

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

Dinair Couto-Lima¹, Cecilia S Andreazzi², Paulo José Leite³, Maria Ignez Lima Bersot¹, Jeronimo Alencar⁴, Ricardo Lourenço-de-Oliveira^{1/+}

¹Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz, Laboratório de Mosquitos Transmissores de Hematozoários, Rio de Janeiro, RJ, Brasil

²Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz, Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Rio de Janeiro, RJ, Brasil

³Secretaria Municipal de Saúde de Nova Iguaçu, RJ, Brasil

⁴Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz, Laboratório de Diptera, Rio de Janeiro, RJ, Brasil

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

From 2016-2019, yellow fever has caused an unprecedented outbreak in Brazil.⁽¹⁾ It was consequence of a southward epizootic spreading wave of Yellow Fever virus. (YFV) started in the Amazon in 2014.^(2,3) Almost 98% of the recorded 2,259 human cases and 773 deaths occurred in the Southeast region, in sites under influence of the Cerrado and Atlantic Forest biomes.⁽⁴⁾ The urban *Aedes aegypti* (Linnaeus)-borne transmission has been eradicated for more than 70 years from the country.^(5,6) Thus, human infections in this outbreak were typically acquired in the zoonotic sylvatic transmission cycle where non-human primates (NHPs) are the amplification vertebrate hosts and *Haemagogus* and *Sabethes* mosquitoes the primary and secondary vectors, respectively.^(1,7,8)

Haemagogus leucocelaenus (Dyar & Shannon) (Diptera: Culicidae: Aedini) is a sylvatic primatophilic mosquito distributed from Panama to northern Argentina and Uruguay, whose immature stages develops essentially in water contained in tree-holes.^(9,10) The eggs of *Hg. leucocelaenus* are laid on moist vegetal substrates near water level, where they resist to desiccation and keep the embryos viable outside the water for months^(9,11) Females of *Hg. leucocelaenus* are active during daytime, when they may attack NHPs at the tree canopies and humans on the forest ground or modified open fields.^(12,13,14) The species has been suggested as YFV vector in previous transmission emergences in Brazil and Colombia.^(12,15,16,17,18,19,20)

Entomological surveys conducted during the 2016-2019 outbreak in the southeast Brazil incriminated *Hg. leucocelaenus* as YFV primary vector together with *Hg. janthinomys* Dyar, while some *Aedes* and *Sabethes* species were considered to play a secondary or local role in transmission.^(14,21) It was noticed that *Hg. leucocelaenus* was widespread and abundant in the YFV foci, with detections of high rates of natural infections.^(14,21) In laboratory, a population of *Hg. leucocelaenus* from southeast Brazil has proven to be competent to transmit YFV belonging to distinct lineages of the South America I as well as the West-Africa genotypes.⁽²²⁾ Besides its role in

doi: 10.1590/0074-02760200218

Financial support: CNPq (grant no. 312446/2018), FAPERJ (grants no. E-26/203.064/2016 and E-26/201.335/2016), Institut Pasteur, Transversal Research Program (PTR grant no. 528).

DC-L and CSA contributed equally to this work.

+ Corresponding author: lourenco@ioc.fiocruz.br

Ⓞ <http://orcid.org/0000-0003-0423-5694>

Received 07 May 2020

Accepted 30 June 2020



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.⁽²⁸⁾ 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.^(14,29) 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®, Brazil) as oviposition support was suspended on tree branches at a height of 3-12 m. Ovitrap 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 - Xerém (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 ovitrap 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

