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Density-Dependent Intraspecific Competition in the Larval Stage of *Aedes aegypti* (Diptera: Culicidae): Revisiting the Current Paradigm

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Abstract

Density-dependent intraspecific competition has been considered an important determinant of the dynamics of larval stages of *Aedes aegypti*. A model was published in 1984 providing a mathematical description of this density dependence, based on field data, that has since been widely used. This description, however, is based on the strong assumption that all mortality is density-dependent. We re-examine the data without this premise and find a reduced importance of density dependence, as well as a different functional form. Based on these discrepancies, we emphasize that the characterization of density dependence in the larval stages of *Ae. aegypti* should be based on a more complete dataset, and we use artificially generated data to explore how such additional information could help developing a better description of this density dependence. We review other empirical studies on larval competition, discuss the need for further dedicated studies, and provide a few simple guidelines for the design of such studies.

Keywords

Aedes aegypti; density dependence; intraspecific competition; mathematical model; parameter estimation

Aedes aegypti is the major vector of dengue and yellow fever viruses. Its close association with human populations makes it a particularly competent vector in urban environments and therefore an important target for population control.

A key prerequisite to the design of any population control program is an understanding of the mechanisms underlying the dynamics of the pest population, and, in particular, whether the population is regulated by density-dependent processes. Three types of density dependence can be defined, based on the relationship between initial density at the stage at which density dependence occurs and the final number of individuals surviving to later stages. Density dependence is exactly compensatory when this final number is independent of the initial density. If the final number of survivors increases when initial density increases, density dependence is undercompensatory. Finally, if the final number of survivors decreases when initial density increases, density dependence is overcompensatory.

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These types of density dependence are crucial to determine the ultimate outcome of control strategies (Juliano 2007). If a control effort does reduce densities at a given developmental stage, and if later stages are regulated by density-dependent processes, the most desirable result (an increase in overall mortality) will only be achieved if density dependence is undercompensatory, although the impact of control would still be reduced. Control efforts would be useless if density dependence is compensatory and could even be counterproductive if density dependence is overcompensatory, resulting in an increase in density at later stages. Evidence for such counterproductive control has been shown in laboratory and field studies of mosquito populations (Agudelo-Silva and Spielman 1984, Lounibos 1985, Washburn et al. 1991). If overcompensatory density dependence occurs, the conventional wisdom is to apply control methods at later stages than those on which density dependence acts (Service 1985).

Density dependence is a major component of larval mortality in container-breeding mosquitoes (Service 1985, Washburn 1995) such as *A. aegypti*. In this species, density dependence is mostly driven by indirect competition among larvae for access to nutritional resources within containers rather than direct competition by interference (Gilpin and McClelland 1979; Dye 1982, 1984a). Characterizing these density-dependent processes, the life stage(s) at which they operate, and the type of regulation, if any, that they cause in natural populations (undercompensatory, compensatory, or overcompensatory) is key to the development of successful control efforts.

A critical step toward this understanding is the development of accurate life tables that describe development rates and sources of mortality. Unfortunately, life tables based on field data are rare. Southwood et al. (1972) conducted a study in Bangkok, Thailand, that established a life table of the immature stages of *Ae. aegypti* by conducting a series of nine monthly assays of stage-specific mortalities at different initial densities, using representative containers from this location and their natural water and food contents. The data from this study have been the main source of parameter values used to describe density dependence in population models of *Ae. aegypti* (Dye 1984b, Focks et al. 1993, Phuc et al. 2007) and epidemiological models of dengue (Focks et al. 1995, Atkinson et al. 2007).

Visual inspection of the data of Southwood et al. suggests that the most important components of pre-imaginal mortality are k_1, the mortality from egg to beginning of second instar, and k_4, the mortality from beginning of fourth instar to pupation. k_1 seemed to be density dependent, whereas k_4 seemed to be density independent.

One of the most important models of *Ae. aegypti* dynamics based on these data was developed by Dye (1984b) and mathematically characterizes the density dependence caused by intraspecific competition. Southwood et al.'s data were analyzed to find the equations and parameter values that best describe the relationship between larval density and mortality, particularly in the early stages (eggs and first and second instars) that seemed to show the strongest density dependence.

Dye's equations, and the accompanying parameter values, have since been frequently used to describe density dependence in various models of *Ae. aegypti* populations (Phuc et al. 2007, Yakob et al. 2008). Given the importance of the immature stages in the population dynamics of this species, population models should rely on extensive knowledge of the amount and type of density-dependent intraspecific competition occurring in natural habitats. In this article, we emphasize that the estimates obtained by Dye (1984b) are constrained by very strong assumptions and therefore do not necessarily constitute a general description of density-dependent larval competition. These assumptions, as well as the lack of other field data describing density-dependent processes in a natural environment, stress the need for further studies on this important topic.

The Current Paradigm: Dye's Model

If defined broadly, density dependence in the larval stage can have effects on many life history traits of *Ae. aegypti* such as mortality, fecundity, and development rates (Gilpin and McClelland 1979, Agnew et al. 2002) that can influence population regulation. The effects of density dependence on each of these traits could therefore influence the overall population dynamics. The data provided by Southwood et al. (1972) do not allow for such a general assessment of density dependence; therefore, following the approach of Dye (1984b), we define density dependence strictly as the change in mortality during specific immature life stages in response to a change in initial density.

