# Regionality in vector control: effect of fluctuating temperature in the susceptibility of Aedes aegypti (Diptera: Culicidae) larvae to Pyriproxyfen

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# Abstract

Using Pyriproxyfen to control *Aedes aegypti* populations shows great potential considering its high competence in low dosages and environmental safety. As an endocrine disruptor, temperature can interfere in its efficiency, related to a decrease in larval emergence inhibition in hotter environments. However, previous studies have been performed at constant temperatures in the laboratory, which may not precisely reflect the environmental conditions in the field. This study aimed to assess the effect of the fluctuating temperatures in pyriproxyfen efficiency on controlling *Aedes aegypti* larvae. We selected maximum and minimum temperatures from the Brazilian Meteorological Institute database from September to April for cities grouped by five regions. Five fluctuating temperatures (17–26; 20–28.5; 23–32.5; 23–30.5; 19.5–31 ºC) were applied to bioassays assessing Pyriproxyfen efficiency in preventing adult emergence in *Aedes aegypti* larvae in five concentrations. The proportion of emergence inhibition was compared among treatments and within treatment. In thermal conditions with the lowest temperatures, Pyriproxyfen was efficient to prevent the emergence of twice the larvae than in the hottest temperatures with the lowest concentration applied (average ± SD: 0.61 ± 0.09 in coldest treatment; average ± SD: 0.65 ± 0.12 in the hottest treatment, p value = 0.00015). The concentration that inhibits the emergence of 50% of the population was lower than that preconized by the World Health Organization (0.01 mg/L) in all treatments, except for the hottest temperatures, for which we estimated 0.010 mg/L (SD ± 0.017). Applying fluctuating temperatures in laboratory bioassays provides a more realistic result for vector surveillance strategies. For a country with continental proportions such as Brazil, considering regionalities is crucial for a rational use of insecticides.


# 1. Introduction

Arboviruses transmitted through the bite of infected *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) are still a huge public health concern, especially in tropical and subtropical regions. As a fast growing mosquito-borne viral disease, dengue fever is one of the most frequent infections throughout the tropics and has been considered endemic in Brazil since 1986, when serotype 1 was introduced in the country (MAYER; TESH; VASILAKIS, 2017; LUNA et al., 2020). Cólon-Gonzalez et al. (2021) estimated that the incidence of Dengue fever alone has increased 30-fold in the last 50 years.

The dynamics of mosquito-borne illnesses are climatic driven, and recent work suggests that increasing global temperatures will lead to an expansion of *Aedes aegypti* into temperate regions and dramatically increase *Aedes*-borne virus transmission within the next century (CALDWELL et al., 2021; RYAN et al., 2019, 2021). There is no medical treatment or specific medications for diseases transmitted by this mosquito and prevention through vaccination is accessible only by urban yellow fever (Rodhain 2022). Although there is a prospect of an effective and accessible Dengue vaccine for the mid-term future, *Ae. aegypti* will continue to be a threat to public health due to the possibility of transmission of other arboviruses such as CHKV (Chikungunya virus) and ZKV (Zika virus) (WILDER-SMITH, 2022). Furthermore, Teixeira et al. (2021) described that *Ae. aegypti* mosquitoes can be simultaneously infected by both dengue and Zika virus. Therefore, controlling mosquito populations through mechanical removal

of potential breeding sites associated with applying insecticides as a supplementary measure are still important tools to prevent epidemics. Currently, there is a growing recognition that the solutions to control such arbovirus transmission surpass the health sector and rely on a diversity of structural actions, such as adequate sewage treatment, effective waste management programs, and water supply maintenance, as well as community participation (Valle et al. 2019).

Controlling these insects in their immature phases (egg, larva and pupa) is more feasible, considering that the development occurs in restricted and specific locations, unlike the adult phase, which can disperse throughout various environments and can escape from an insecticide dose (Campos et al. 2020). However, the continuous and intensive application of a compound can lead to developing resistant mosquito populations, considering that the larvicide presents an evolutive pressure in the environment for the individuals exposed to it. A sustainable and effective chemical control strategy must be based on detailed planning considering the mosquito populational distribution, the species susceptibility to compounds and possible mechanisms involved in resistance selection to decrease vector infestation and prevent epidemics (Roush 1989).