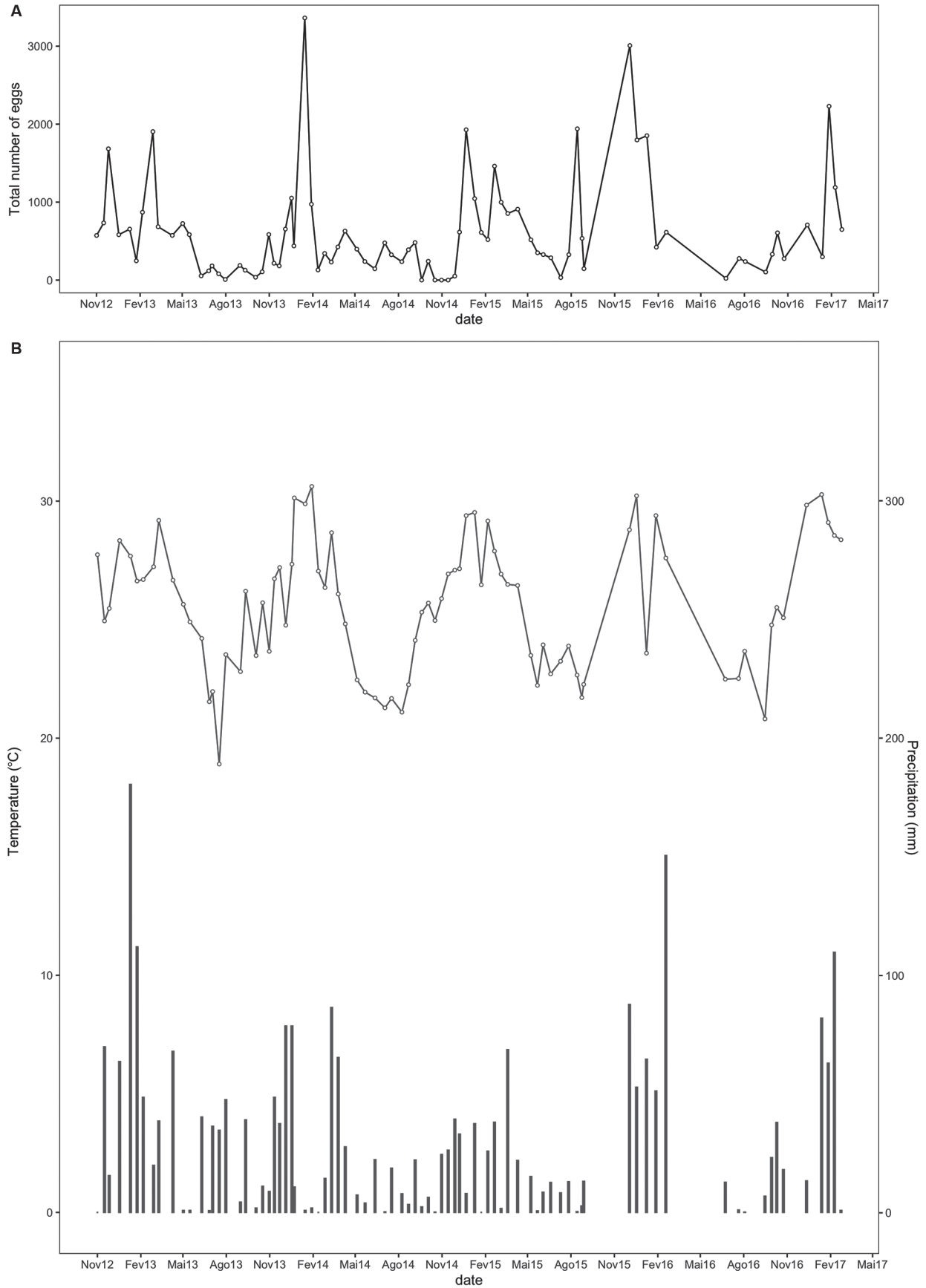


Fig. 1: time series with (A) Sum of the total number of eggs collected in the 20 trap stations on each sampling event. (B) Time series of the mean temperature and accumulated rainfall considering the period in-between the sampling events.

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.

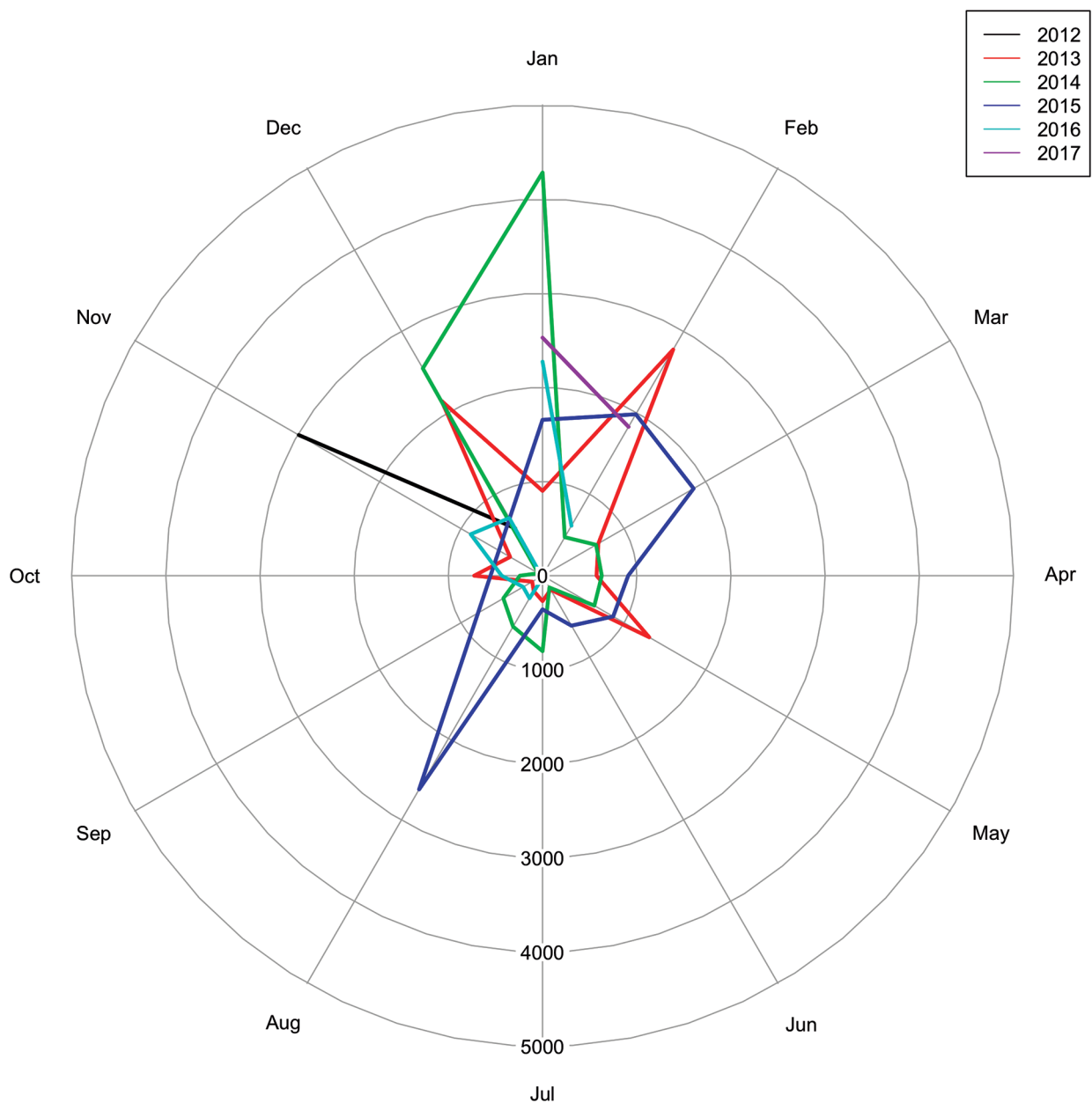


Fig. 2: circular histogram for the sum of the total number of eggs collected per month during the studied period. Each colour represents the sampled year.

