

## The influence of local environment on the aging and mortality of *Aedes aegypti* (L.): Case study in Fortaleza-CE, Brazil

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**ABSTRACT:** It is generally assumed that the daily probability of survival of mosquitoes is independent of age. To test this assumption we have conducted a three-year experimental fieldwork study (2005-2007) at Fortaleza-CE in Brazil, determining daily survival rates of the dengue vector *Aedes aegypti* (L.). Survival rates of adult *Ae. aegypti* may be age-dependent and the statistical analysis is a sensitive approach for comparing patterns of mosquito survival. The mosquito survival data were better fit by a Weibull survival function than by the more traditionally used Gompertz or logistic survival functions. Gompertz, Weibull, or logistic survival functions often fit the survival, and the tails of the survival curves usually appear to fall between the values predicted by the three functions. We corroborate that the mortality of *Ae. aegypti* in semi-natural conditions may no more be considered as a constant phenomenon during the life of adult mosquitoes but varies according to the age and environmental conditions under a tropical climate. This study estimates the variability in the survival rate of *Ae. aegypti* and environmental factors that are related to such variability. The statistical analysis shows that the fitting ability, concerning the hazard function, was in decreasing order: Seasonal Cox, the three-parameter Gompertz, and the three-parameter Weibull, that was similar to the three-parameter logistic. The advantage of using the Cox model is that it is convenient for exploring the relationship between survival and several explanatory variables. The Cox model has the advantage of preserving the variable in its original quantitative form and of using a maximum of information. The survival analyses indicate that mosquito mortality is both age- and environment-dependent. *Journal of Vector Ecology* 37 (2): xxx-xxx. 2012.

**Keyword Index:** Dengue, Kruskal-Wallis test, hazard rate, Gompertz function, logistic function, Weibull function, Cox's proportional hazard function.

### INTRODUCTION

Dengue fever, together with associated dengue hemorrhagic fever, is the most important vector-borne viral disease affecting humans. *Aedes aegypti* (L.), the urban yellow fever mosquito, is also the principal dengue-carrying vector. A secondary vector is *Aedes albopictus* (Skuse). *Ae. aegypti* (L.) is distributed in the majority of tropical and subtropical cities (Hopp and Foley 2001) with its wild form living in tropical Africa (Diallo et al. 2005). In most urban areas, the vector of the dengue fever virus (DEN) causes dramatic epidemics (Gubler 1997). As there is yet no available vaccine, vector control remains the only means to prevent epidemics, but it is actually very difficult to maintain the mosquito populations at a suitable level, especially when resistance to insecticides occurs (Carvalho et al. 2004, Lima et al. 2003, Luna et al. 2004).

Social and environmental factors, including increased urbanization (particularly of poor populations lacking basic health services), as well as expansion of international travel and trade, are linked to the resurgence of dengue disease (Hales and van Panhuis 2005). Climate change also may affect transmission, as dengue mosquitoes reproduce more quickly and bite more frequently at higher temperatures (McMichael 2003, Hales et al. 2002).

Dengue currently occurs in nearly 100 tropical and subtropical countries. Epidemics have become progressively larger. In 2002, the disease was responsible for an estimated 19,000 deaths, as well as the loss of 616,000 disability-adjusted life years. The World Health Organization (WHO) currently estimates there may be 50 million dengue infections worldwide every year. Therefore, available scientific knowledge about vector ecology and disease epidemiology may potentially be harnessed to improve environmental management and

community action as a means for combating a most dreaded disease.

In Brazil, since some older historical reports published when clinical descriptions were available (Pedro 1923), DEN re-emerged with ever growing incidence since the epidemics in Boa Vista-RR in 1982 and Rio de Janeiro-RJ in 1986 (Degallier et al. 1996). In this country, seasonal and interannual variations of DEN epidemics are clearly linked to seasonal and interannual variability of regional climate (Tauil 2002). Yearly peaks of dengue cases are generally noted during and just after the wet season (Degallier et al. 1996). That is the case for Fortaleza-CE, a 3-million-inhabitant city located at the oceanic bordure of the semi-arid region of northeastern Brazil (Figure 1) and where several epidemics occurred over the last decades (Vasconcelos et al. 1989, Vasconcelos et al. 1995).

Knowledge of the relations between meteorological parameters and mosquito survival/mortality need both field evaluation and regional validation. Here a difficulty arose, as some authors have shown that during the life of the mosquito, the mortality rate does not remain constant, contrary to the generally accepted assumption (Styer et al. 2007). These authors showed that younger mosquitoes have a lower mortality rate than old ones and thus may have a higher vectorial capacity.

Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas. The region of Fortaleza (Figure 1) has a sub-tropical climate, specifically a tropical wet and dry climate, with high temperatures and high relative humidity throughout the year, with a large seasonal-to-interannual variability in the rainfall regime. The wet season is roughly limited to four months, February to May, with more than 60% of the annual total amount, about 960 mm/year on average (Hastenrath and Greishar 1993), with much rainfall particularly in March and April. The weekly DEN case number recorded for the city of Fortaleza remained very low (<100 cases/week) during the first four months of the years 2005 to 2007. This number rapidly grew to 1,000 cases per week in July, 2005 and June, 2006, a few months after the end of the wet season. The year 2007 was somewhat different with an earlier slow increase, including a lower seasonal peak (around 600 cases/week in May-June) and a continuation of a significant number of cases during the second semester (around 150 cases/week).

This study is built on data obtained from extensive fieldwork. In this light, it is an important contribution to the literature and can help to improve current understanding of mosquito distribution. Daily mortality is an important determinant of a vector's ability to transmit pathogens. It has long been accepted that mosquito mortality rate varies according to its age, but there is no published work describing the relationship between the survival rate of *Ae. aegypti* and environmental factors. Although different statistical models, such as exponential, Weibull, and Gompertz models are used in the literature to describe the relationship between mortality rate and age, in this study we apply the proportional hazards model, also called the Cox model, to relate time to death to a number of environmental explanatory variables (covariates)

as a robust alternative to the previously developed models. The Cox model has the advantage of preserving the variables in their original quantitative form while using a maximum of information.

We tested whether *Ae. aegypti* do senesce and if there are associations and interactions among some environmental conditions, such as locality, survey surroundings, and dry (average precipitation below 60 mm) and wet (average precipitation above 180 mm) seasons, and the daily mortality of the mosquito. For that, we developed a three-year fieldwork, 2005-2007, in Fortaleza-CE, combining an experimental study with a statistical analysis of survival. The goal of mortality/survival analysis is to gain a deeper understanding of the mechanisms involved in aging according to different local environments and meteorological conditions that modulate mortality rate.