In Brazil, insecticide resistance in *Ae. aegypti* populations was detected for different compounds that were applied, such as temephos (organophosphate) and deltamethrin (pyrethroid) (Valle et al. 2019). The intense application of temephos between 2003 to 2014 is worth noting, showing the relation between long time exposition and resistance development in *Ae. aegypti* mosquito populations (Rahman et al. 2021). Currently, temephos resistance is so widespread in Brazil that this compound is no longer considered as the first-choice larvicide for use against *Ae. aegypti*, and it has been suggested to replace it by another, preferentially using non-neurotoxic products (Valle et al. 2019).

To strategically avoid the development of resistance to insecticides, the Brazilian Ministry of Health (MoH) adopted a larvicide rotation approach, changing the compound applied every four years (SVS, 2012). Between 2014 and 2018, MoH deliberated the application of Pyriproxyfen to control *Ae. aegypti* larvae. Pyriproxyfen is a non-neurotoxic compound, classified within the insect growth regulator (IGR) class of insecticides. Pyriproxyfen is a juvenile hormone analogue that acts inside the organism preventing moulting into the adult stage, causing death because of this endocrine disruption.

As a larvicide, Pyriproxyfen shows great efficiency in laboratory and semi-field settings demonstrating high emergence inhibition for larvae exposed to low concentrations (VYTHILINGAM et al., 2005; DE RESENDE; GAMA, 2006; LAU et al., 2015; SAMUEL et al., 2017; HUSTEDT et al., 2020; FANSIRI et al., 2022). However, environmental factors known to interfere with the developmental aspects of the larvae (e.g.: temperature, organic matter loads, pH) can also affect the larvicide efficiency, considering its mode of action as a non-neurotoxic compound (CARRINGTON; ARMIJOS; et al., 2013; CARRINGTON; SEIFERT; et al., 2013; DE NADAI et al., 2021; OHASHI, 2017; DURANT; DONINI, 2018; TALAGA et al., 2020; HUZORTEY et al., 2022). Considering the impacts of temperature, insect responses to fluctuating temperatures contrast with responses to constant temperature at multiple levels of organization, from physiology and stress tolerance to life history traits and fitness (Colinet et al. 2015). Previous research

testing insecticide susceptibility in field populations of mosquitoes have demonstrated that there is seasonal variability in sensitivity, suggesting that environmental interference is important to mosquito control programs (Hernandez et al. 2022). In this respect, few previous studies have addressed the impact of the fluctuating temperature in response to insecticides (Salinas et al. 2021).

Considering the continental proportions of Brazilian territory, with an area comprising 8.516.000 km², fluctuations of temperature follow distinct patterns considering different regions. This, in turn, produces different temperature fluctuations in daily cycles, as a response to climatic factors (e.g. latitude, vegetation and continentality). We hypothesized that different patterns of temperature fluctuation grouped by Brazilian regions produces differences in Pyriproxyfen susceptibility to *Ae. aegypti*. In this study, we report the differences in susceptibility of *Ae. aegypti* larvae exposed to Pyriproxyfen under the daily temperature ranges simulated.

# 2. Materials And Methods

## 2.1 Regional temperatures

We tested the effect of temperature over *Ae. aegypti* susceptibility to Pyriproxyfen combining five concentrations of the larvicide and two different temperatures: one designed for the day cycle and the other for the night cycle to simulate natural conditions of daily temperature regimes. The temperatures were based on registers from automatic meteorological stations provided by the Brazilian Meteorological Institute database (BRAZILIAN METEOROLOGICAL INSTITUTE, 2022). We chose records of maximum and minimum temperatures from 1988 and 2018 of all of the capital cities and 3 more cities from each Brazilian state, selected through simple random sampling. After the sampling, we selected the time horizon from September to March for the calculations, comprising spring and summer in Brazil. The location of the cities sampled for the calculations can be seen in the Supplemental Files. We calculated the mean value of both the maximum and minimum temperatures grouped by region. The mean maximum and minimum temperatures for each region were programmed for light and dark cycles of the experiments, respectively (Table 1).

The experiments were conducted inside incubator chambers (Eletrolab®, Model EL212/4LED) with a light/dark cycle of 14/10 h.