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

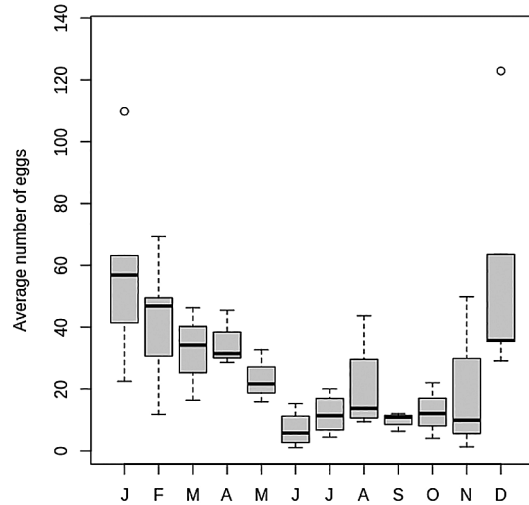


Fig. 3: distribution of the average number of eggs collected in the 20 ovitraps for each month considering the entire study (2012-2017). Thick lines within boxes represent median values for the average number of eggs collected per month in each year. Upper and lower limits of boxes represent 1st and 3rd quartiles, respectively.

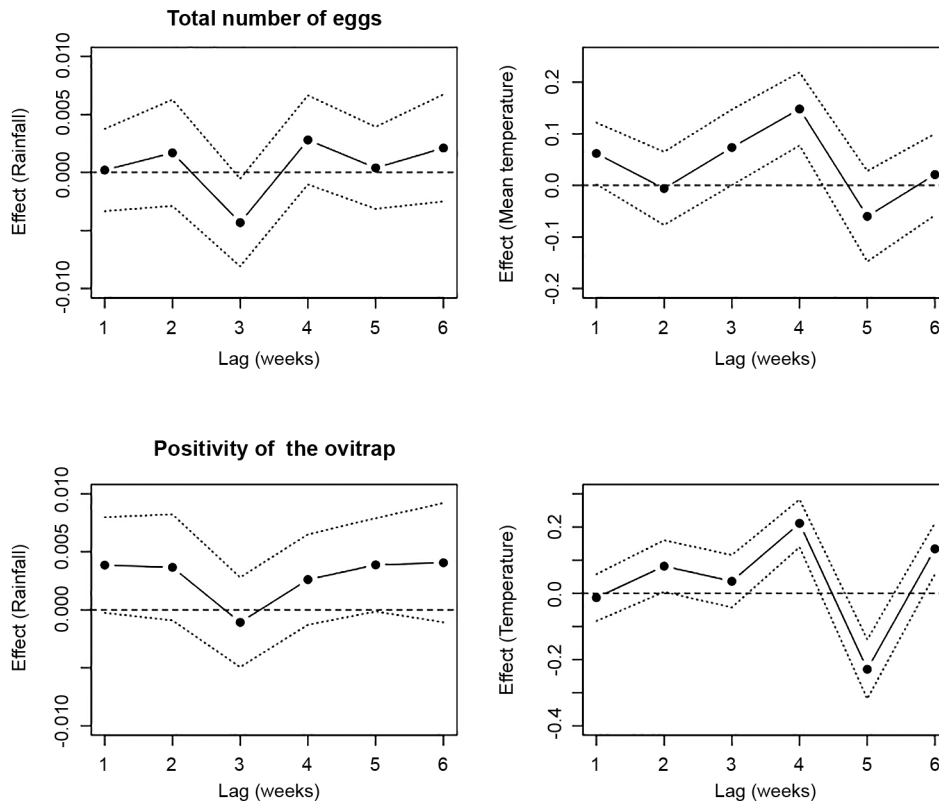


Fig. 4: lag distributed effect of temperature and rainfall on considering the full models the total number of eggs produced (top) and the positivity of the ovitraps (bottom). Dotted lines indicated 95% confidence interval.