## MATERIALS AND METHODS

### Experimental procedure

Twelve experiments (EXP1 to EXP12) were conducted in Fortaleza during the three years of the study. They were distributed on seven sites (#1 to #7) distanced from each other by 3 to 10 km (Figure 1c and Table 1). Site #1 to Site #6 were operated in private or institutional locations with a natural air environment, while Site #7 was operated inside a closed air-conditioned room at the Ceará State Secretary of Health (SESACE). A programmable automatic recorder for air temperature and relative humidity sensor was located near a small cage with about 50 *Ae. aegypti* mosquitoes (see details below). Six experiments were conducted during the wet season: EXP1, EXP2, EXP6, EXP7, EXP10, EXP11 and the six others during the dry season: EXP3, EXP4, EXP5, EXP8, EXP9, EXP12 during the years 2005-2007.

For Sites #1 to #6, submitted to natural air conditions, significant seasonal differences were noted between the experiments operated during the dry seasons and the experiments operated during the wet seasons. The daily variations recorded a higher variability for averages and daily ranges during the wet seasons. However, the climate parameters were quite stable during the dry seasons. Independently of a specific season, the daily variations of the three variables were somewhat different among the sites. For instance, they were systematically higher for Site #2 than for Site #1.

In order to observe mosquito mortality under conditions which may be as close as possible to natural ones, 40-50 mosquitoes (35-45 females and about five males) were released in netted wooden cages (30x30x30 cm). The mosquitoes were drawn from their rearing cage two days after hatching. Just before release in the experimental cages, the females were blood fed for 2 h on anaesthetized quail. In the same day of blood feeding, the cages were installed in some of the experimental sites (Figure 1c). Ten percent sugar-imbibed cotton plugs were changed and dead mosquitoes were counted daily. A small tube with filter paper and tap water was renewed daily in each cage to collect the eggs (not studied here). Most of the twelve experiments lasted until the

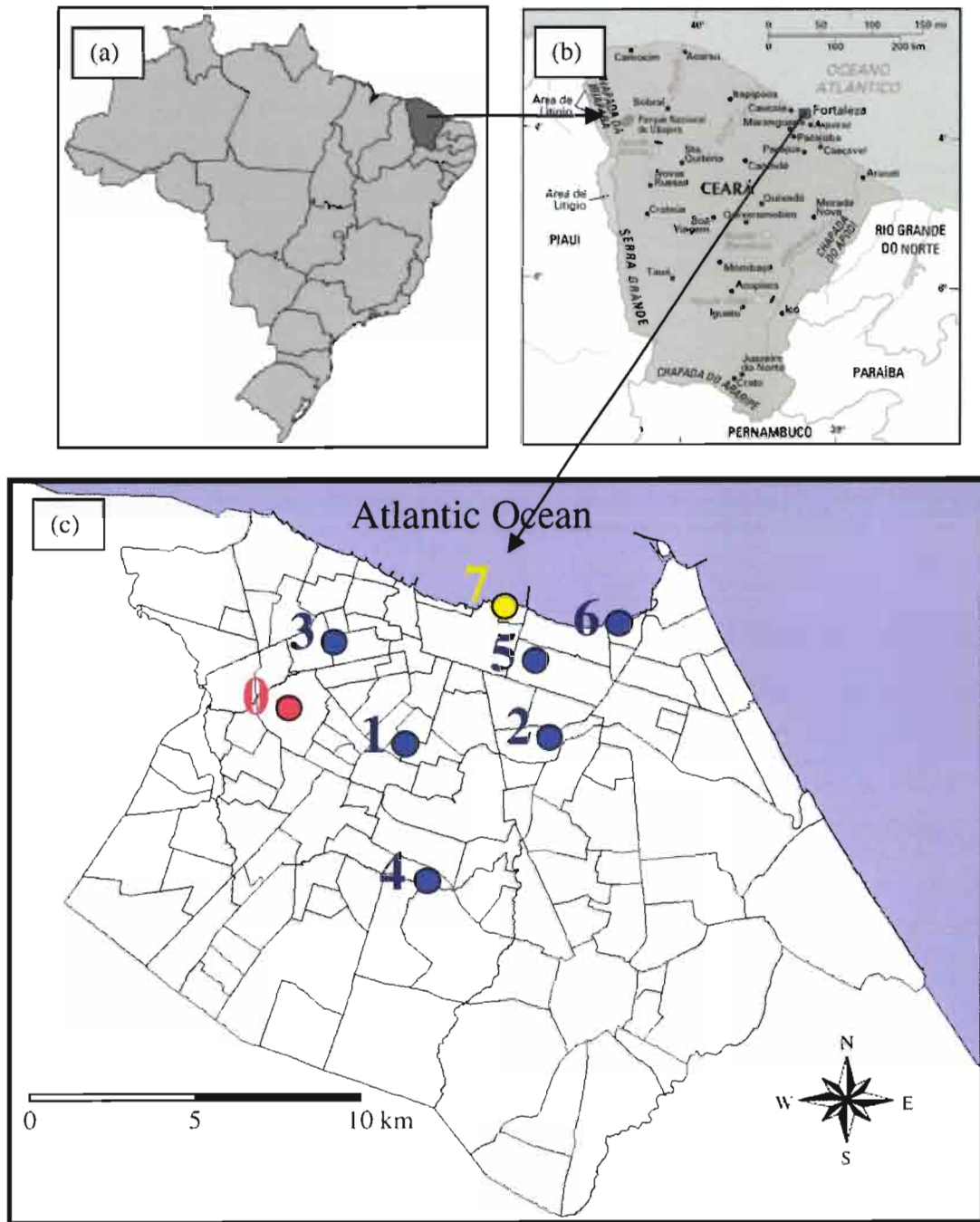


Figure 1. (a) Map of Brazil, (b) map of Ceará State, (c) map of Fortaleza with the areas of experiments: (0) UFC, (1) Montese, (2) Serrinha, (3) São Geraldo, (4) Pio XII, (5) Aldeota, (6) Mucuripe, (7) Iracema (SESACE).

Table 1. Durations (in number of days summarized from the first day of experiment (EXP) until the day of death of the last mosquito in the cages of the 12 EXP (lines 1 to 12) for the experimental sites (columns with local district names; see Figure 1 for exact locations). For a very limited number of cases, the EXP was interrupted when one (or maximum two) mosquito(es) was (were) still alive. The first column indicates the first day of each EXP (the same day for all sites). The last column is related to the SESACE reference site (with air-conditioning during most of the time). Various experimental problems are reported here : Pb1 - the total number of dead mosquitoes was significantly different (>3) from 50 (EXP 01-11) or 80 (EXP 12); Pb2 - presence of ants during the experiment, and/or discovering of hole(s) in the cage, and/or several mosquitoes drowned; Pb3 - technical problem with the AT/RH sensor; Pb4 - air-conditioning problem (SESACE site only); and Pb5 - handling problem; see the text for explanations. NA is "not available" (site not operated). The duration averages are computed (i) according to the EXP number (yellow column at right, where dry and wet seasons are differently hatched), or (ii) according to a same site (last line in yellow). For all the EXP, the initial number of mosquitoes in each cage was 50, except for the EXP 12 when this number was 80.

