Seasonal population dynamics of the primary yellow fever vector *Haemagogus leucocelaenus* (Dyar & Shannon) (Diptera: Culicidae) is mainly influenced by temperature in the Atlantic Forest, southeast Brazil

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BACKGROUND Southeast Brazil has recently experienced a Yellow Fever virus (YFV) outbreak where the mosquito *Haemagogus leucocelaenus* was a primary vector. Climatic factors influence the abundance of mosquito vectors and arbovirus transmission.

OBJECTIVES We aimed at describing the population dynamics of *Hg. leucocelaenus* in a county touched by the recent YFV outbreak.

METHODS Fortnightly egg collections with ovitraps were performed from November 2012 to February 2017 in a forest in Nova Iguaçu, Rio de Janeiro, Brazil. The effects of mean temperature and rainfall on the *Hg. leucocelaenus* population dynamics were explored.

FINDINGS *Hg. leucocelaenus* eggs were continuously collected throughout the study, with a peak in the warmer months (December-March). The climatic variables had a time-lagged effect and four weeks before sampling was the best predictor for the positivity of ovitraps and total number of eggs collected. The probability of finding > 50% positive ovitraps increased when the mean temperature was above 24°C. The number of *Hg. leucocelaenus* eggs expressively increase when the mean temperature and accumulated precipitation surpassed 27°C and 100 mm, respectively, although the effect of rainfall was less pronounced.

MAIN CONCLUSIONS Monitoring population dynamics of *Hg. leucocelaenus* and climatic factors in YFV risk areas, especially mean temperature, may assist in developing climate-based surveillance procedures to timely strengthening prophylaxis and control.

Key words: mosquito ecology - oviposition - rainfall - temperature - yellow fever - Rio de Janeiro
YFV transmission, the abovementioned Hg. leucocelaenus population was experimentally competent to transmit Chikungunya virus,\(^{23}\) and one amplicon of putative DENV-1 was found in one pool of this species collected in northeast Brazil.\(^{24}\)

Climatic factors influence the abundance and activity of mosquito vectors, which in turn affect arbovirus transmission such as YFV.\(^{25,26,27}\) Here, we evaluated the influence of climatic variables such as temperature and rainfall in the seasonal dynamics of Hg. leucocelaenus during a long-term egg collection conducted in an Atlantic Forest area in a county of southeast Brazil touched by the recent YFV outbreak.

**MATERIALS AND METHODS**

**Study area** - The study was conducted in Parque Natural Municipal de Nova Iguaçu (PNMNI) (22°46'45"S 43°27'23"W), a conservation area of 1,100 hectares of the Atlantic Forest biome at the northwest flank of the Gericinó massif, adjacent to the periurban zone of the municipality of Nova Iguaçu, at 35 km from the city of Rio de Janeiro, Brazil.\(^{25}\) As other counties in southeast Brazil, the recent YFV outbreak reached Nova Iguaçu in 2017-2018, with records of autochthonous human cases, numerous epizooties of NHPs and natural infections in sylvatic mosquitoes.\(^{25,26,27}\) The forest in PNMNI cover an essentially mountain area, with altitude varying from 150-956 m. The local climate is classified as Aw (Köppen-Geiger classification) with rainy summer (December to March) and dry winter (June-September); the average temperature and annual rainfall are 23.4°C and 1408 mm, respectively.\(^{30}\)