Table 1
Information about the temperatures used in the larval bioassay

| | Temperature (ºC) | |
|---|---|---|
| Region | Minimum | Maximum |
| Central-West | 19.5 | 31 |
| Northeast | 23 | 30.5 |
| North | 23 | 32.5 |
| Southeast | 20 | 28.5 |
| South | 17 | 26 |

# 2.2 Larval rearing

We used a Rockefeller strain from a laboratory population established since

1996 (ASR – Analytical and Scientific Research Laboratory®) provided by eggs attached into porous paper. We stored the mosquito eggs inside plastic boxes at room temperature (26°C ± 2) and relative humidity of 70% (± 5). To stimulate egg hatching, we immersed 1 cm² of the paper containing the eggs in 1 liter of tap water and 1g. of *Saccharomyces cerevisiae* (MP Biomedicals, France). After 24 h, we separated batches of 20 I instar larvae to avoid effects of intraspecific competition (Steinwascher 2020). We placed the larvae in new plastic vessels containing 250 ml of tap water with 64 mg of *S. cerevisiae* added as a nutritional source (Souza et al. 2019). The batches of larvae were maintained inside an incubator chamber (Eletrolab®, Model EL212/4LED) until they reached late III instar under the temperature regimes of the experimentation interest, considering the region to be simulated (photoperiod 14:10 light:dark, considering higher temperature for the light cycle and low temperature for the dark cycle). We chose the light:dark cycle of 14:10 to simulate the higher sunlight exposition that is typical of the spring and summer in tropical areas (Costanzo et al. 2015). Every two days, we added a new nutritional source (64 mg of *S. cerevisiae*) until the larvae reached III instar.

# 2.3 Insecticide formulation

We utilized Sumilarv 0,5G® (CAS #95737–68 – 1), kindly donated by Epidemiological Surveillance of Araraquara (São Paulo, Brazil), for the experiments. Sumilarv 0,5G® is synthesized by Sumitomo Chemical (Tokyo, Japan) containing 0.5% active ingredient (weight:weight) in a granular formulation. Sumilarv 0.5G® has a slow release formulation due to its constitution with pumice and sand as main solutes (SUMITOMO CHEMICAL, 2012).

# 2.4 Larval bioassay experiments

We prepared a stock solution with Sumilarv 0.5G® following the methodology

described by Sihuincha et al. (2005) and Moura et al. (2021). The final concentrations derived from the stock solution were 0.0025, 0.005, 0.01, 0.02 and 0.04 mg/L, comprising lower and higher concentrations based on the WHO recommendation for *Ae. aegypti* control programs (0.01 mg/L) (WHO, 2005;2016).

For each concentration, we prepared five replicates containing 250 mL into 500 mL beakers and 20 IV instar larvae, based on the WHO protocol (WHO, 2016). We provided 64 mg of *S. cerevisiae* for each beaker. Simultaneously, 5 replicates of beakers with 250 ml of tap water and the same amount of yeast containing 20 larvae each were used as the control experiment. All beakers were covered with netting to prevent emerging adults from escaping. We repeated the experiments five times on different days, using new stock solutions and new batches of larvae each day. We monitored survival on a daily basis by counting and removing dead larvae and pupae until complete emergence of adults in control experiment beakers. During the daily monitoring, we rotated the beakers to reduce the likelihood of a position effect (Gutiérrez et al. 2020).

## 2.5 Data analysis

After the larval bioassay experiments, the quantity of mosquitoes that successfully emerged to the adult stage after Pyriproxyfen exposure was registered to calculate the proportion of emergence inhibition. We then calculated the descriptive statistics of the emergence inhibition for each thermal condition and the average time of exposure, using the software Origin (ORIGIN PRO, 2022). The concentration that inhibits the emergence of 50% of the larvae population ($EI_{50}$) for each thermally simulated region was estimated by fitting log-logistic models to the data. After a model fitting procedure based on the maximum likelihood method, the three-parameter log-logistic model was applied to emergence inhibition dose-response data. The $EI_{50}$ was estimated for each thermal condition with the “estimate_EC50()” function from the package “ec50estimator” using software R (ALVES, 2020; R CORE TEAM, 2022). To test the hypothesis that emergence inhibition in warmer and more stable thermal variations has reduced efficiency of the Pyriproxyfen as larvicide, we applied the ANOVA test and Tukey’s post hoc tests, considering $\alpha = 0.05$.