N° Exp.	Montese	Serrinha	São Gerardo	Pie XII	Aldeota	Mucuripe	Average**	Iracema**** (SESACE)
1 18 Feb 05	67 days	55 days	57 days (Pb1-5)	67 days	NA	NA	63 days	30 days (Pb05)
2 06 May 05	65 days	65 days	49 days (Pb5)	31 days (Pb2-5)	NA	NA	65 days	61 days
3 12 Aug 05	56 days	53 days	NA	NA	45 days (Pb01-05)	NA	54.5 days	56 days
4 18 Nov 05	26 days	24 days	17 days (Pb1-5)	NA	19 days (Pb1-5)	NA	25 days	51 days
5 01 Feb 06	48 days	36 days	27 days (Pb1-5)	NA	NA	NA	42 days	58 days
6 26 Apr 06	57 days	41 days	34 days (Pb5)	NA	52 days (Pb5)	NA	49 days	34 days
7 24 Aug 06	46 days	49 days	NA	NA	20 days (Pb5)	NA	46.5 days	49 days (Pb04-05)
8 31 Oct 06	63 days	57 days	NA	NA	27 days (Pb5)	NA	60 days	48 days
9 23 Jan 07	48 days	54 days	NA	NA	34 days (Pb5)	32 days (Pb2)	51 days	56 days
10 11 May 07	47 days	41 days	NA	NA	20 days (Pb1)	5 days (Pb2)	44 days	41 days
11 24 Jul 07	39 days	38 days	NA	NA	NA	37 (Pb2-3)	38.5 days	41 days
12* 27 Sep 07	20 days (Pb2)	30 days	NA	NA	NA	58 days (Pb1-2-3)		8 days (Pb04)
Average***	51.1 days	46.6 days					48.9 days HS=49.8 d DS=48.2 d	49.5 days

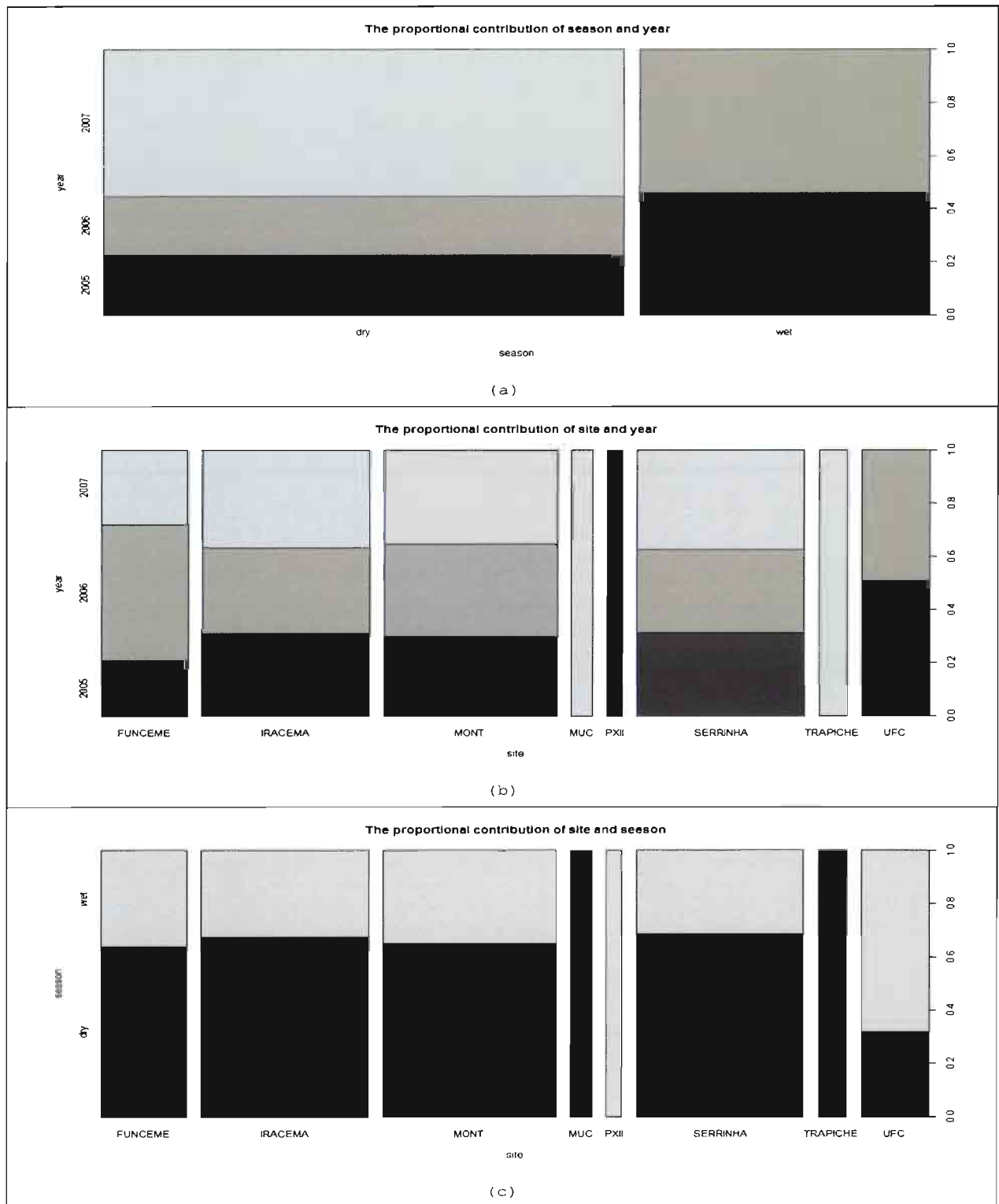


Figure 2. The proportional contribution of the (a) season and year, (b) site and year, and (c) site and season effects to the mosquito mortality experiment.

death of the last mosquito in the last cage.

Some additional information about local conditions, the proportional contributions of age, season, and sites, are presented in Figure 2 and Table 1. Figure 2a shows that 2/3 of the data were obtained during the dry season and that there were no data from the wet season in the third year (2007). In Figure 2b, it appears that the three years were evenly represented in five sites, but only Sites #1, #2, and #7 were equally represented in the data. In these same sites, we obtained more data during the dry season than during the wet season: Sites #4, #5, and #6 were sampled only during one season (Figure 2c). Figure 3 shows the proportion of relevant data obtained in each environmental condition, even with the experimental difficulties encountered in Sites #4, #6, and #7. All experiments were conducted only in Sites #1, #2, and #7.