**Mosquito collection** - Mosquito collections were approved by local environmental authorities (PNMNI license 001/14-15; SISBIO-MMA licenses 37362-2 and 012/2016). A total of 20 ovitraps\(^{31}\) containing ~ 300 mL of water from a local source and leaf litter, and three plywood paddles (Eucatex\(^{6}\), Brazil) as oviposition support was suspended on tree branches at a height of 3-12 m. Ovitraps were distributed in the forest at different distances from each other (3.3-22.9 m) and from the edge of a narrow path (0-173.2 m) that runs roughly parallel to a non-perennial stream. The number of used ovitraps per sampling and their locations did not change during the entire study. Paddles were sampled from November 2012 to February 2017. At each fortnightly sampling, the used paddles were changed by new ones, and brought to the laboratory, allowed to dry slowly for ten days in an insectary (26 ± 1°C, 70 ± 10% RH) and examined to egg counting. Eggs were then hatched by immersing the paddles twice in dechlorinated tap water for two consecutive days. Larvae were reared in pans (~ 50 larvae/pan measuring 25 x 25 x 10 cm) containing 1 L of dechlorinated tap water, supplemented with yeast powder and shed leaves, renewed every 2-3 days. Emerged adults were transferred to cubic (30 cm) mesh cages supplied with 10% honey solution in the mentioned insectary, and soon morphologically identified to species according to Consoli and Lourenço-de-Oliveira.\(^{25}\)

**Climate data** - To evaluate the influence of temperature and rainfall in the seasonal dynamics of Hg. leucocelaenus oviposition in the ovitraps we used data obtained by the closest meteorological stations of Instituto Nacional de Meteorologia (INMET) with availability of the required climate data for the study period. Accordingly, daily mean temperatures were available from the Rio de Janeiro station (A636; OMM: 83743, 18 km from PNMNI), while regarding daily rainfall we used the average of the records of the two nearest stations from the collecting site: the Duque de Caxias - Xerêm (A603; OMM: 86877, 28 km from PNMNI) and Ecologia Agrícola - Seropédica (A601; OMM: 86878; 42 km) stations.

**Data analysis** - We calculated two indices: (a) the proportion of positive ovitraps per sampling (“positive” means at least one egg was found on paddles) and (b) the total number of eggs collected in all ovitraps per fortnightly sampling. Exploratory data analysis and model validation was conducted following Zuur et al.\(^{32}\)

We explored the effects of mean temperature and rainfall (fixed effects) on the proportion of positive ovitraps using generalised linear mixed models (GLMMs)\(^{32}\) with a binomial error structure and logit link. We considered as fixed predictor variables the mean temperature and total rainfall accumulated in 1-6 weeks prior to the sampling event as well as the height of the ovitrap and its distance to the edge of the forest. The ovitrap was considered as a random factor to control for variation among ovitraps. Such variation could be related to the height of the ovitrap and its distance from the edge of the forest, so we specified them as random slopes of the random effect.

Since the variance of the total number of eggs (2932) was much greater than its mean (31), characterising overdispersion, the Negative Binomial probability distribution was chosen instead of the Poisson distribution, the typical probability distribution for modeling counting data. The effects of mean temperature and rainfall (fixed effects) on the total number of eggs were analysed using GLMMs\(^{32}\) with a negative binomial error structure and logarithm link. In the same way as for the proportion of positive ovitrap models (see above), we considered as fixed predictor variables the mean temperature and total rainfall accumulated in 1-6 weeks prior to the sampling event and the height of the ovitrap and its distance to the edge of the forest. The ovitrap was also considered as a random factor.

In all cases, we started by building a full GLMM, then created nested GLMMs by evaluating the significance of predictors with Wald chi-square tests and dropping the non-significant individual predictors (p > 0.05) based on differences in model fit.\(^{32}\) We ensured the predictors were not correlated with each other.\(^{33}\) We ranked all candidate models by the lowest Akaike information criterion (AIC) and evaluated their relative likelihoods using AIC weights,\(^{34}\) considering a null model with only the intercept as a benchmark. The most parsimonious model was selected as the one with the lowest AIC. Models with the difference in AIC < 2 were considered equally plausible. We also calculated the area under the receiver operating characteristic (ROC) curves - the area under the ROC
Fig. 1: time series with (A) Sum of the total number of eggs collected in the 20 trap stations on each sampling event. (B) Time series of the mean temperature and accumulated rainfall considering the period in-between the sampling events.
curve (AUC)\(^{35}\) - to find the combination of predictor variables that maximises the probability of finding positive ovitraps. Finally, we followed the protocol to validate the most parsimonious GLMMs by inspecting Q-Q plots and plots of residuals against fitted data and deviance residuals against predicted data\(^{36}\). The GLMMs were carried out with the “glmer.nb” and “glmer” functions in “lme4” package in software R version 3.6.2.