# 3. Results

## 3.1 Pyriproxyfen regional efficiency

In general, in lower temperatures conditions, the larvae took longer to develop into an adult stage in the control experiments, and therefore the time of exposure in treatments with Pyriproxyfen was also longer (Table 2). The colder condition, corresponding to the South region of Brazil, presented the longest time of exposure of 10.5 days on average. On the other hand, the thermal simulation for the Northeast region of Brazil presented the shortest duration for the experiments, with 5 days of larval exposure to Pyriproxyfen.

**Table 2**

Quantification of larvae exposed and duration of the bioassays in each

experimental condition

| Region simulated | Number of larvae exposed | Experimental days (mean) | Standard deviation |
|---|---|---|---|
| Central-West (19.5–31 ºC) | 400 | 7 | 1.6 |
| Northeast (23–30.5 ºC) | 400 | 5 | 0.95 |
| North (23–32.5 ºC) | 300 | 8.2 | 0.8 |
| Southeast (20–28.5 ºC) | 300 | 8 | 1.3 |
| South (17–26 ºC) | 300 | 10.5 | 2.19 |

Among the combinations of temperature applied in the bioassays, we found that the efficiency of the larvicide increased along with the time of exposure. For the South region simulated thermally, the larval sensitivity to Pyriproxyfen was high even at lower concentrations, for which we found an emergence inhibition of 60% of the population tested. In the experiments simulating the Southeast region, the emergence inhibition was significantly higher in a dose-dependent model, but there was no evidence that the emergence inhibition was different for exposure to 0.01 and 0.02 mg/L ($p = 0.9013$). In addition, the emergence inhibition in 0.01 and 0.02 mg/L was higher than 80% of the exposed larvae population which represents an increase of efficiency of 37% from the emergence inhibition to a concentration two times lower. Regarding the simulation for the Central-West region of Brazil, the larval sensitivity was lower in all concentrations, except for the highest (0.04 mg/L). Considering the simulation in the North region, the emergence inhibition of the larvae exposed to 0.02 mg/L was significantly higher than in 0.005 mg/L ($p = 0.012403$). The same relation was observed in Northeast thermal simulations, which can be seen in Fig. 1.

Considering multiple comparisons with the Tukey's post hoc test, we found that the conditions simulated for the South region exhibited the highest sensitivity to 0.0025 mg/L of Pyriproxyfen than in other conditions ($p < 0.0001$ in all comparisons). On the other hand, Central-West thermal conditions had lower inhibition emergence. We observed the same pattern for the 0.005 mg/L exposition, showing evidence of significantly higher emergence inhibition for the larvae in South thermal conditions than in the Central-West ($p = 0.004768$). For the concentration recommended by WHO (2016), we observed that the emergence of at least 50% of the population was inhibited by Pyriproxyfen exposition in all the conditions tested (Fig. 2). However, we found evidence that emergence inhibition for the larvae in the Southeast conditions was significantly higher than in the Central-West and Northeast ($p = 0.001070$ and 0.045222, respectively). In the two highest concentrations (0.02 and 0.04 mg/L), all conditions showed emergence inhibition rates equal or above 80% of the population of larvae, except for the Northeast region. In the highest concentration, there was evidence that the Northeast region exhibited a lower emergence

inhibition proportion when compared with the South and Southeast conditions (p = 0.000173 and 0.002835, respectively).

### 3.2 Pyriproxyfen concentrations that inhibit the emergence of 50% of the population in different thermally simulated regions

Among the five thermally simulated Brazilian regions, the $EI_{50}$ did not surpass that recommended by WHO (0.01 mg/L) even in the hottest treatments (North and Northeast), which can be seen in Fig. 3. We found higher sensitivity in the individuals in the South conditions, where the $EI_{50}$ was three times lower ($\bar{x}$=0.004175; σ = 0.144) than the concentration preconized by the WHO (2016) followed by the Southeast treatments, which was two times lower ($\bar{x}$=0.00575; σ = 0.0137). For individuals exposed to Central-West temperatures, the $EI_{50}$ was 1.5 times lower than the concentration recommended by the WHO ($\bar{x}$=0.007; σ = 0.0158). The simulations for temperature conditions for the North and Northeast both showed $EI_{50}$ close to the concentration preconized by the WHO ($\bar{x}$=0.01; σ = 0.0178 and $\bar{x}$=0.00859; σ = 0.0012, respectively).