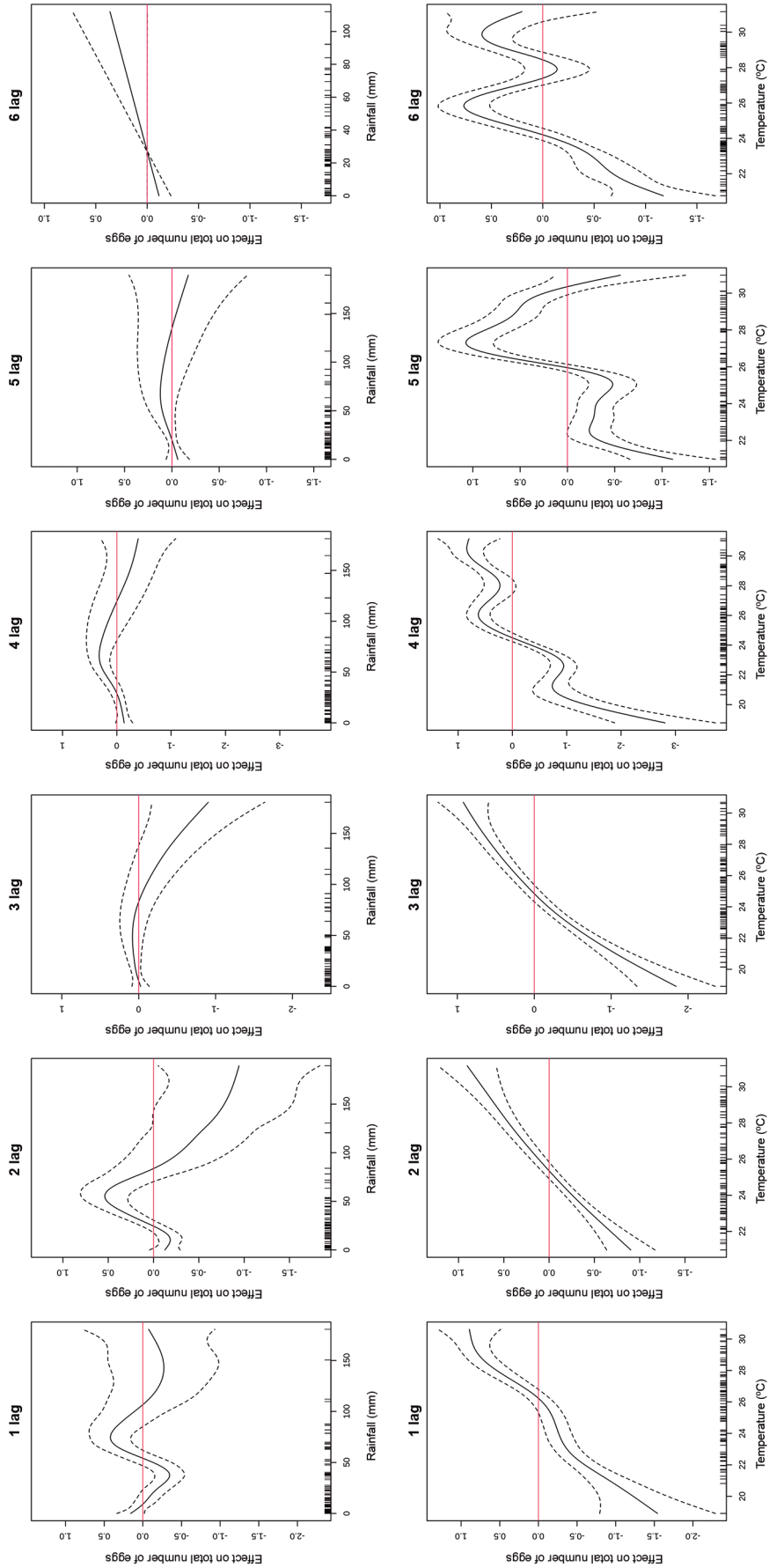


Fig. 5: smooth effect of temperature and rainfall at lag 1–6 weeks on the total number of collected eggs. Dotted lines indicated 95% confidence interval.

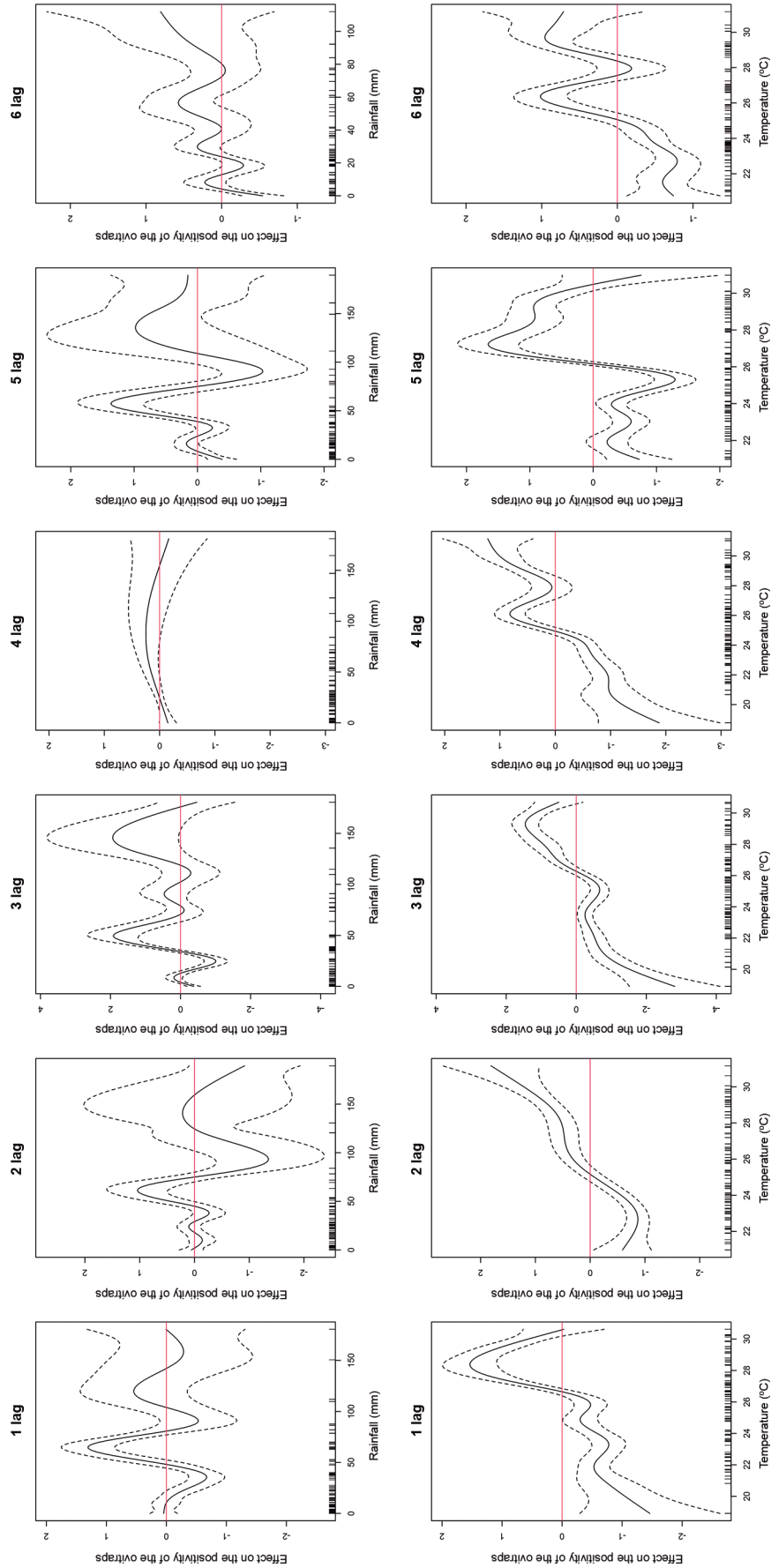


Fig. 6: smooth effect of temperature and rainfall at lag 1-6 weeks on the positivity of the ovitraps. Dotted lines indicated 95% confidence interval.

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

Model	Lag	AIC	Δ 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 of the climatic variable (in weeks); AIC: Akaike information criterion; k: number of model parameters; Δ 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 (Δ 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	SDev		
Ovitrap	0.31	0.56		

SE: standard error; SDev: standard deviation

the sampling event had a significant and positive effect on the positivity of the ovitrap, 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 ovitrap at 1-6 weeks' time lags of samplings (Figs 5, 6). In contrast, temperature had a linear or relatively linear effect on the number of laid eggs in the ovitrap at 1-4 weeks, but became nonlinear after at 5-6 weeks' time lag (Fig. 5). Considering positivity of ovitrap, temperature had a nearly linear effect only in the interval of 2-4 weeks' time lag of a samplings (Fig. 6).