To investigate potential nonlinearities on the effects of rainfall and temperature on total number of eggs, we also fitted a set of generalised additive mixed models (GAMMs) with log link function and negative binomial distribution (for the total number of eggs) and binomial distribution (for the positivity of the ovitrap). GAMMs are an extension of GLMMs that allows for the inclusion of nonparametric smoothing terms in the place of the constant parameters. By plotting the fitted smooth terms versus the predictor, one may investigate the nature of the relationship between the predictor and the outcome variable, detecting potential nonlinearities.

RESULTS

*Haemagogus leucocelaenus* was the only species of *Haemagogus* detected in the area and by far the predominant mosquito ovipositing in the settled ovitraps throughout the collection period. The other two species occasionally found [*Aedes albopictus* (Skuse) and *Aedes terrens* (Walker)] were not considered.

![Fig. 2: circular histogram for the sum of the total number of eggs collected per month during the studied period. Each colour represents the sampled year.](image)
Oviposition of *Hg. leucocelaenus* was recorded in every sampling throughout the years (Fig. 1), gathering a total of 50,921 eggs the entire study. Higher egg amounts were usually recorded in the warmer months (December-March) than in those with lower mean temperatures (June-October) (Fig. 2), which respectively coincide with the periods of higher and lower rainfalls (Fig. 1). The exception was a peak reported in August 2015 (Figs 1, 2). Regardless of the sampling year, the mean number of eggs collected from April to October was consistently low, although the data for August differed from the pattern influenced by the apparently atypical collections in 2015 (Fig. 2). This general distribution tendency is confirmed when we analysed the monthly pattern of average number of eggs gathered in each month from 2012 to 2017 (Fig. 3). Again, the number of collected eggs was higher in January and December than in the rest of the year (particularly in June). Greater amplitude in egg counting was observed in the months of transition between summer and autumn (March) and between spring and summer (November).

We evaluated the influence of accumulated rainfall and mean temperature recorded from one to six weeks before sampling events in both GLMMs and GAMMs models. The accumulated rainfall of three weeks before a sampling event had a significant negative effect on the total number of collected eggs (Fig. 4). No significant positive influence of rainfall accumulated during any time lag on the amount of laid egg in the ensemble of ovitraps was found (Fig. 4). In contrast, the mean temperature recorded during the three and four weeks before a sampling event had a significant and positive effect on the total number gathered eggs (Fig. 4). When considering the probability of finding a positive ovitraps, it was noticed that the accumulated rainfall considering 1-6-weeks’ time lags did not affected positivity (Fig. 4). On the other hand, the mean temperature of four and six weeks before
Fig. 5: Smooth effect of temperature and rainfall at lag 1-6 weeks on the total number of collected eggs. Dotted lines indicated 95% confidence interval.
Fig. 6: Smooth effect of temperature and rainfall at lag 1-6 weeks on the positivity of the ovitraps. Dotted lines indicate 95% confidence interval.
the sampling event had a significant and positive effect on the positivity of the ovitraps, while a negative effect was found when considering a five-week time lag (Fig. 4).

Rainfall had a nonlinear effect either on the number of collected eggs or the positivity of ovitraps at 1-6 weeks’ time lags (Figs 5, 6). In contrast, temperature had a linear or relatively linear effect on the number of laid eggs in the ovitraps at 1-4 weeks, but became nonlinear after a 5-6 weeks’ time lag (Fig. 5). Considering positivity of ovitraps, temperature had a nearly linear effect only in the interval of 2-4 weeks’ time lag of a samplings (Fig. 6).