#### Analysis of the influence of local environment on mortality

According to the experimental design, we quantified the influence of two local environmental factors on mosquito mortality: season (dry or wet) and site. Since these environmental factors are assumed not to change during each experiment, their influence can be measured straightforwardly by comparing the average lifetime obtained in each instance of each factor. As the number of instances and resulting sample sizes used for this comparison vary and are relatively small, it was clearly necessary to cautiously assess the statistical significance of obtained differences. To perform the latter, we used  $p$ -values of the classical Kruskal-Wallis (KW) test.

#### Analysis of the influence of age on mortality

The modeling of survival data centers on the hazard function (the instantaneous death rate), which is used to express the risk or hazard of death at time  $t$ . Mortality rates and survival are related to one another by the equation  $ds/dt = -ms$ , describing change on time, where  $s$  is the fraction of a population surviving and  $m$  is the mortality rate. This equation is used to determine the survival function corresponding to a mortality function.

The survival function, conventionally denoted by  $S$ , is defined as  $S(t) = 1 - F(t)$ , where  $F(t) = P(T \leq t)$  is the cumulative distribution function (cdf), with  $T$  the random variable denoting the time of death. The survival function must be non-increasing. This reflects the notion that survival to a later age is only possible if all younger ages are attained. The survival function is usually assumed to approach zero as age increases without bound, i.e.,  $S(t) \xrightarrow{t \rightarrow \infty} 0$ . The hazard function (also known as hazard rate), is defined as the event rate at time  $t$  conditional on survival until time  $t$  or later:  $P(t \leq T < t + \delta t | T \geq t)$ . So, the hazard function can be defined as  $h(t) = f(t)/S(t)$ , where  $f(t)$  is the probability density function (pdf) and  $S(t)$  is the survival function (Lawless 2003). Because of censoring, it is convenient to model survival times through the hazard function or the log hazard function.

Some of the most common hazard functions applied in survival analysis were used to explore the underlying mosquito survivorship. These included a constant death rate, the Gompertz function (the rate of mortality increases with

age in such a manner that its logarithm is linearly proportional to age), the Weibull function (the rate of mortality increases or decreases monotonically with age depending on the values of a shape and a scale parameter), and the the Cox proportional-hazards regression model (a statistical technique for exploring the relationship between survival and several explanatory variables).

In the Gompertz model, one states that the law of geometric progression influences mortality after a certain age. In this model, the hazard rate is given by the expression  $h(t) = \alpha \exp[-\beta \exp(-\gamma t)]$ , where the parameter  $\beta$  is the senescent component or the rate of increasing during lifetime, and  $\beta$  is proportional to the initial relative growth rate, when  $t=0$ , characterizing the exponential mortality increase with age. The parameter  $\alpha$  is the final mortality rate, reached with  $t$  going to infinity; the parameter  $\gamma$  is the relative growth rate at inflection point. Thus the curve is monotonously increasing in  $t$ . Note that since the Gompertz model is for a mortality hazard, one can integrate it to obtain the survival function. Also note that log-mortality is a linear function of age:

$$\log[h(t)] = \log(\alpha) - \beta \exp(-\gamma t).$$

This suggests a regression in time (age) approach which may be useful. The Gompertz survival function thus corresponds to exponential mortality rate increases with time.

The three-parameter Weibull hazard function (power mortality function) is determined by:

$$h(t) = \alpha \exp[-\exp(\beta \log(t) - \mu)]$$

where  $\beta$  is the shape parameter,  $\alpha$  is the scale parameter, and  $\mu$  is the location parameter. When  $\beta < 1$  the hazard function is decreasing; once  $\beta = 1$  the mortality rate is constant; and when  $\beta > 1$  the mortality rate is increasing.

In the three-parameter log-logistic model, the hazard rate for a specific age is characterized by the expression:

$$h(t) = \alpha \{1 + \exp[\beta \log(t) - \log \mu]\}^{-1}$$

where the parameter  $\beta$  is the senescent component, the parameter  $\alpha$  is the final mortality rate, reached for  $t$  going to infinity, and the parameter  $\mu$  is the relative growth rate at the inflection point. In all these hazard rate models one can add a *constant* term (*shift* parameter) representing an age-independent mortality parameter (Makeham-based models, cf. Comfort 1979).

Survival data is fitted to age-dependent models for mosquitoes (Styer et al. 2007, Dawes et al. 2009), and some are better fitted by a logistic survival function than by the more traditionally used Gompertz or Weibull functions (Wilson 1994). Thus, we fitted our experimental survival data to three parametric mortality models, namely the Gompertz hazard function, Weibull hazard function, and logistic hazard function, the latter being a particular case of the Cox Regression.

Inside the framework of hazard rate, we estimated an empirical hazard function, with a constant hazard in the case of an exponential distribution of survival times, with  $h(t) = \alpha$ , a log-linear hazard in the case of the Gompertz distribution of survival times, with  $\ln(h(t)) = \alpha + \gamma t$ , leading to the Weibull distribution of survival times, with  $\ln(h(t)) = \alpha + \gamma \ln(t)$  (Cox and Oakes 1984). The Gompertz survival function corresponds to exponential mortality rate increasing with time. The Weibull