## 4. Discussion

In the larval bioassay, juvenile hormone analogues such as Pyriproxyfen offer excellent potential for controlling *Ae. aegypti* larvae by preventing their successful development into viable adults (Fansiri et al. 2022). In addition, Pyriproxyfen is considered as a pesticide that is non-toxic to birds or animals, with no genotoxic or carcinogenic effects (Suman et al. 2014). Taking this into consideration, Pyriproxyfen is recommended to be applied in drinking water at a concentration of 0.01 mg/L (WHO, 2016). However, considering that Pyriproxyfen is an endocrine disruptor, environmental factors that affect the development of *Ae. aegypti* can interfere with the emergence inhibition.

Temperature is one of the factors that directly affects the responses to insect growth regulators, because it alters the life-history traits and the sensitivity of the target-organisms to Pyriproxyfen (Alomar et al. 2021). Higher temperatures were associated with the decrease in emergence inhibition in *Ae. aegypti* treated with Pyriproxyfen in laboratory conditions (Moura et al. 2021). However, experiments with constant temperatures fail to represent what happens in natural conditions when compared with bioassays that apply different temperatures according to the photoperiod phase. Higher temperatures during the daylight phase of the photoperiod and lower temperature in the dark phase are more likely to mimic what happens in natural conditions, with the natural fluctuation of temperature between day and night. Temperature fluctuation between day and night can interfere with the regulation of heat shock proteins and, consequently, with the thermal tolerance which can influence the metabolic resistance involved with detoxification mechanisms and, consequently, with sensitivity to insecticides (Colinet et al. 2015).

Salinas, Ferria-Arroyo and Vitek (2021) showed that *Ae. aegypti* susceptibility to deltamethrin and permethrin decreased significantly in higher thermal regimes (ranging between 36 ºC in the light phase and 24.6 ºC in the dark phase) when compared to treatments with lower temperature regimes. Despite the different mode of action, the results found by our study with Pyriproxyfen are very similar, in which *Ae. aegypti* individuals showed lower sensitivity in scenarios with higher temperatures in the light and dark phases, such as those simulated for North and Northeast conditions.

It is important to highlight the relationship between larval susceptibility with exposure time in the different thermal regimes. Given that all individuals were from the same strain and, therefore, had the same susceptibility status, it is worth mentioning that the longer the larval were exposed to pyriproxyfen, the less opportunities of emerging successfully as adult mosquitoes. As observed by Alomar, Eastmond and Alto (2021), lower temperatures have been correlated to longer larval development in *Ae. aegypti* exposed to Pyriproxyfen, and this corroborates with the results found in our study. Moreover, longer exposition to the larvicide in the colder treatment (South thermal simulation) provide more chances of Pyriproxyfen intake. As a consequence, the concentration for $IE_{50}$ in this condition (South – 17 to 26 ºC) is lower than hotter treatments.

The application of lower concentrations of larvicide, in those thermal scenarios where the susceptibility was higher, can present a potential strategy to Epidemiological Surveillance to reduce the threat to non-target species. Moreover, low concentrations of Pyriproxyfen can be associated with the application of other compounds with different modes of action, such as Spinosad. A recent study has shown that the association of Spinosad (0.0125 mg/L) with Pyriproxyfen (0.00063 mg/L) resulted in high efficiency in larval control of *Ae. aegypti* (Santos et al. 2020). Santos, Limongi and Pereira (2020) also reported that the effective concentrations did not impair reproductive parameters or increase mortality of *Daphnia magna*.

Environmental parameters, such as fluctuating temperatures, are important to larvicide efficiency as are the characteristics of local mosquito populations. Sylvatic strains are under constant evolutive pressures that can be particular of a given environment and from a particular mosquito population that can present different susceptibility status to the compound. Although no previous studies have tested the effects of temperature fluctuation on emergence inhibition of *Ae. aegypti* treated with Pyriproxyfen, this compound is known to present high efficiency in larval control in semi-field conditions with low concentrations (GÓMEZ et al., 2011; DEVILLERS, 2020; HUSTEDT et al., 2020). Recently, Campos et al. (2020) demonstrated that 126 out of 132 *Ae. aegypti* populations in different Brazilian regions are susceptible to Pyriproxyfen in low dosages. Only six populations from Northeast cities demonstrated moderate resistance to the compound (Campos et al. 2020). Therefore, maintaining the efficiency of the compound in a sustainable way is crucial to enhance *Ae. aegypti* control.