Dye (1984b) used the following equation (Thomas et al. 1980, Bellows 1981) to describe the changes in density between two specific life stages of *Ae. aegypti*:

$$N' = \lambda N e^{-\alpha N^{\beta}}, \tag{1}$$

where N and N' are the initial and final densities, respectively. In this model, λ represents the finite rate of increase (net fecundity after lifetime density-independent mortalities). Because only immature stages are considered here, λ accounts for density-independent mortality only and, by definition, $\lambda \leq 1$. Two distinct parameters, α and β, describe the mortality caused by density-dependent competition. In particular, β describes the qualitative characteristics of density dependence at that stage: higher values of β mean that overcompensatory regulation occurs at lower densities (for a given α) and acts more sharply (a slight increase in initial density causes a large decrease in the number of survivors). The choice of this model is motivated by the fact that models in which density dependence is described by a two-parameter function typically have great flexibility and can describe a large variety of forms of density dependence (Bellows 1981).

Stage-specific mortalities are expressed as k values, defined by:

$$k = -\ln \frac{N'}{N}. \tag{2}$$

This can be combined with equation 1 to give:

$$k = \alpha N^{\beta} - \ln \lambda. \tag{3}$$

Dye fit the above model to the data from Southwood et al. (1972) through nonlinear regression analysis, using a least-squares approach to estimate values for the parameters λ, α, and β, specifically focusing on mortality between eggs and second-instar larvae and between second- and third-instar larvae (k_1 and k_2, respectively). However, he reports that, when considering all three parameters, this fit does not yield significant density dependence:

"If the density-independent components are extracted from k_1 and k_2 […], no density-dependent component remains! These data do not pass any of the statistical tests for density dependence […]; the only evidence for density-dependent mortality comes from visual inspection of the data." (Dye 1984b, p. 252).

He then postulates that k_1 and k_2 are strictly density dependent (i.e., $\lambda = 1$ for these stages) and extracts the corresponding values for α and β under this assumption. The model used becomes:

$$k=\alpha N^{\beta}. \quad (4)$$

The results of this estimation procedure are given in Dye (1984b, p.253: Fig. 3 and Table 1). When mortality from eggs to third-instar larvae (i.e., the summed variable $k_1 + k_2$, denoted here k_{12}) is considered, estimates of α and β are as follows (all estimates are given as estimate ± SE):

$$\alpha_{12}=0.229 \pm 0.251;$$
$$\beta_{12}=0.302 \pm 0.129. \quad (5)$$

Including the Density-independent Component

The ideal approach for obtaining estimates for all three parameters (α, β, and λ) would be to fit equation 1 to the data of Southwood et al. using nonlinear regression. Unfortunately, as Dye found, use of this straightforward approach gives a large set of (α,β,λ) parameter combinations that provide very similar fits. The model-fitting procedure has difficulty in converging toward the optimal parameter set, and the large set of parameters leading to statistically indistinguishable fits means that the resulting estimates are associated with very large statistical errors.

The only possible approach to fitting the three-parameter model is to restrict the parameter space, for instance by fixing a value for one of the parameters to get estimates of the other two. This is equivalent to exploring a two-dimensional parameter subset within the three-dimensional (α,β,λ) parameter space.

Dye's approach is an example of such a restriction, exploring only the plane described by $\lambda = 1$, and finds the (α,β) parameter set corresponding to the best fit within this plane. This parameter combination, however, is only a local optimum. In a different subset of the parameter space, for instance, another plane based on a different assumption about the value of λ, the local optimum, and therefore the (α,β) estimates, might be different. Biologically, this means that the amount of density-independent mortality assumed affects the amount and shape of density dependence estimated from the data. In particular, Bellows (1981) argues that:

“if some degree of density-independent mortality is present, the omission of this parameter [λ] would result in an overestimate of the amount of density dependence present.” (Bellows, 1981, p. 148).

To be able to fit the model to the data of Southwood et al., Dye made the assumption that there is no density-independent mortality, which predicts that mortality would be virtually absent at very low densities. This is unrealistic in the case of *Ae. aegypti*, where eggs, in particular, are considered to experience high density-independent mortality (Gilpin and McClelland 1979). Therefore, we use an approach that allows us to get estimates for α and β but with a different assumption about λ.

This approach is based on two successive steps. First we use a simpler two-parameter model that includes density independence but assumes a fixed shape of density dependence. Visual

inspection suggests that the data could be consistent with a linear relationship between k and N. We therefore assume, in this first step, that $\beta = 1$, that is:

$$k=\alpha N - \ln(\lambda). \tag{6}$$

Nonlinear regression analysis was carried out in JMP, version 7 (SAS Institute, Cary, NC) and resulted in the following estimates:

$$\begin{aligned}\lambda_{12}&=0.126 \pm 0.052;\\ \alpha_{12}&=1.798 \times 10^{-4} \pm 0.827 \times 10^{-4}.\end{aligned} \tag{7}$$

This simpler model shows a significant level of density dependence when it is described by a single parameter (α significantly different from zero), as well as a value of λ significantly different from 1, which suggests that density independence is quantitatively important.

We then use the value of λ obtained in this first step, and fit the model to the data with α and β as unknown parameters. This second step is thus similar to the approach used by Dye, but with a different initial assumption on λ. We obtain the following estimates of α and β:

$$\begin{aligned}\alpha_{12}&=3.518 \times 10^{-4} \pm 14.36 \times 10^{-4};\\ \beta_{12}&=0.922 \pm 0.474.\end{aligned} \tag{8}$$

The differences between these two approaches (assuming $\lambda = 1$ or using the two-step method and assuming $\lambda = 0.126$) are shown by the resulting density-dependence curves shown in Fig. 1 (top). In the bottom panel, these same curves are plotted against the logarithm of the initial number of eggs. On this plot, the type of density dependence is easily visualized and directly related to the slope of the curve: density dependence is undercompensatory if the slope is <1, exactly compensatory if the slope = 1, and overcompensatory if the slope is >1.