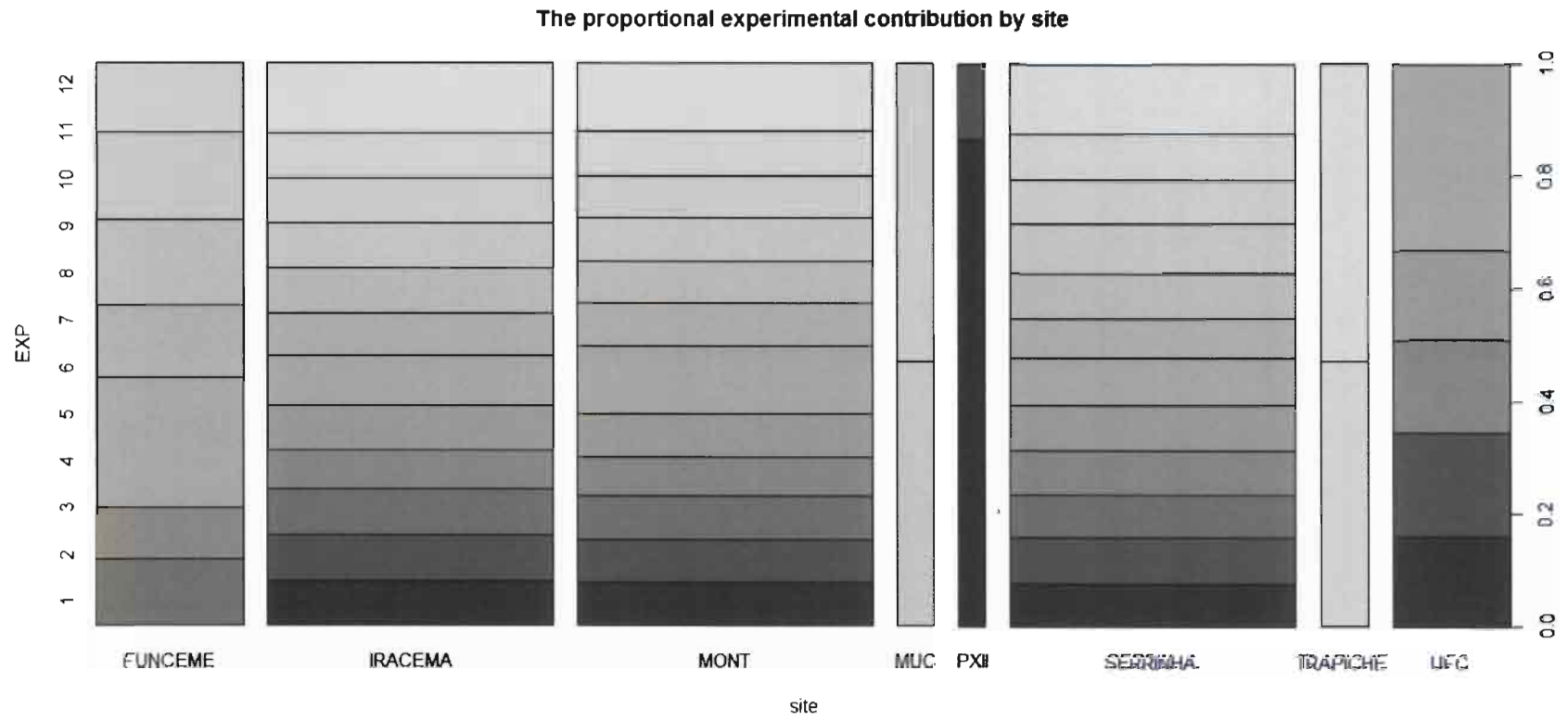


Figure 3. The proportional contribution of the site and experimentation (EXP) effects to the mosquito mortality experiment.



survival function corresponds to mortality rates that increase as a power function of time. A three-parameter logistic mortality function reflects mortality rates that rise and then become constant with age.

The purpose of using those three distinct models is to detect senescence i.e., the increase of hazard rate with age. We thus compared the fit obtained with a constant hazard rate (no senescence) to the fit obtained with two increasing hazard functions (some senescence) that proved appropriate in previous attempts to model mosquito mortality. Therefore, to be familiar with survival analysis models, we will briefly review the basic model concepts, mainly to establish terminology and notation.

It is worthwhile to note that Gompertz and Weibull functions imply contrasting biological causes of demographic aging - the terms describing increasing mortality can result from an increase in the vulnerability of individuals to extrinsic causes in the Gompertz model and the predominance of intrinsic causes at older ages in the Weibull model. They thus provide biologically meaningful parameters.

#### **Analysis of the influence of both local environment and age on mortality**

Introduced by Cox (1972), the proportional hazards model was developed to estimate the effects of different covariates influencing mortality. Cox proportional hazard allows the analysis of the effect of several risk factors on survival. The proportional hazard model is the most general of the regression models because it is not based on any assumptions concerning the nature or shape of the underlying survival distribution. The model assumes that the underlying hazard rate is a function of the independent variables (covariates); no assumptions are made about the nature or shape of the hazard function. Thus, Cox's regression model may be considered to be a nonparametric method. According to this model, the hazard rate of an individual is affected not only by its lifetime, but also by the covariates under which it lives, for instance, a combination of different factors such as air relative humidity, air temperature, and local environment. This function shifts up or down by an order of proportionality with changes in each  $X_i$ . As in the exponential or Weibull models, the hazards remain proportional (Cox 1972, Cox and Oakes 1984). This model has a number of useful and attractive characteristics. For instance, under a log-linear model assumption for  $f(X)$  and without any further assumptions about the life distribution model, it is possible to analyze experimental data and compute maximum likelihood estimates.

## **RESULTS**

#### **Experimental procedure**

Each of the twelve experiments from 2005 to 2007 lasted until the death of the last mosquito. Though the operational procedure appears simple, it does not prevent practical difficulties. Those difficulties are indexed according to five categories which appear in Table 1 and are listed in its associated caption. Most of the problems occurred within Sites #3 to #7. Site #1 and Site #2 were generally free from

these technical difficulties. Thus, using only Sites #1 and #2, the total average of the 11 first experiment durations (the 12th experiment was operated with 80 mosquitoes) is 48.9 days (Table 1), with an average during the dry season (48.2 days) a bit less than during the wet season (49.8 days). The difference is larger when comparing the averages of the first 11 experiments (dry and humid seasons together), either for Site #1 (51.1 days) or for Site #2 (46.6 days). Indeed, the mortality of the mosquitoes seems higher when the daily range of climatic conditions is also higher, as discussed previously. However, that hypothesis seems diminished by the following result. The total average of the experiment durations for Site #7, supposed to be a seasonal effect-free site where the air conditioning was (generally) operated during the daytime, is very close to the general average for Site #1 and Site #2 (49.5 days vs 48.9 days). Finally, the 12th experiment, the only one with 80 mosquitoes in the cages, does not allow for conclusions about a significant difference between the filling conditions of the cages. In spite of all these uncertainties, we processed the experimental data analyses according to statistical techniques, the results of which are described in the following subsections.

#### **The influence of local environment on mortality**

Analyzing the outcome of the Kruskal-Wallis test, we rejected at a significance level of 5% the independence assumption for the two environmental factors analyzed (seasonal and location effects). Thus, at least one factor has a statistically significant influence on mosquito mortality. Statistically significant year effect was detected, but this analysis was only exploratory. Besides, we observed that for 2007 the mortality was below average, whereas in 2005 and 2006 the mortality was above average. However, there is some interplay between season and year effect. The reason why 2007 has the major part below average might simply be that the experimental survey was carried out only in the dry season, despite efforts to capture the wet season during this atypical year. If one considers the season effect, one detects that the wet season has a clear positive influence on lifetime and therefore on survival (Figure 4b). Overall, lifetime is increased by 20% during the wet season as compared to the dry season. This positive influence is perceived overall, but also year by year and site by site (Figure 2). Seasonal effect varies from year to year, and the positive lift during the wet season was much stronger in 2005 than it was in 2006. Sites also influence lifetime significantly (Figure 4c). The site effects vary somewhat with year and season but are directionally identical. However, it is not clear which elements in the sites cause such variations. Figure 5 illustrates this fact contrasting the three sites: Montese (Site #1), Serrinha (Site #2), and Iracema (site #7). Site #1 shows a significant difference between mortality during the dry and wet seasons (Figure 5a), but there were no seasonal effects concerning the two other sites.