The estimates of the most plausible models for total number of eggs and positivity of ovitraps are in Tables II and IV, respectively. When evaluating the effect of temperature and rainfall with four weeks the model predicted a greater increase in the number of eggs when the mean temperature was above 27°C and when the accumulated rainfall was above 100 mm (Fig. 7). However, the effect of rainfall was less pronounced than the effect of mean temperatures. In the same direction, we found that the probability of finding more than 50% of ovitraps containing eggs was higher when the mean temperatures during the 4-weeks’ time lag before sampling was above 24°C (Fig. 8). This model also showed that we would expect to find more than 75% of the ovitraps with at least one egg when the accumulated rainfall during the 4-weeks’ time lag before sampling was above 100 mm (Fig. 8). The height of the ovitrap was included in the second most plausible model for the total number of eggs, but with a non-significant effect ($\beta = -0.06, p = 0.36$).

| TABLE I |
| --- |
| Comparison of the top five candidate generalised linear mixed models (GLMMs) for the total number of eggs |

| Model | Lag | AIC | $\Delta$AIC | k | wAIC |
| --- | --- | --- | --- | --- | --- |
| rainfall + temperature + (1 | ovitrap) | 4 | 12230.34 | 0 | 5 | 0.54 |
| rainfall + temperature + height + (height | ovitrap) | 4 | 12231.2 | 0.87 | 8 | 0.35 |
| rainfall + temperature + distance + (distance | ovitrap) | 4 | 12235.1 | 4.76 | 8 | 0.05 |
| temperature + (1 | ovitrap) | 4 | 12236.34 | 5.99 | 4 | 0.03 |
| temperature + height + (height | ovitrap) | 4 | 12270.3 | 6.8 | 7 | 0.02 |
| 1 + (1 | ovitrap) | null | 12354.9 | 124.6 | 3 | < 0.001 |

Lag: the time lag og the climatic variable (in weeks); AIC: Akaike information criterion; k: number of model parameters; $\Delta$AIC: difference between the AIC of a given model and that of the best model; wAIC: Akaike weights. Marked in gray are the most plausible models ($\Delta$AIC < 2).

| TABLE II |
| --- |
| Estimated parameters of the most parsimonious generalised linear mixed models (GLMMs) (Table I) describing the effects of temperature and rainfall with four weeks’ time lag on the total number of eggs, considering ovitrap as random variable |

| Estimate | SE | z-value | p-value |
| --- | --- | --- | --- |
| Intercept | -2.23 | 0.53 | -4.23 | < 0.0005 |
| Fixed effects | Temperature | 0.20 | 0.02 | 9.99 | < 0.0005 |
| | Rainfall | 0.004 | 0.001 | 2.71 | 0.007 |
| Random effects | Variance | 0.31 | 0.56 |
| Ovitrap | SDev | |

SE: standard error; SDev: standard deviation.

In Brazil, humans are contaminated by YFV during epizooties, by the bite of infected sylvatic mosquitoes, primarily *Hg. leucocelaenus* and *Hg. janthinomys*.[14,17] From the entomological point of view, only preventive measures to avoid mosquito biting when into or near epizootic forests by using repellents and personal protective equipment are plausible. These forest mosquitoes breed essentially in rather cryptic tree holes.[9,25] Thus, YFV control strategy based on the fight against their adult and immature forms is unfeasible.

On the other hand, understanding the population dynamics of YFV primary vectors such as *Hg. leucocelaenus* and the climatic variables influencing this dynamic may help in defining expanded risk areas, predicting silent virus circulation and NHP epizooties, timely implementing adequate prophylaxis and control strategies such as intensification of local vaccination campaigns in risk areas. In the present study, we described population dynamics of *Hg. leucocelaenus* based on a long-term egg collection in a forest located in a municipality of southeastern Brazil affected by the recent YFV out-
Variance break. In summary, our data evidenced that the egg counting in January was significantly higher in the PNMNI forest, which coincided with the peak month of human case records within the 2017-2018 epidemic wave in the southeast areas under influence of the Atlantic Forest.

