simplified version of the model. The difference in lower bounds is 0.75 °C, which is not negligible, if we take into account the global warming up and vector controlling efforts. With respect to the global warming up, the basic offspring number Q_0 increases monotonically until 29 °C increasing the risk of dengue disease in sub-tropical regions. Another consequence is an increasing in the expectation (probability) of invasion and colonization by mosquitoes *A. aegypti* in temperate regions. Hence the global warming up should be considered seriously by the public health authorities because those regions are heavily populated. Restricting only in the variation of temperature, the gains with global warming up seem very restrictive, since the temperature at which mosquito population is naturally eradicated must be higher than 36 °C.

Finally, we will point out some further works related to this paper. First, we can allow to the entomological parameters of the model, Eqs. (6) and (7), to vary with calendar year, instead of the assumption of the annual mean values. For instance, we can retrieve collected temperature data during several years from a selected city. Then the varying temperature with time can be matched with the temperature varying entomological parameters ϕ and μ_f (Table 1), and π_l, μ_l, π_p and μ_p (Figs. 1 and 2). By doing this we can assess the time varying number of mosquitoes and incidence of dengue disease. These results obtained by simulating the model can be compared with real data (Lu et al., 2009; Chen et al., 2010 fitted incidence data considering time lagged temperature to assess the effect of meteorological factors with dengue transmission). Another important aspect is the possibility of evaluating optimal time to introduce some kind of intervention, as mechanical control and chemical controls as insecticide and larvicide (Yang and Ferreira, 2008). Further study is, taking into account the estimated entomological parameters, to assess how the uncertainties affect the epidemiological parameters. To address this question, the sensitivity analysis of the basic offspring number and the basic reproduction number with respect to the entomological parameters can be performed taking into account the high level of variability in the estimated parameters (Yang, 2001). Besides the sensitivity of the parameters, stochastic version of the deterministic model can be simulated by considering the entomological parameters behaving as random variables.

References

Barbazan, P., Guiserix, M., Boonyuan, W., Tuntaprasart, W., Pontier, D., Gonzalez, J.-P., 2010. Modelling the effect of temperature on transmission of dengue. Med. Vet. Entomol. 24, 66–73.

Chen, S.C., Liao, C.M., Chio, C.P., Chou, H.H., You, S.H., Cheng, Y.H., 2010. Lagged temperature effect with mosquito transmission potential explains dengue variability in Southern Taiwan: insights from a statistical analysis. Sci. Total Environ. 408, 4069–4075.

Farnesi, L.C., Martins, A.J., Valle, D., Rezende, G.L., 2009. Embryonic development of *Aedes aegypti* (Diptera: Culicidae): influence of different constant temperatures. Mem. Inst. Oswaldo Cruz. 104 (1), 124–126.

Focks, D.A., Haile, D.G., Daniels, E., Mount, G.A., 1993a. Dynamic life table model for *Aedes aegypti* (Diptera:Culicidae): analysis of the literature and model development. J. Med. Entomol. 30, 1003–1017.

Focks, D.A., Haile, D.G., Daniels, E., Mount, G.A., 1993b. Dynamic life table model for *Aedes aegypti* (Diptera:Culicidae): simulation results and validation. J. Med. Entomol. 30, 1018–1028.

Frank, P.M., 1978. Introduction to System Sensitivity Theory. Academic Press, New York, 385 pp.

Hale, J.K., 1969. Ordinary Differential Equations. John Wiley and Sons, New York, 332 pp.

Kroeger, A., Horstick, O., Christine, R., Kaiser, A., Becker, N., 1995. The potential for malaria control with the biological larvicide *Bacillus thuringiensis israelensis* (Bti) in Peru and Equador. Acta Trop. 60, 47–57.

Leite, M.B., Bassanezi, R.C., Yang, H.M., 2000. The basic reproduction ratio for a model of directly transmitted infections considering the virus charge and the immunological response. IMA J. Math. Appl. Med. Biol. 17, 15–31.

Lindsay, S.W., Birley, M.H, 1996. Review: climate change and malaria transmission. Ann. Trop. Med. Parasitol. 90 (6), 573–588.

Lu, L., Lin, H., Tian, L., Yang, W., Sun, J., Liu, Q., 2009. Time series analysis of dengue fever and weather in Guangshou, China. BMC Public Health 9, 395.

Maidana, N.A., Yang, H.M., 2008. Described the geographic propagation of dengue disease by traveling waves. Math. Biosci. 215, 64–77.

Mandell, G.L., Bennett, J.E., Dolin, R., 2005. Mandell, Douglas and Bennett's principles and practice of infectious diseases. Elsevier Inc., Philadelphia, 3662 pp.

Monath, T.P. (Ed.), 1989. The Arboviruses: Epidemiology and Ecology, vol. V. CRC Press, Boca Raton, FL, 241 pp.

Nelson, M.J., 1986. Aedes aegypti: Biologia y Ecologia. Organización Panamericana de la Salud, Washington, DC, 50 pp.

Regis, L., Monteiro, A.M., Melo-Santos, M.A.V., et al., 2008. Developing new approaches for detecting and preventing *Aedes aegypti* population outbreaks: basis for surveillance, alert and control system. Mem. Inst. Oswaldo Cruz. 103 (1), 50–59.

Rueda, L.M., Patel, K.J., Axtale, R.C., Stimer, R.E., 1990. Temperature development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 27 (5), 892–898.

Thu, H.M., Aye, K.M., Thein, S., 1998. The effect of temperature and humidity on dengue virus propagation in *Aedes aegypti* mosquitoes. Southeast Asian J. Trop. Med. Public Health 29 (2), 280–284.

Vector Topics, 1980. Biologia y Control del *Aedes aegypti*. U.S. Department of Health and Human Services, 80 pp.

Yang, H.M., 1998. Modelling vaccination strategy against directly transmitted diseases using a series of pulses. J. Biol. Syst. 6 (2), 187–212.

Yang, H.M., 2001. A mathematical model for malaria transmission considering global warming and local socio-economic conditions—the sensitivity analysis. Rev. Saúde Pública 35 (3), 224–231.

Yang, H.M., Della Negra, M., Lian, Y.C., Queiroz, W., Hotta, L.K., 2003. The serore-vertion and the survival related to HIV infection among children: statistical modeling applied to retrospective data collection. Math. Comput. Modell. 38, 251–267.

Yang, H.M., Ferreira, C.P., 2008. Assessing the effects of vector control on dengue transmission. Appl. Math. Comput. 198, 401–413.

Yang, H.M., Macoris, M.L.G., Galvani, K.C., Andrighetti, M.T.M., Wanderely, DMV, 2009a. Assessing the effects of temperature on the population of *Aedes aegypti*, the vector of dengue. Epidemiol. Infect. 137 (8), 1188–1202.

Yang, H.M., Macoris, M.L.G., Galvani, K.C., Andrighetti, M.T.M., Wanderely, D.M.V., 2009b. Assessing the effects of temperature on dengue transmission. Epidemiol. Infect. 137 (8), 1179–1187.