#### **The influence of age on mortality**

A common question concerning the survival times analysis is given two or more samples, is there a difference



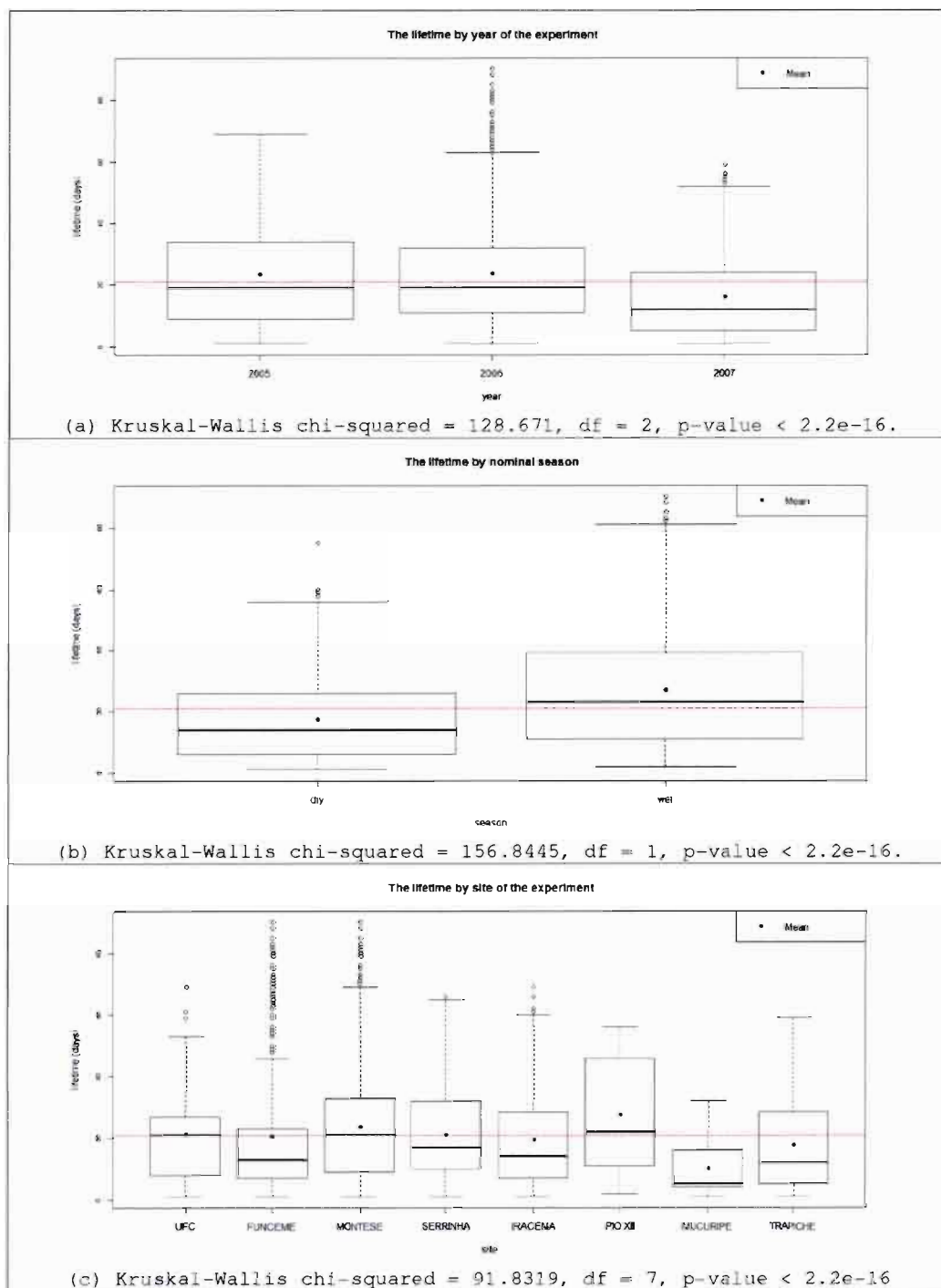


Figure 4. The box-plot representation and the Kruskal-Wallis tests for (a) year, (b) season, and (c) location.