Comparison of the Two Methods

It is clear that the two approaches presented above give very different outcomes. These differences have three major implications.

First, they indicate that, when density-independent processes are taken into consideration in our two-step approach, they account for a large part of the mortality of immature stages. Our estimate of $\lambda_{12} = 0.126$ corresponds to a density-independent mortality of 87.4% from egg to third instar, when the actual mortalities between these stages in the data of Southwood et al. range from 86.8 to 97.4%. Under this assumption, density dependence is reduced to a much lower level, weakly significant when it is described by a single parameter (equation 7) and nonsignificant when described by the complete model (equation 8). Furthermore, when we perform the same analysis on mortality from egg to pupa, density-independent mortality is estimated at 98%, and no density dependence can be extracted from the data.

More generally, even without consideration for the respective validities of the two initial assumptions ($\lambda = 1$ or $\lambda = 0.126$), the comparison between the outcomes of both approaches clearly shows that the choice of assumption about λ greatly affects the estimation of the other two parameters, and, more generally, the shape of the density-dependence curve. As shown in Fig. 1 (top), when assuming $\lambda = 1$, the curve (dotted line) is forced to go through the ($k_{12} = 0$,

$N = 0$) origin, affecting both the general slope and the shape of that curve (α and β, respectively). With our two-step method, the $\beta = 1$ assumption made in the first step corresponds to a linear relationship between k and N (dashed line). The density-dependence curve obtained after the second step (solid line) does not depart substantially from this first linear relationship. As a result, curves from the two different approaches (dotted and solid lines) are very different, with corresponding differences between the sets of estimates for α and β (see equations 5 and 8).

Within the range of initial densities contained in the dataset, these two very different functions provide a fairly similar description of the data. If the primary goal of the analysis is to interpolate the characteristics of density dependence within a particular range of densities, these two approaches lead to roughly equivalent conclusions. In this case, it is worth noting that the only model raising significantly non-null estimates for density-dependent parameters is the model used in the first step of the two-step method. This model is also the most parsimonious one, in which density dependence is described with only one parameter.

However, the curves in Fig. 1 differ markedly outside the range of the data of Southwood et al., particularly when initial densities are low—a situation for which no information is available in this dataset. The curves plotted against the logarithm of initial densities (Fig. 1, bottom) show that the two assumptions correspond to very different patterns of density dependence at these low densities. When $\lambda = 1$ is assumed (no density independence), the strength of density dependence, represented by the slope of the curve, increases rapidly as initial density increases. With the two-step method, where density independence is included, there is a large range of initial densities in which there is no, or weak undercompensatory, density dependence acting. Only at higher densities can a stronger density dependence be observed. In other words, if the purpose of the analysis is extrapolation and full characterization of density dependence at any initial density, it is crucial to obtain reliable estimates for all three parameters to discriminate between these patterns.

The inability of the data of Southwood et al. alone to support estimation of all three parameters calls for more data to address this issue. Characterizing the dynamics at low initial densities seems especially important for capturing the whole functional form of density dependence. In the following section, we investigate whether additional data could aid the characterization of density dependence, and, if so, what type of data is needed.

Parameter Estimation with Additional Data

Parameter estimation should be more reliable if based on more than the nine data points of the dataset of Southwood et al. For this reason, we examine how our ability to estimate parameters with the model used here (equation 1) could improve if additional data were provided. To that end, we create artificial data-sets that augment the nine points from the study of Southwood et al. with 500 artificial data points of the form (N, k_{12}). These points are generated as follows:

- N is the initial number of eggs, chosen uniformly at random between 0 and 8,000.
- $k_{12} = K_{12} + \varepsilon$, where:
- K_{12} is the value predicted by the model (equation 1)
- ε is a random noise added to the calculated value.

Because we want to consider additional data of the same quality as in the study of Southwood et al., ε is generated according to a normal distribution of mean = 0 and SD equal to the SD of the original set of nine points.

Calculation of K_{12} requires a defined set of parameter values to be used. The previous sections show that two such sets, corresponding to two quite different biological processes, are

consistent with the observed data: the first one (A) is based on the assumption $\lambda = 1$ (no density independence), with parameter values given in equation 5, whereas the second one (B) is based on our two-step estimate, with parameter values given in equations 7 and 8. We therefore generate two artificial datasets, A and B, based on these two sets of assumptions. We fit the model in equation 1 to both of these artificial datasets (because of the large number of data points, a single fitting procedure of the three-parameter model is now possible). In both cases, this fit is expected to result in parameter estimates that correspond to the values used to generate the artificial points.

The results of these new fits are presented in Fig. 2, with the descriptive statistics of these fits (parameter estimates, confidence intervals, and pairwise correlation coefficients) summarized in Table 1. We observe that, with this greatly increased number of data points, it is now possible to directly extract estimates of all three parameters with reasonable statistical support. The quality of the estimation, however, seems to differ between the two artificial datasets, A and B. More specifically, comparison shows that the parameter estimates are closer to their correct values in the case where the artificial points are generated assuming no density independence (case A), than when density independence is assumed (case B). In particular, the confidence interval for the estimate of α_{12} is much larger in case B (close to 100% of the estimate) than in case A (<50%). This most likely results from a stronger correlation between parameter estimates in case B. A strong correlation between two parameters means that a small change in one of them can be almost exactly compensated by a small change in the other one. In this case, it can be impossible to distinguish the best fit (and thus the best parameter estimates) within this range of changes, unless the amount of data available is very large. In general, the stronger the correlations between parameters, the more data are needed to obtain individual estimates of each of them.