TABLE III
Comparison of the top five candidate generalised linear mixed models (GLMMs) for the positivity of the ovitraps

| Model | Lag | AIC | ΔAIC k | wAIC | AUC ROC |
|-------|-----|-----|--------|------|---------|
| rainfall + temperature + (1 | ovitrap) | 4 | 1988.3 | 0 | 0.79 | 0.7393147 |
| rainfall + temperature + height + (height | ovitrap) | 4 | 1992.06 | 3.75 | 7 | 0.12 | 0.7389699 |
| rainfall + temperature + distance + (distance | ovitrap) | 4 | 1993.36 | 5.06 | 7 | 0.06 | 0.7390462 |
| temperature + (1 | ovitrap) | 4 | 1995.09 | 6.79 | 3 | 0.02 | 0.73572 |
| rainfall + temperature + height + distance + (height | ovitrap) + (distance | ovitrap) | 4 | 1998.48 | 10.18 | 11 | 5 | 0.7389089 |
| 1 + (1 | ovitrap) | null | 2161.4 | 173.1 | 2 | < 0.001 | 0.6512692 |

Lag: the time lag of the climatic variable (in weeks); AIC: Akaike information criterion; k: number of model parameters; ΔAIC: difference between the AIC of a given model and that of the best model; wAIC: Akaike weights; AUC ROC: area under the ROC curve. Marked in gray are the most plausible models (ΔAIC < 2).

TABLE IV
Estimated parameters of the most parsimonious generalised linear mixed models (GLMMs) (Table III) describing the effects of temperature and rainfall with four weeks’ time lag on the positivity of the ovitraps, considering ovitrap as random variable

| Parameter          | Estimate | SE   | z-value | p-value |
|--------------------|----------|------|---------|---------|
| Intercept          | -5.73    | 0.56 | -10.31  | < 0.0005|
| Fixed effects      |          |      |         |         |
| Temperature        | 0.24     | 0.02 | 10.95   | < 0.0005|
| Rainfall           | 0.005    | 0.001| 2.9     | 0.004   |
| Random effects     |          |      |         |         |
| Variance           | 0.33     | 0.57 |         |         |
| Ovitrap            |          |      |         |         |

SE: standard error; SDev: standard deviation.

firmed YFV epizooties in NHPs were recorded every month, except September in the 2017-2018 outbreak in southeast Brazil, although reports peaked during the rainy summer, December-March. Coincidentally, regardless the year of sampling, higher numbers of eggs of *Hg. leucocelaenus* were usually recorded from December to March than in those months with lower mean temperatures and rainfall, from June to October in PNMNI, with larger amplitudes in the number of gathered eggs in March and November, months of transition between summer and autumn and spring and summer, respectively. This pattern of monthly egg collection and biting activity of *Hg. leucocelaenus* has been described in other sites of Atlantic Forest in southeast and in the Cerrado in centre-west Brazil as well as in Trinidad. Moreover, egg counting in January was significantly higher in the PNMNI forest, which coincided with the peak month of human case records within the 2017-2018 epidemic wave in the southeast areas under influence of the Atlantic Forest.

Noteworthy, collections of *Hg. leucocelaenus* made simultaneously in three Atlantic Forest sites, in 2015-2016, that is, before the arrival of the YFV epizootic wave in this part of southeast Brazil, curiously revealed distinct dynamics from the aforementioned pattern. Accordingly, in PNMNI and Jacarapuá (~20 km away), unexpected peaks were respectively recorded in August 2015 and October 2015, in the dry-cold season, while in Casimiro de Abreu (~130 km from PNMNI) the egg counting peaked in December 2015, in the rainy season as expected. Thus, variations in the population dynamics of *Hg. leucocelaenus* can occur in the same type of biome a few kilometers apart. Coincidentally, an El Niño phenomenon categorised as very strong was recorded in 2015-2016.