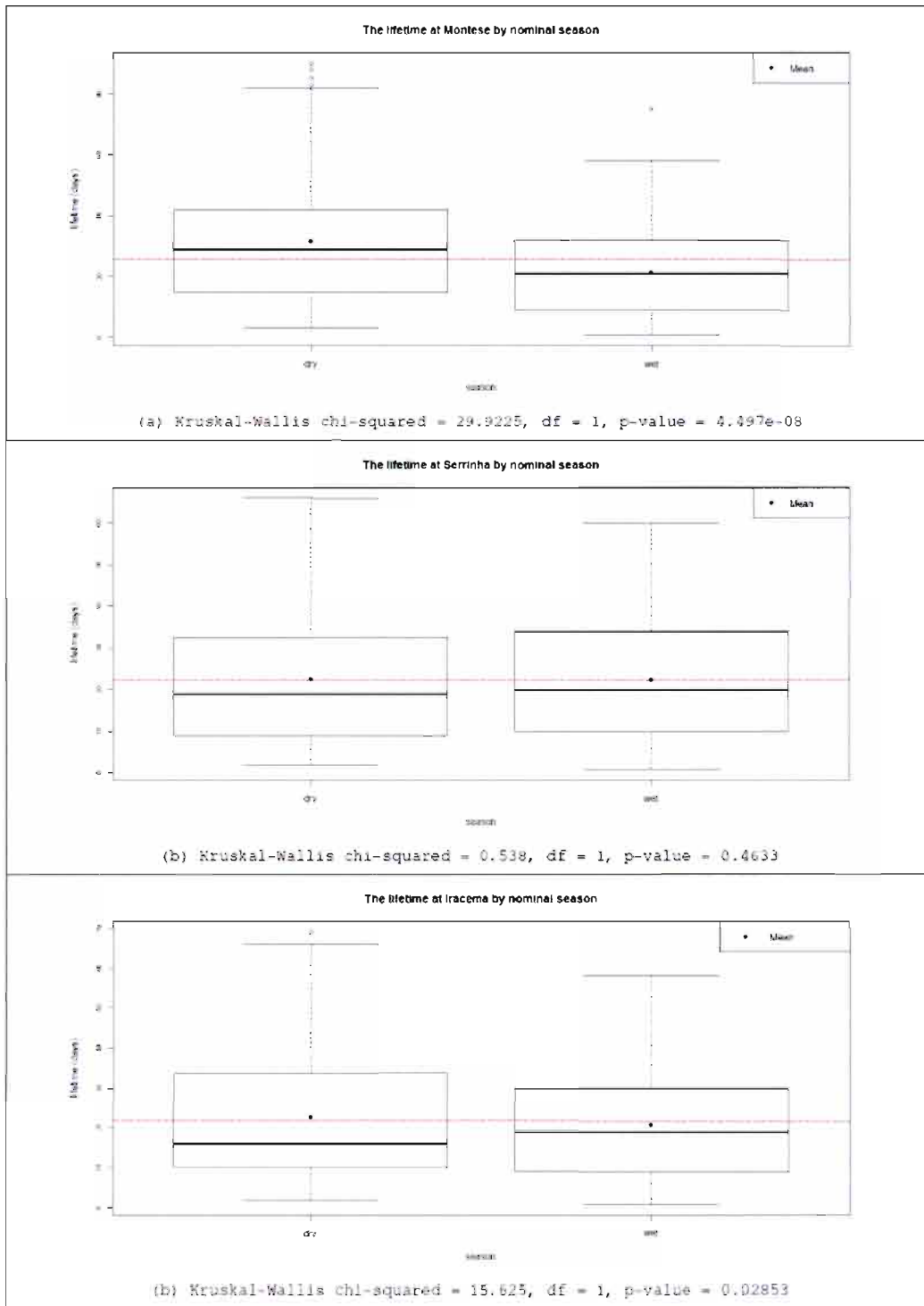


Figure 5. The box-plot representation and the Kruskal-Wallis tests for three selected locations by season.

between the empirical survival times? Thus, we have applied a test available in the R "survival" library (R Development Core Team 2012). It tests if there is a difference between two or more survival curves using the *G-rho* family of tests (Harrington and Fleming 1982), where the null hypothesis ( $H_0$ ) states that there is no difference between groups or strata. Taking into account the EXP1 to EXP11 that had produced the results displayed in Figure 6 (a naive estimation method of the hazard function  $h(t)$  by getting the negative of the log of the survival function  $S(t)$ ), to check the null hypothesis, there is a statistical significant difference between the ages and the seasons on the mosquito mortality, considering overall experimental sites.

The survival time average was 20.6 days for Site #1 and 21.5 days for Site #2, with a survival average during the dry season of about 13.9 days for Site #1 and 14.5 for Site #2, less than the wet season: 32.5 days for Site #1 and 31.7 days for Site #2. Taking into account the Kruskal-Wallis test results, we conclude that for Site #2 and Site #7 there was no seasonal effect. On the contrary, for Site #1, there was a statistically significant difference between the seasons on the mosquito mortality. We note that the total average of the experiment durations (directed related to the survival) for Site #7 throughout the dry season of 2007, where the air conditioning was operated during the duty day time, is very close to the general average for Sites #1 and #2. Thus, it seems difficult to assert that the climatic daily conditions have a significant influence on mosquito mortality.

It is interesting to observe that for the 12th experiment (realized during the dry season of 2007 with a starting number of 80 mosquitoes), there was a significant difference between the climatic conditions at Iracema (Site #7), Montese (Site #1), and Serrinha (Site #2). For the EXP1 to EXP11 at Iracema (Site #7), Montese (Site #1), and Serrinha (Site #2), the estimated hazard function showed a clear overall increasing pattern (Figure 6), being linear during most of the lifecycle, though reaching a plateau for old individuals. This pattern is mainly consistent with the results of Styer et al. (2007).

To assess the significance of the hazard function's increasing pattern (Site #1 and Site #2) vs a near-constant (Site #7) mortality, one observe that the plateau parameters of the Gompertz model are significantly positive. Thus, the increasing pattern is very unlikely to be an effect of estimation noise. Our results therefore confirm senescence, consistently with those of Styer et al. (2007). To assess the significance of the plateau pattern vs a constant growth of hazard rate, one notes that parameter  $\gamma$  of the log-Logistic and Weibull models that drive the plateau effect is again significantly positive (Figure 6). Thus, the plateau pattern is also unlikely to be caused by noise and is rather likely to be a structural feature of mosquito life cycle. This finding is also consistent with the results of Styer et al. (2007).

A common research question in biological or entomological research is to determine whether or not certain variables are correlated with survival times. Hence, alternatively, one can consider the methodology of proportional hazards model.

### The influence of both age and local environment on mortality

On behalf of the Cox proportional model (Figure 7) for Serrinha (Site #2), where mortality was more discriminated by seasonality, only the covariate seasons have highly statistically significant coefficients at a 5% significance level. The power exponential coefficients in the second column of the first panel and in the first column of the second panel of the R output are interpretable as multiplicative effects on the hazard. Thus, as illustration, we consider the case of holding the other covariates constant; an additional wet season increases the daily mosquito survival by a factor of about 1.69 on average, or 69%. The likelihood-ratio, Wald, and score chi-square statistics at the bottom of the output are asymptotically equivalent tests of the omnibus null hypothesis that all of the  $\beta$ s are zero. In this instance, the test statistics are in close agreement, and the hypothesis is rejected.

## DISCUSSION

Mosquito mortality is an important parameter of vectorial capacity, especially in the case of arboviruses for which no vaccine is available, and thus the elimination of the vectors remains the only means of prevention (Barbazan et al. 2010). Natural mortality of adult mosquitoes may be due either to intrinsic (aging, genetic) or extrinsic factors (predation, control measures, weather). The present work showed that mortality of *Ae. aegypti* in semi-natural conditions may no more be considered a constant phenomenon during the life of adult mosquitoes. It varies according to the age of the mosquitoes and to local environment, even in tropical climates.

Most authors have considered that only the exponential distribution of the survival may be sufficient and that tropical mosquito mortality rates are independent of age (Focks et al. 1993a, Focks et al. 1993b, Focks et al. 1995). However, for a more realistic description of various survival time data, other distributions are required. The more frequently used are the Gompertz or the Weibull distributions. Another commonly used distribution in survival time analysis is the log-logistic distribution (Lee and Go 1997), but this distribution does not have the proportional hazards property. Among the known parametric distributions, only the exponential, the Weibull, and the Gompertz models share the assumption of proportional hazards with the Cox proportional hazard function (Lee and Go 1997). Our statistical analysis showed that the fitting ability was in the order: seasonal Cox proportional hazard function > three-parameter Gompertz > three-parameter Weibull > three-parameter logistic.

Including these results in models that will assist early warning systems would allow better predictions of risks of epidemics (Degallier et al. 2010). In fact, the age-dependent and local-environment-dependent mortality encourages research on mosquito population dynamics. Predictive computer models have been developed to determine more accurately a limit for epidemic risk in a particular locale.