All the five most plausible models explaining the total number of eggs gathered considered a time lag of four weeks and included mean temperature, rainfall and the height of the ovitrap (Table I). The distance from the forest edge was not included in any of the plausible models. For the positivity of ovitrap we also found that temperature and rainfall considering a time lag of four weeks were the explanatory variables included in all the most plausible models (Table III).

The estimates of the most plausible models for total number of eggs and positivity of ovitrap 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 ovitrap 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 ovitrap 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 ($\beta = -0.06$, $p = 0.36$).

DISCUSSION

In Brazil, humans are contaminated by YFV during epizootics, 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 epizootics, 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-

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 ovitraps)	4	1988.3	0	4	0.79	0,7393147
rainfall + temperature + height + (height ovitraps)	4	1992.06	3.75	7	0.12	0,7389699
rainfall + temperature + distance + (distance ovitraps)	4	1993.36	5.06	7	0.06	0,7390462
temperature + (1 ovitraps)	4	1995.09	6.79	3	0.02	0,73572
rainfall + temperature + height + distance + (height ovitraps) + (distance ovitraps)	4	1998.48	10.18	11	5	0,7389089
1 + (1 ovitraps)	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 ovitraps as random variable

	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				
Ovitraps	Variance	SDev		
	0.33	0.57		

SE: standard error; SDev: standard deviation.

break. In summary, our data evidenced that *Hg. leuocolaenus* has a pronounced seasonal population dynamics expressively influenced by variations of climatic factors, where temperature has a major role.

Some *Aedinii* species whose eggs are resistant to desiccation may disappear in adulthood stage during some months in the unfavorable season, persisting as embryos. It is not the case of *Hg. leuocolaenus* in the Atlantic Forest. Interestingly, in the present study eggs of *Hg. leuocolaenus* were detected in every sampled month from November 2012 to March 2017. *Hg. leuocolaenus* females need to take at least one blood meal to sustain its anautogenous reproduction and may retain eggs of a previous gonotrophic cycle in their ovaries when seeking for a new blood meal.⁽³⁷⁾ So, even though fluctuating in number, the uninterrupted encounter of eggs of *Hg. leuocolaenus* in the area indirectly illustrates that this species maintains haematophagic activity throughout the months in the PNMNI forest, regardless of climate conditions.

Climate in the southeast Brazil, particularly mean temperature and rainfall are adequate for maintenance of YFV transmission throughout the year in almost anywhere in this region.⁽³⁸⁾ Indeed, human cases and con-

firmed YFV epizootics 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.⁽³⁹⁾ Coincidentally, regardless the year of sampling, higher numbers of eggs of *Hg. leuocolaenus* 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. leuocolaenus* has been described in other sites of Atlantic Forest in southeast^(11,40,41) and in the Cerrado in centre-west Brazil^(42,43) as well as in Trinidad.^(37,44) 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.⁽³⁹⁾

Noteworthy, collections of *Hg. leuocolaenus* 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 Jacarapaguá (~ 20 km away),⁽⁴⁵⁾ 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)⁽⁴¹⁾ the egg counting peaked in December 2015, in the rainy summer as expected. Thus, variations in the population dynamics of *Hg. leuocolaenus* 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.⁽⁴⁶⁾

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.^(25,26) 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 influenced the amount of eggs laid by *Hg. leuocolaenus* at PNMNI. Moreover, but unlike Casimiro de Abreu,⁽⁴¹⁾

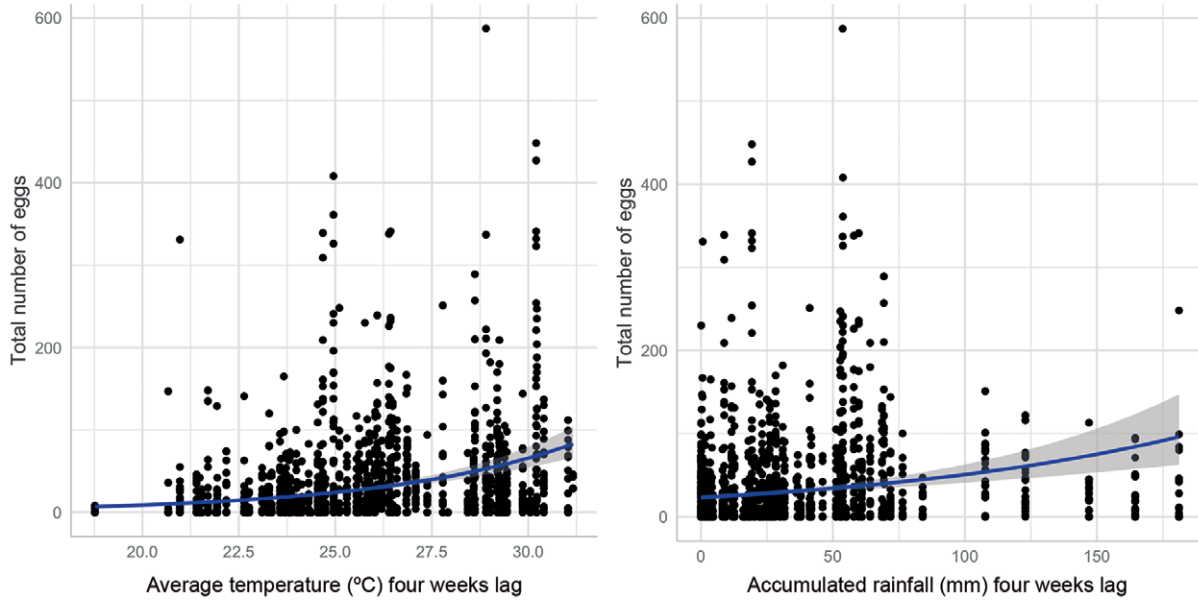


Fig. 7: effect of temperature and rainfall (four weeks lag) on the total number of eggs collected considering the 20 ovitrap. The line represents the predicted number of eggs.