The effects of the correlation between estimates of α_{12} and β_{12} are presented in Fig. 3, showing the overall goodness-of-fit of the model, measured as the sum of the squared differences between data and model predictions, when α and β vary. Nonlinear regression aims to minimize the sum of the squared errors and therefore corresponds to finding the minimum point of this surface. The generally strong interaction between these two parameters (see Table 1) is represented by the valley formed similarly by both surfaces in cases A and B. Along the floor of this valley, the quality of the fit is very similar, and it is very difficult to locate the values that correspond to the best overall fit (hence the high values of the correlation coefficients). However, the loss in quality of fit when parameter values move up the walls of the valley is much higher in case A (Fig. 3A) than in case B (Fig. 3B), where the surface is flatter, especially along the α-axis. This shows why the uncertainty of the estimate of α_{12} is higher in case B.

The inability to obtain good parameter estimates for Dye's model in case B, even when a very large dataset is considered, suggests that the model is inadequate to describe density dependence in such instances, i.e., when density-independent mortality is high. This is easily understandable in this case because, when mortality is expressed as a *k*-value, it is almost linearly related to initial density, as seen in Fig. 1. A model that uses three parameters to describe this relationship is obviously redundant, preventing parameters from being independently estimated. However, the strong curvature in case A justifies the use of three distinct parameters and results in lower uncertainty in their estimates.

From an applied perspective, this stresses the importance of using an appropriate model for the available data. The choice of model used here (equation 1) is biologically justified by its versatility in describing density-dependent, potentially overcompensatory, intraspecific competition. However, obtaining parameter estimates with this model requires a set of data that encompasses the entirety of these dynamics and that covers a range of densities across which the compensatory effects of density dependence change markedly. Under the assumption

of no density independence (case A), our artificial dataset showed more of such a change than when density independence was included (case B), resulting in a better ability to estimate parameters. More generally, this leads to two important guidelines:

1. Experiments that aim to estimate parameters describing density dependence should be designed to include the biologically appropriate range of initial densities, a range large enough to encompass changes in mortality with increasing density.
2. Given a particular data set, an appropriate model should be selected, and the number of parameters should be chosen parsimoniously. If the number of parameters is too high, parameter estimates are more likely to be affected by strong correlations, reducing their reliability.

Only when these two conditions are met can a proper characterization of density dependence be extracted from experimental data.

In the following sections, we explore the existing literature on density dependence and intraspecific competition in search of field studies that are relevant for the characterization of density dependence.

Existing Field Studies on Density Dependence

To the extent of our knowledge, there is no field study of *Ae. aegypti* in the literature that is similar to Southwood et al., reporting a detailed life table of the immature stages from which parameters describing density dependence could be estimated in the way described above. However, other ecological studies of *Ae. aegypti* populations provide insights on the relationship between larval density and survival.

Seawright et al. (1977) studied a population of *Ae. aegypti* in Florida and observed a strong negative relationship between the percentage of surviving larvae in containers and their initial densities. However, two important factors should be noted. First, because the original containers in the field (discarded washing machines) were no longer available, these larval studies were carried out in an experimental site and in a different type of container (plastic buckets). Second, the nutritional conditions in these containers were obviously very harsh, because the developmental time (until pupation) at all densities ranged from 43 to 49 d. This duration is much longer than the 13–16 d observed by Southwood et al. in Thailand, which are themselves longer than the typical developmental time of ≈6–8 d under favorable conditions (Christophers 1960). Because natural populations are likely exposed to very different environmental conditions, it is difficult to extrapolate the results of this study to such populations.

Romero-Vivas and Falkonar (2005) surveyed a population in an urban area in Colombia, calculating various density indexes at all life stages of *Ae. aegypti* in different houses. They found a negative correlation between the ovitrap density index (ODI; average number of eggs found in ovitraps per house) and the larval density index (LDI; average number of fourth-instar larvae per house), a result that could be interpreted as evidence for strong density dependence. However, the values of ODI cannot be considered as a direct reflection of the number of eggs in the house, because the choice of the females to oviposit in ovitraps is influenced by many other factors, such as the availability of other containers in the house. Houses with many attractive natural containers may therefore have the fewest eggs laid in ovitraps but high numbers of larvae in these natural containers, which could by itself explain the observed negative correlation. Moreover, the different experimental points consist of identical surveys carried out at different times (1 mo per point), leading to seasonal variation being a potential confounding factor (it should be noted that the same confounding effect might occur in the study of Southwood et al. 1972).

Other studies, undertaken in field conditions and using representative productive containers from those locations, studied how survival relates to initial density. Juliano (1998), in Florida, and Braks et al. (2004), in Brazil, presented data in which survival is significantly lower for higher initial densities. Maciá (2006), in Argentina, observed no such difference. However, the limited number of data points in these studies does not allow a detailed characterization of the density dependence involved, if any, and of the possible compensatory mechanisms involved. This again stresses the need for comprehensive field studies providing detailed life tables of the immature stages of *Ae. aegypti*.

Food as a Limiting Factor

Although there is a lack of comprehensive studies on the relationship between density and survival, numerous studies have investigated the effects of food availability in natural habitats of *Ae. aegypti*. Because density dependence in this species is mostly driven by indirect intraspecific competition for food, these studies could provide insights into the details of these density-dependent processes. However, most of them do not differentiate between density-dependent and density-independent effects of low levels of food in containers. For example, in a study by Subra and Mouchet (1984) in Kenya, the effect of an additional input of food, or of first-instar larvae, to the pupal production of containers in the field was measured. It was observed that adding food promoted a significant increase in the number of pupae, whereas adding larvae did not significantly affect that number. The authors concluded that the natural population was very close to the carrying capacity and that food was a limiting factor. However, because 400 first-instar larvae were added to each container on each day in the density manipulation experiment, it is hard to assess the meaning of that manipulation relative to natural conditions.