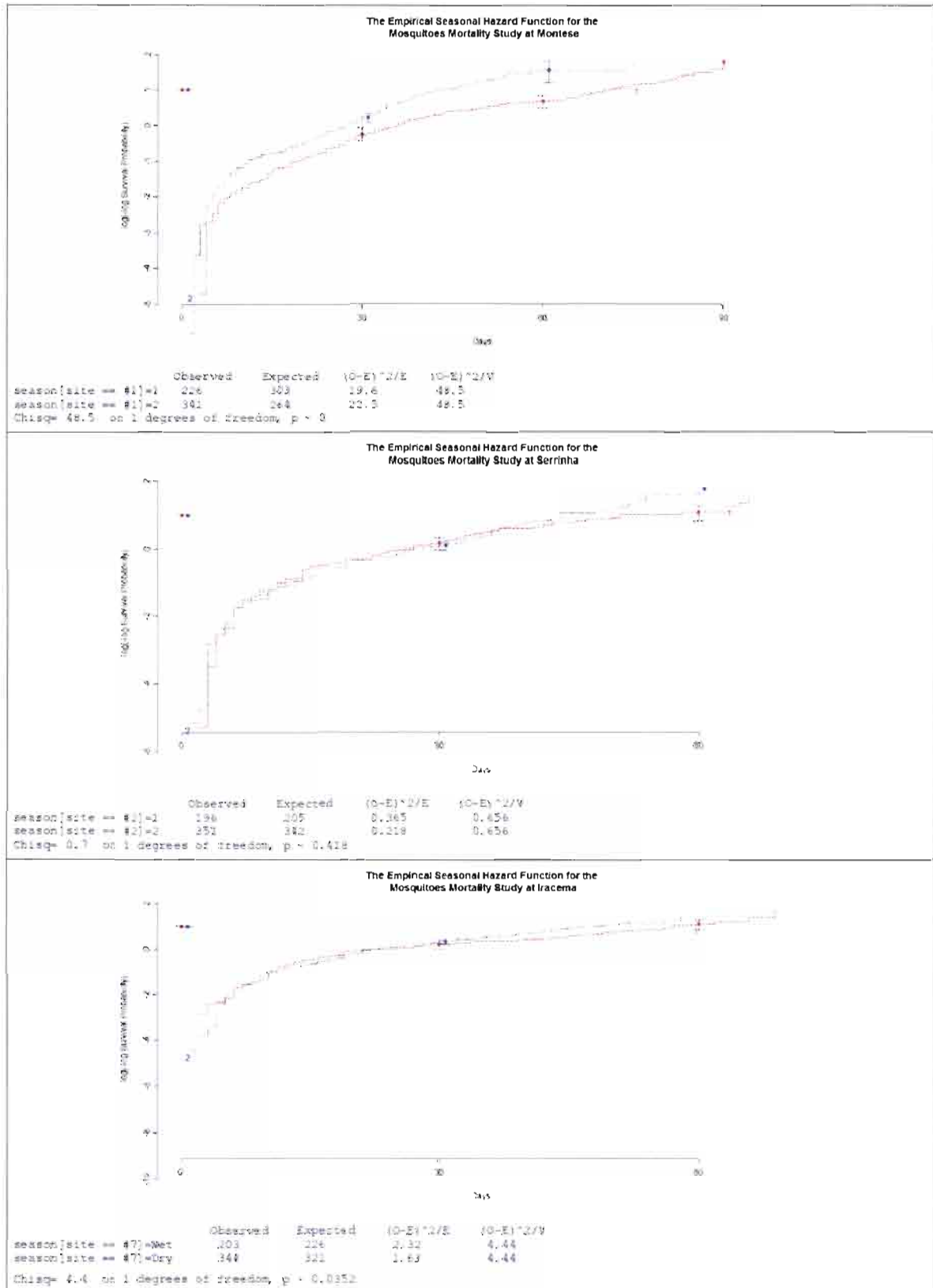


Figure 6. The seasonal empirical hazard functions for three selected locations. The blue line represents the rainy season and the red line the dry season.

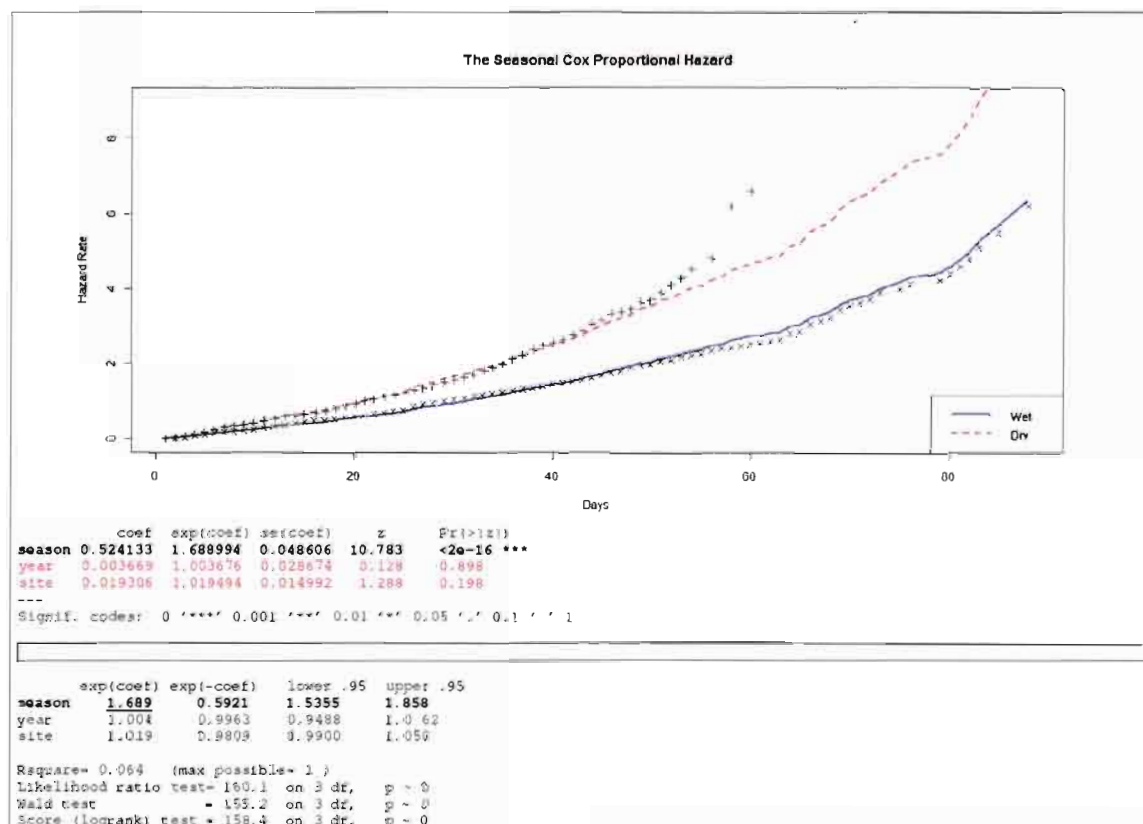


Figure 7. The estimated Cox proportional hazard functions for seasonal effect at Serrinha (Site #2). The response is survival time.

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