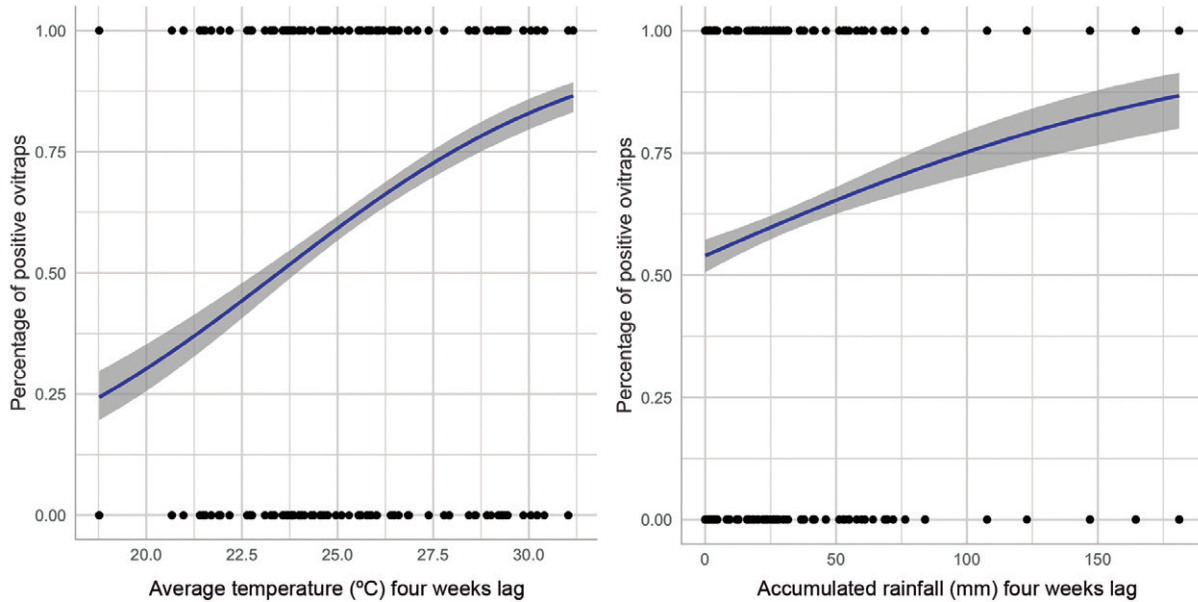


Fig. 8: effect of temperature and rainfall (four weeks lag) on the positivity of the ovttraps. The line represents the predicted percentage of positive ovttraps.

precipitation had a nonlinear effect either on the number of collected *Hg. leucocelaenus* eggs or the detection of eggs in ovttraps at any time lag in PNMNI. The height of the ovttrap 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 ovttrap. Also, temperature had a linear or relatively linear effect on the number of collected eggs and the positivity of ovttraps 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.⁽¹¹⁾

It has been demonstrated that higher mean temperatures induce greater mosquito abundance and biting activity,⁽²⁵⁾ 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.^(47,48,49) 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.

ACKNOWLEDGEMENTS

To Sebastião Loures de Miranda Filho, José Romero C de Oliveira e Souza, Marcelo Celestino dos Santos, Mauro M Muniz, Renato Carvalho, Marcelo Quintela Gomes, Tacilane Divina Cardoso for the help in mosquito collections; Anselmo for the climate data; Marco Venício Silva de Barros (Secretaria Municipal de Saúde de Nova Iguaçu) and Edgar José

Silva Martins (Parque Natural Municipal de Nova Iguaçu, Secretaria de Meio Ambiente e Urbanismo de Nova Iguaçu) for the support for the field work; Nildimar A Honório, Filipe Vieira S de Abreu and Aline Tátia-Ferreira for the critical reading of the manuscript.

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.

REFERENCES

1. Possas C, Lourenço-de-Oliveira R, Tauil PL, Pinheiro FP, Pissinatti A, da Cunha RV, et al. Yellow fever outbreak in Brazil: the puzzle of rapid viral spread and challenges for immunisation. Mem Inst Oswaldo Cruz. 2018; 113(10): 1-12.
2. MS/SVE - Ministério da Saúde/Secretaria de Vigilância Epidemiológica. Boletim epidemiológico - Reemergência da Febre Amarela silvestre no Brasil, 2014/2015: situação epidemiológica e a importância da vacinação preventiva e da vigilância intensificada no período sazonal. Bol Epidemiol. 2015; 46: 1-10.
3. Delatorre E, de Abreu FVS, Ribeiro IP, Gómez MM, dos Santos AAC, Ferreira-de-Brito A, et al. Distinct YFV lineages co-circulated in the Central-Western and Southeastern Brazilian regions from 2015 to 2018. Front Microbiol. 2019; 10: 1-12.
4. MS/SVE - Ministério da Saúde/Secretaria de Vigilância Epidemiológica. Saúde Brasil 2019: uma análise da situação de saúde com enfoque nas doenças imunopreveníveis e na imunização [Internet]. Brasília: Ministério da Saúde; 2019. Available from: <https://www.saude.gov.br/images/pdf/2019/dezembro/05/Saude-Brasil-2019-imunizacao.pdf>.
5. Franco O. História da Febre Amarela no Brasil. Vol. 1. Rio de Janeiro: Ministério da Saúde, Departamento Nacional de Endemias Rurais; 1969. 210 pp.
6. Possas C, Martins RM, Lourenço-de-Oliveira R, Homma A. Urgent call for action: avoiding spread and re-urbanisation of yellow fever in Brazil. Mem Inst Oswaldo Cruz. 2018; 113(1): 1-2.
7. Vasconcelos PFC. Yellow fever. Rev Soc Bras Med Trop. 2003; 36(2): 275-93.
8. de Abreu FVS, Ferreira-de-Brito A, Azevedo ADS, Linhares JHR, Santos VO, Miranda EH, et al. Survey on non-human primates and mosquitoes does not provide evidences of spillover/spillback between the urban and sylvatic cycles of yellow fever and Zika viruses following severe outbreaks in southeast Brazil. Viruses. 2020; 12(4): 364.
9. Arnell JH. Mosquito studies (Diptera, Culicidae) XXXII. A revision of the genus *Haemagogus*. Contrib Am Entomol Inst. 1973; 10(2): 1-174.
10. Walter Reed Biosystematics Unit. Systematic Catalog of Culicidae - [Internet]. [cited 2020 May 3]. Available from: <http://www.mosquitocatalog.org/default.aspx>.
11. Alencar J, de Mello CF, Gil-Santana HR, Guimarães AÉ, de Almeida SAS, Gleiser RM. Vertical oviposition activity of mosquitoes in the Atlantic Forest of Brazil with emphasis on the sylvan vector, *Haemagogus leucocelaenus* (Diptera: Culicidae). J Vector Ecol. 2016; 41(1): 18-26.
12. Bates M. The natural history of mosquitoes [Internet]. J Am Med Assoc. 1949; 141: 956 pp. Available from: <http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.1949.02910130070038>.