Another interesting study by Arrivillaga and Barrera (2004) in Venezuela assessed the effect of competition through a different variable: the starvation resistance of third-instar larvae, i.e., the number of days a given larva stays alive without food. The comparison of the starvation resistance of larvae collected from the field to curves obtained in the laboratory under controlled food regimens showed that the amount of food available for this population is on the low side of the range of known laboratory regimens. Similar nutritional conditions were observed in Puerto Rico by Barrera et al. (2006), although this nutritional stress seems to be limited to smaller containers in this study.

These studies, among others, suggest that, in most populations, the availability of food is an important limiting factor. However, food limitation can cause mortality in different manners. Density-dependent mortality occurs with indirect competition, as described above, when all individuals have access to the entire amount of the resource but that amount is such that each individual obtains, or is likely to obtain, a suboptimal share. However, in a situation where each individual does not have access to the entire food supply (highly diluted nutritional resources), mortality is merely caused by this scarcity, regardless of how many individuals could theoretically share the total amount of resource, and can be mostly density independent. Therefore, it is impossible to infer the relative importance of density dependence or, a fortiori, derive any estimation of descriptive parameters (such as β in the above study), solely from information on food limitation.

Importance of Density Dependence in Applied Control Programs

The potential existence of overcompensatory density dependence in the immature stages of *Ae. aegypti* is a major concern for the implementation of control methods in natural populations. If such processes are actually regulating the dynamics of the population, it is easy to envision that any applied program that would successfully decrease larval densities could eventually fail to decrease the number of adult mosquitoes and could even increase that number. From

the perspective of an effort to reduce transmission of diseases carried by *Ae. aegypti*, this would make these programs virtually useless, or worse, counterproductive. This has, for instance, been observed by McDonald et al. (1977). In that work, the authors released genetically altered male *Ae. aegypti* carrying chromosomal translocations that can lead to nonviable offspring production. Although the release experiment successfully introduced a high level of sterility among adults in the natural population, the overall pupal production remained unchanged. They conjectured that density-dependent larval mortality was responsible for this compensatory process, although lack of data about the actual larval densities in the immature habitats prevented confirmation of this hypothesis.

Theoretical investigation of the effect of density dependence on the outcome of a control strategy has been well studied in the case of control approaches involving late-acting dominant lethal genetic systems (Phuc et al. 2007). This idea is a refinement of the release of insects carrying a dominant lethal (RIDL) population suppression strategy (Thomas et al. 2000), in which insects are genetically engineered to carry a dominant lethal gene, causing the carriers of that gene to die before reaching reproductive stages. However, if there is strong compensatory or overcompensatory density dependence, early death of a certain fraction of the offspring in a given cohort because of RIDL might only reduce intraspecific competition in the larval stages but not affect (or even increase) the number of reproductive adults emerging from that cohort. However, if the lethal gene is genetically programmed to act late in larval development, carriers of this gene will compete with their conspecifics throughout early larval stages. The lethal gene prevents them to emerge as reproductive adults, effectively reducing the density of adults. Theoretical studies of this late-acting approach (Phuc et al. 2007, Yakob et al. 2008) showed that, compared with its early-acting equivalent, population suppression can be achieved faster, with lower initial release ratios, and without the risk of undesired population increase caused by overcompensatory density dependence. Epidemiological modeling shows that, because of this effect, late-acting RIDL is also a better strategy to combat dengue (Atkinson et al. 2007). The benefits of late-acting RIDL depend on the density-dependent regulation of the larval stages incorporated in the models and become more important as this density dependence increases, particularly as β increases (Phuc et al. 2007). From an applied perspective, this means that the outcome of a control program based on this type of population suppression approach will rely heavily on our knowledge of the extent and type of density dependence acting in natural populations.

General Conclusions

The biotic and abiotic factors acting on immature stages are assumed to be a major component of *Ae. aegypti* population dynamics, as well as predominant factors determining important adult traits, such as size and biting rate, that could impact disease epidemiology. In that context, we make the following observations:

- Most of the current views on density dependence in the larval stages are based, directly or indirectly, on the only empirical study (Southwood et al. 1972) that included enough detail to fit a population dynamics model. However, it is clear that no general understanding of this phenomenon can come from a single study.
- Dye's final interpretation of this study (Dye 1984b) uses an approach that is expected to overestimate the importance of density dependence. Our analysis, which does not mathematically eliminate density-independent components in larval mortality, suggests that the importance of density dependence is not predominant in early immature stages. Moreover, when survival of all instars is considered, there is no evidence of density dependence.

- More generally, regardless of the approach, the data of Southwood et al. alone do not provide the information needed to estimate the parameters of interest using Dye's model. Additional data are needed for proper parameter estimation, as is care in the choice of an appropriate model.
- There have been, to our knowledge, very few studies that rigorously investigate density-dependent mortality in larval stages of *Ae. aegypti* in natural populations, and none of the existing studies provided a precise description of these processes.

Future Directions

Overall, it is striking to realize how little is known about the density dependence acting in natural populations of *Ae. aegypti*. Therefore, we emphasize the need for dedicated field studies that measure the density-dependent dynamics of larval populations. More precisely, we feel that the issues developed here call for studies that follow a few simple guidelines.