The combination of high average temperature and precipitation recorded during the rainy summer favors YFV transmission and geographical spread of epizootic waves by positively influencing mosquito egg hatching and accelerating larval development. When we evaluated the influence of accumulated rainfall and mean temperature recorded from one to six weeks before samplings, rainfall accumulated during any time lag did not influence the amount of eggs laid by *Hg. leucocelaenus* at PNMNI. Moreover, but unlike Casimiro de Abreu,...
precipitation had a nonlinear effect either on the number of collected *Hg. leucocelaenus* eggs or the detection of eggs in ovitraps at any time lag in PNMNI. The height of the ovitrap in the tree canopies in PNMNI had a negative but very small effect on the total number of *Hg. leucocelaenus* eggs all 1-6 weeks’ time lags. It seems that this species may lay eggs and bite in a large range of heights in the Atlantic Forest.\(^{11,40,44}\) This ability to move vertically in the forest favors zoonotic transmission of pathogens from infected arboreal animals such as infected NHPs to humans.

In contrast to other tested variables, temperature showed to considerably influence in the population dynamics of *Hg. leucocelaenus*. The mean temperature recorded during four weeks before samplings had a significant and positive effect both on the total number eggs and the probability of finding a positive ovitrap. Also, temperature had a linear or relatively linear effect on the number of collected eggs and the positivity of ovitraps specially in the interval of 2-4 weeks’ time lag in PNMNI. These results are similar to observations made in another Atlantic Forest area, where
mean temperature but not rainfall recorded in the same month of sampling was positively related to number of collected *Hg. leucocelaenus* eggs.\(^{(51)}\)

It has been demonstrated that higher mean temperatures induce greater mosquito abundance and biting activity,\(^{(29)}\) besides reducing the duration of the extrinsic incubation period of YFV, that is the time elapsed between taking an infective bloodmeal and the delivery of viral particles in the saliva of a competent mosquito vector.\(^{(47-49)}\) An increase in temperature, but also of rainfall was noticed the month preceding the 2000 YFV outbreak in Brazil.\(^{(50)}\) Thus, temperature has an important influence in YFV circulation, and monitoring mean temperature in risk areas can help in predicting enhancement of YFV activity. Accordingly, when analysing the model predictions, we found that the probability of having more than 50% of ovitraps containing at least one egg of *Hg. leucocelaenus* was higher when the mean temperatures during the 4-weeks’ time lag before sampling is above 24°C. This data may be taken as an indirect sign of the start of increased biting activity of *Hg. leucocelaenus* in the area. Moreover, we verified an expressive increase in the number of eggs of *Hg. leucocelaenus* four weeks after the mean temperature surpasses 27°C. Mean weekly temperatures above 22-24°C was found to be strongly associated with high *Ae. aegypti* abundance and consequently with an increased risk of dengue transmission in Rio de Janeiro.\(^{(51)}\)

Although the effect of rainfall is less pronounced than that of mean temperatures in *Hg. leucocelaenus* population dynamics, we found that more than 75% of the ovitraps became positive and the number of eggs increased when the accumulated rainfall in four weeks’ time lag was above 100 mm. Very distinctly from the eggs of *Hg. janthinomys*, those of *Hg. leucocelaenus* hatch mostly after the first immersion in water.\(^{(52)}\) Hence, we assume that rainfall above 100 mm would raise the volume of water in the tree holes sufficiently to cover and lead to the immediate hatching of most of the existing viable eggs of *Hg. leucocelaenus*. We suppose that with the simultaneous increase in mean temperatures (≥ 27°C), the generation of adults that emerged in this batch would rapidly develop and increase the number of accumulated eggs detected four weeks later.

In conclusion, monitoring population dynamics of *Hg. leucocelaenus* in risk areas and expanded risk areas is an important component in the YFV surveillance system. Moreover, our data suggest that, besides monitoring mean temperature, and secondarily rainfall may assist in constructing climate-based surveillance procedures to timely making alerts of YFV activity, start emergency risk communication in risk communities and strengthening vaccination campaigns in target areas.

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**AUTHORS’ CONTRIBUTION**

DCL, JA and RLO conceived and designed the research; DCL and PL collected mosquitoes in the forest; MILB, DCA and RLO performed egg counting and mosquito rearing in the laboratory; CSA conducted data analysis; RLO and CSA wrote the manuscript. All authors reviewed and approved the manuscript.

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