13. Pinheiro FP, Travassos da Rosa APA, Moraes MAP. An epidemic of yellow fever in central Brazil, 1972-1973. Vol. II. Ecological studies. *Am J Trop Med Hyg.* 1981; 30(1): 204-11.
14. de Abreu FVS, Ribeiro IP, Ferreira-de-Brito A, dos Santos AAC, de Miranda RM, Bonelly IS, et al. *Haemagogus leucocelaenus* and *Haemagogus janthinomys* are the primary vectors in the major yellow fever outbreak in Brazil, 2016-2018. *Emerg Microbes Infect.* 2019; 8(1): 218-31.
15. Soper FL. Yellow fever: the present situation (October 1938) with special reference to South America. *Trans R Soc Trop Med Hyg.* 1938; 32(3): 297-322.
16. Kumm HW, Cerqueira NL. The role of *Aedes leucocelaenus* in the epidemiology of jungle yellow fever in Brazil. *Bull Entomol Res.* 1951; 42(1): 195-9.
17. Vasconcelos PFC, Sperb AF, Monteiro HAO, Torres MAN, Sousa MRS, Vasconcelos HB, et al. Isolations of Yellow Fever virus from *Haemagogus leucocelaenus* in Rio Grande do Sul State, Brazil. *Trans R Soc Trop Med Hyg.* 2003; 97(1): 60-2.
18. Cardoso JC, de Almeida MAB, dos Santos E, da Fonseca DF, Salum MAM, Noll CA, et al. Yellow Fever virus in *Haemagogus leucocelaenus* and *Aedes serratus* mosquitoes, southern Brazil, 2008. *Emerg Infect Dis.* 2010; 16(12): 1918-24.
19. Mascheretti M, Tengan CH, Sato HK, Suzuki A, de Souza RP, Maeda M, et al. Yellow fever: reemerging in the State of São Paulo, Brazil, 2009. *Rev Saude Publica.* 2013; 47(5): 881-9.
20. de Souza RP, Petrella S, Coimbra TLM, Maeda AY, Rocco IM, Bissordi I, et al. Isolation of Yellow Fever virus (YFV) from naturally infected *Haemagogus* (Conopostegus) *leucocelaenus* (Diptera, Culicidae) in São Paulo State, Brazil, 2009. *Rev Inst Med Trop São Paulo.* 2011; 53(3): 133-9.
21. Cunha MS, da Costa AC, Fernandes NCCA, Guerra JM, dos Santos FCP, Nogueira JS, et al. Epizootics due to Yellow Fever virus in São Paulo State, Brazil: viral dissemination to new areas (2016–2017). *Sci Rep.* 2019; 9(1): 5474.
22. Couto-Lima D, Madec Y, Bersot MI, Campos SS, Motta MA, dos Santos FB, et al. Potential risk of re-emergence of urban transmission of Yellow Fever virus in Brazil facilitated by competent *Aedes* populations. *Sci Rep.* 2017; 7(1): 4848.
23. Lourenço-de-Oliveira R, Failloux A-B. High risk for Chikungunya virus to initiate an enzootic sylvatic cycle in the tropical Americas. *PLoS Negl Trop Dis.* 2017; 11(6): e0005698.
24. de Figueiredo ML, Gomes AC, Amarilla AA, Leandro AS, Orrico AS, de Araujo RF, et al. Mosquitoes infected with dengue viruses in Brazil. *Virology.* 2010; 7(1): 152.
25. Consoli RAGB, Lourenço-de-Oliveira R. Principais mosquitos de importância sanitária no Brasil. Rio de Janeiro: Fiocruz; 1994. 228 pp.
26. Hamrick PN, Aldighieri S, Machado G, Leonel DG, Vilca LM, Uriona S, et al. Geographic patterns and environmental factors associated with human Yellow Fever presence in the Americas. *PLoS Negl Trop Dis.* 2017; 11(9): 1-27.
27. Hamlet A, Jean K, Perea W, Yactayo S, Biey J, Van Kerkhove M, et al. The seasonal influence of climate and environment on Yellow Fever transmission across Africa. *PLoS Negl Trop Dis.* 2018; 12(3): e0006284.
28. Oliveira FL, Da Costa NMC. Parque Natural Municipal de Nova Iguaçu: um peculiar patrimônio geológico-geomorfológico na Baixada Fluminense, RJ. *História, Nat e Espaço.* 2014; 2(2). Available from: Available from: <https://www.e-publicacoes.uerj.br/index.php/niesbf/article/view/12116/9493>.
29. SES - Secretaria de Estado de Saúde do Rio de Janeiro. Informe epidemiológico 020/2018. Monitoramento das emergências em saúde pública - Febre Amarela. [Internet]. 2018. Available from: <http://www.riocomsaude.rj.gov.br/Publico/MostrarArquivo.aspx?C=L4wqOoj4OVw%3D>.
30. Climate data. Clima Nova Iguaçu: temperatura, tempo e dados climatológicos Nova Iguaçu - Climate-Data.org [Internet]. 2020 [cited 2020 May 3]. Available from: <https://pt.climate-data.org/america-do-sul/brasil/rio-de-janeiro/nova-iguacu-4044/>.
31. Fay RW, Eliason DA. A preferred oviposition site as surveillance method for *Aedes aegypti*. *Mosq News.* 1966; 26(4): 531-5.
32. Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. Mixed effects models and extensions in ecology with R. Springer; 2009. 574 pp.
33. Zuur AF, Ieno EN, Elphick CS. A protocol for data exploration to avoid common statistical problems. *Methods Ecol Evol.* 2010; 1: 3-14.
34. Burnham KP, Anderson DR. Model selection and multimodel inference: a practical information-theoretic approach. Fort Collins: Springer-Verlag; 2002. 208 pp.
35. Seshan VE, Gönen M, Begg CB. Comparing ROC curves derived from regression models. *Stat Med.* 2013; 32(9): 1483-93.
36. Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, et al. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol.* 2009; 24(3): 127-35.
37. Chadee DD, Ganesh R, Hingwan JO, Tikasingh ES. Seasonal abundance, biting cycle and parity of the mosquito *Haemagogus leucocelaenus* in Trinidad, West Indies. *Med Vet Entomol.* 1995; 9(4): 372-6.
38. de Paiva CA, Oliveira APS, Muniz SS, Calijuri ML, dos Santos VJ, Alves SC. Determination of the spatial susceptibility to Yellow Fever using a multicriteria analysis. *Mem Inst Oswaldo Cruz.* 2019; 114(3): 1-8.
39. MS/SVE - Ministério da Saúde/Secretaria de Vigilância Epidemiológica. Monitoramento do período sazonal da Febre Amarela - Informe 26 [Internet]. 2018. Available from: <https://portal.arquivos2.saude.gov.br/images/pdf/2018/maio/18/Informe-FA-26.pdf>.
40. Alencar J, Morone F, de Mello CF, Degallier N, Lucio PS, de Serra-Freire NM, et al. Flight height preference for oviposition of mosquito (Diptera: Culicidae) vectors of sylvatic Yellow Fever virus near the hydroelectric reservoir of Simplicio, Minas Gerais, Brazil. *J Med Entomol.* 2013; 50(4): 791-5.
41. Silva SOF, de Mello CF, Figueiró R, Maia DA, Alencar J. Distribution of the mosquito communities (Diptera: Culicidae) in oviposition traps introduced into the Atlantic Forest in the State of Rio de Janeiro, Brazil. *Vector-Borne Zoonotic Dis.* 2018; 18(4): 214-21.
42. Ribeiro ALM, Miyazaki RD, Silva M, Zeilhofer P. Spatial and temporal abundance of three sylvatic Yellow Fever vectors in the influence area of the Manso Hydroelectric Power Plant, Mato Grosso, Brazil. *J Med Entomol.* 2012; 49(1): 223-6.
43. Lira-Vieira AR, Gurgel-Gonçalves R, Moreira IM, Yoshizawa MAC, Coutinho ML, Prado PS, et al. Ecological aspects of mosquitoes (Diptera: Culicidae) in the gallery forest of Brasília National Park, Brazil, with an emphasis on potential vectors of Yellow Fever. *Rev Soc Bras Med Trop.* 2013; 46(5): 566-74.
44. Chadee DD, Tikasingh ES. Seasonal incidence and diel oviposition periodicity of *Haemagogus* mosquitoes (Diptera: Culicidae) in Trinidad, WI. Part III. *Haemagogus celeste* (Dyar and Nunez Tovar) and *Haemagogus leucocelaenus* (Dyar and Shannon). *Ann Trop Med Parasitol.* 1991; 85(5): 543-50.