- Most importantly, studies have to be carried entirely in the field, investigating larval dynamics in natural environmental conditions and in containers that are representative of the local container distribution and pupal productivity, to characterize how the amount and type of density dependence varies between populations and field sites.
- These studies should be carried using natural food type and availability, ideally by using the water and nutrients found in actual containers. Studies have shown that manipulating the amount of nutritional resources available in the containers can change the outcome of density-dependent processes (Washburn et al. 1991, Juliano 1998).
- Estimating the parameters that describe density-dependent dynamics (typically α and β) will be facilitated if studies encompass a large portion of the possible range of initial densities. In particular, it is critical to know what happens at very low larval densities, a situation that is frequently encountered in natural populations. This range of densities was not represented in the experiment of Southwood et al., forcing any subsequent analysis to make unverifiable assumptions about mortality at low density.
- Experiments must be designed to discriminate purely density-dependent processes, driven by intraspecific exploitation competition, from simple food scarcity that would affect the dynamics in a density-independent manner. Using starvation resistance as a proxy of the actual amount of food ingested might be a good way to distinguish between these two assumptions: if local dynamics are regulated in a density-dependent manner, one would expect that increasing density would decrease the amount of fat reserves per larva, whereas under density-independent regulation, this amount would be independent of the initial density.
- The design of the life table experiment of Southwood et al. specifically excludes interactions and competition between different instars and only considers density-dependent competition within a given instar. In natural conditions, however, different instars coexist in the same containers and compete with each other for access to nutritional resources. This interaction between instars is therefore likely to be an important component of density dependence acting in natural habitats, especially on density-dependent mortality of early instars.
- Finally, the analyses presented here have only considered the effects of density dependence on larval mortality in *Ae. aegypti*. Although this mortality is certainly a major component of *Ae. aegypti* population dynamics, it is important that studies of natural populations also characterize the effects of density dependence on important adult life history traits such as size, sex ratio, and female lifespan for two main reasons. First, these adult traits are known to greatly impact *Ae. aegypti* competency as a vector

of dengue or other diseases. Second, these traits can also affect population dynamics and therefore constitute important feedback loops that impact the overall effects of density dependence on population regulation (Livdahl and Sugihara 1984, Juliano 1998). Characterizing the overall importance of density-dependent regulation of *Ae. aegypti* populations requires that studies incorporate these traits as well.

Given the importance of density dependence in the immature stages for large-scale control strategies of *Ae. aegypti* and diseases such as dengue, we believe that it would be dangerous to overstate our understanding of these dynamics and that additional information from field studies is needed.

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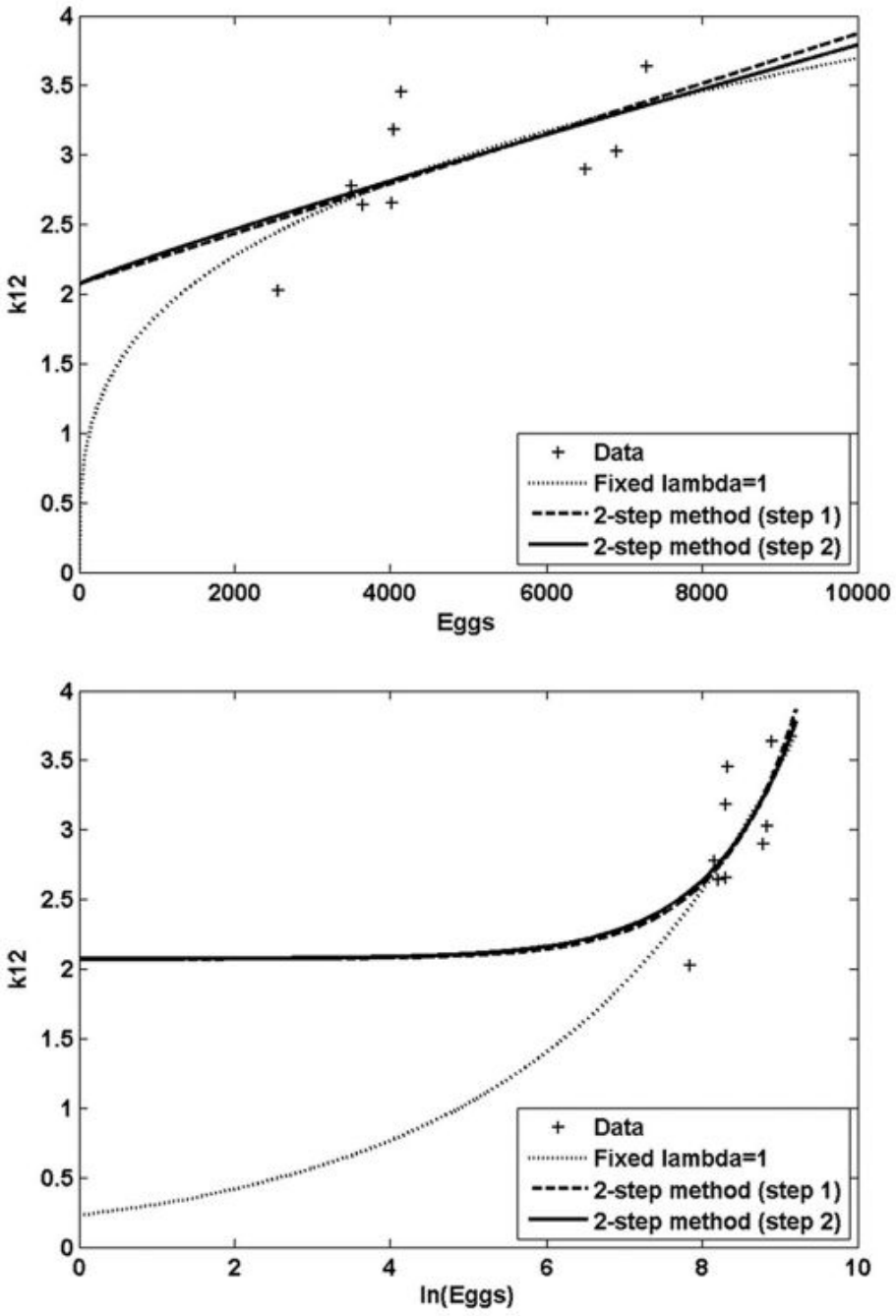

Fig. 1.

k_{12} mortality from the Southwood et al. (1972) dataset (symbols), together with best-fitting functional forms for the dependence of k_{12} on egg density (N) obtained using two assumptions about the density-independent parameter λ. Dotted lines: assuming no density independence ($\lambda = 1$), the curve is forced to go through ($k_{12} = 0$, $N = 0$), showing strong density dependence with $\alpha_{12} = 0.229 \pm 0.251$ and $\beta_{12} = 0.302 \pm 0.129$ (Dye 1984b). Dashed lines: first step of the two-step approach, assuming a linear relationship between k and N to estimate $\lambda = 0.126 \pm 0.052$. Solid lines: second step using the value of $\lambda = 0.126$ to estimate $\alpha_{12} = 3.518 \times 10^{-4} \pm 14.36 \times 10^{-4}$ and $\beta_{12} = 0.922 \pm 0.474$, showing weaker (nonsignificant) density dependence. (Top) k_{12} plotted against N. (Bottom) k_{12} plotted against ln(N). On the bottom panel, density

dependence is undercompensatory if the slope of the curve is <1, exactly compensatory if the slope = 1, and overcompensatory if the slope is >1.

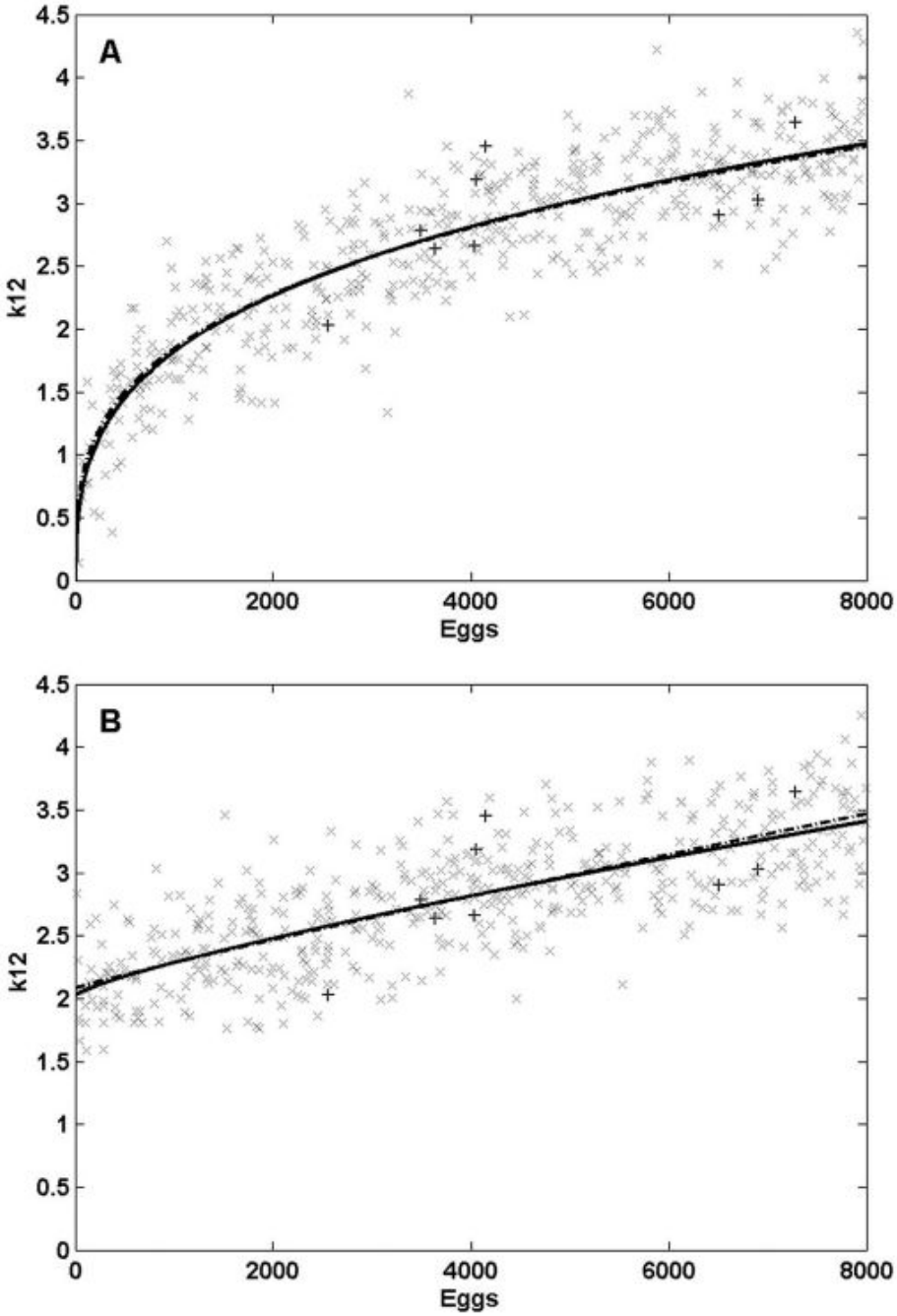

Fig. 2.