45. Tátila-Ferreira A, Maia D de A, Abreu FVS de, Rodrigues WC, Alencar J. Oviposition behavior of *Haemagogus leucocelaenus* (Diptera: culicidae), a vector of wild yellow fever in Brazil. Rev Inst Med Trop Sao Paulo. 2017;7;59(0).
46. Golden Gate Weather Services. El Niño and La Niña years and intensities [Internet]. [cited 2020 May 3]. Available from: <https://ggweather.com/enso/oni.htm>.
47. Davis NC. The effect of various temperatures in modifying the extrinsic incubation period of the Yellow Fever virus in *Aedes aegypti*. Am J Epidemiol. 1932; 16(1): 163-76.
48. Bates M, Roca-Garcia M. The development of the virus of Yellow Fever in *Haemagogus* mosquitoes. Am J Trop Med Hyg. 1946; 26(5): 585-605.
49. Johansson MA, Arana-Vizcarrondo N, Biggerstaff BJ, Staples JE. Incubation periods of Yellow Fever virus. Am J Trop Med Hyg. 2010; 83(1): 183-8.
50. Vasconcelos PFC, Costa ZG, Travassos Da Rosa ES, Luna E, Rodrigues SG, Barros VLRS, et al. Epidemic of jungle Yellow Fever in Brazil, 2000: implications of climatic alterations in disease spread. J Med Virol. 2001; 65(3): 598-604.
51. Honório NA, Codeço CT, Alves FC, Magalhães MAFM, Lourenço-de-Oliveira R. Temporal distribution of *Aedes aegypti* in different districts of Rio de Janeiro, Brazil, measured by two types of traps. J Med Entomol. 2009; 46(5): 1001-14.
52. Alencar J, Gleiser RM, Morone F, de Mello CF, Silva JS, Serra-Freire NM, et al. A comparative study of the effect of multiple immersions on Aedini (Diptera: Culicidae) mosquito eggs with emphasis on sylvan vectors of yellow fever virus. Mem Inst Oswaldo Cruz. 2014; 109(1): 114-7.