Estimating the parameters describing density dependence in k_{12} mortality with 500 additional artificial data points (see text). + signs mark the original nine points from the study of Southwood et al. × signs mark the artificial added points. Dot-dashed lines are the best-fit curves based on the original nine points, from which the artificial points are generated. Solid lines are the new fits based on all 509 points. (A) Artificial points are generated using the parameter values in equation 5, assuming $\lambda_{12} = 1$. (B) Artificial points are generated using the parameter values in equations 6 and 7, based on the two-step method and assuming $\lambda_{12} = 0.126$. Note that the dot-dashed line in A corresponds to the dotted line in Fig. 1, and the dot-dashed line in B corresponds to the solid line in Fig. 1.

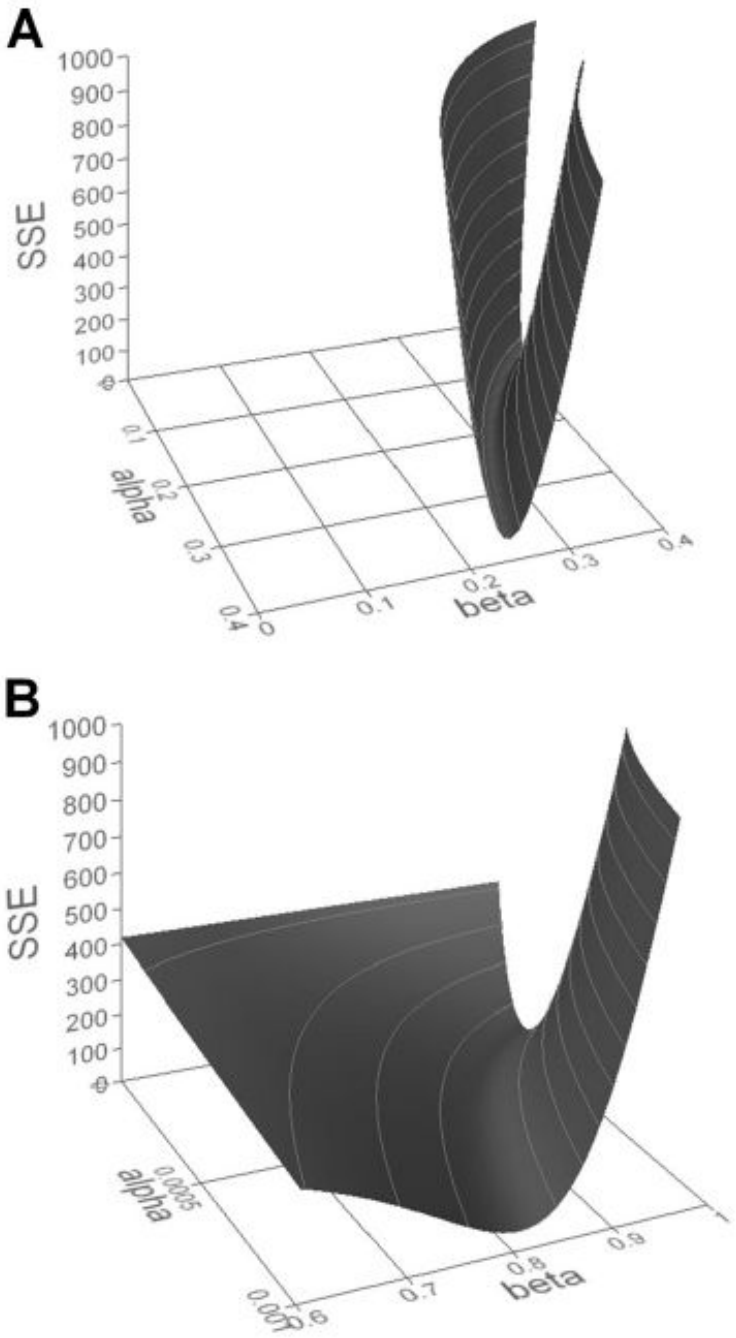

Fig. 3.

Sensitivity of the goodness-of-fit (sum of squared error [SSE]) to the parameters α and β, when the model in equation 1 is fitted to the artificial 509-point datasets. (A) Using the dataset in which artificial points are generated according to equation 5, assuming no density-independent mortality ($\lambda = 1$). (B) Using the dataset in which artificial points are generated according to equations 6 and 7, assuming some level of density-independent mortality ($\lambda = 0.126$). Note that the scale of the SSE axis is the same in both panels. Regression analysis involves finding the lowest point on this surface, that is, the set of parameters that minimizes SSE.

Table 1

Descriptive properties of the fits of the artificial datasets (500 additional data points) to the model from Eq. 1

	A			B		
	α_{12}	β_{12}	λ_{12}	α_{12}	β_{12}	λ_{12}
True values	0.229	0.302	1	3.518×10^{-4}	0.922	0.126
Estimated values	0.289 ± 0.133	0.284 ± 0.041	1.272 ± 0.454	$9.559 \times 10^{-4} \pm 8.531 \times 10^{-4}$	0.810 ± 0.096	0.132±0.009
Correlation						
α_{12}	—			—		
β_{12}	−0.9987	—		−0.9991	—	
λ_{12}	0.9895	−0.9813	—	0.9043	−0.8875	—

True values denote the parameter values used to generate the artificial points (see text): (A) without density-independent mortality ($\lambda = 1$) and (B) with density-independent mortality ($\lambda = 0.126$). Estimated values are the parameter estimates (±SE) obtained from the fit of the 509 data points to the model. Correlation denotes the correlation coefficients between each pair of parameter estimates. These coefficients can range, in absolute value, from 0 (no correlation, parameters act independently) to 1 (complete correlation, the two parameters cannot be separately estimated).