# Declarations

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**Availability of data and material** The data used in the present study can be request by demand

**Author contribution** Lidia Moura: Conceptualization, Methodology, Investigation, Formal Analysis, Visualiztion, Writing - Original Draft

Juliano J. Corbi: Validation, Resources, Data Curation, Writing - Review & Editing, Supervision, Project Administration, Funding Acquisition

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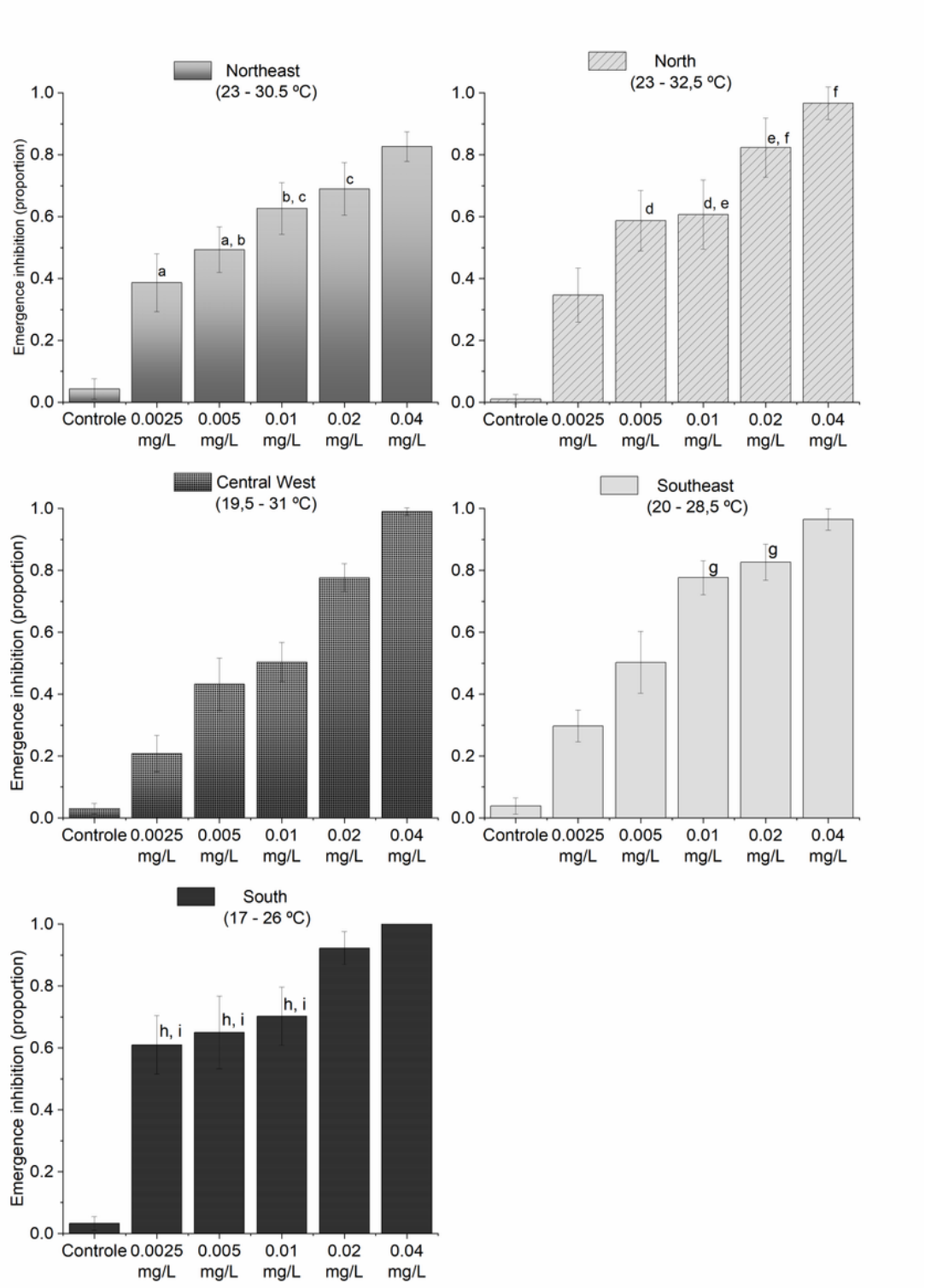
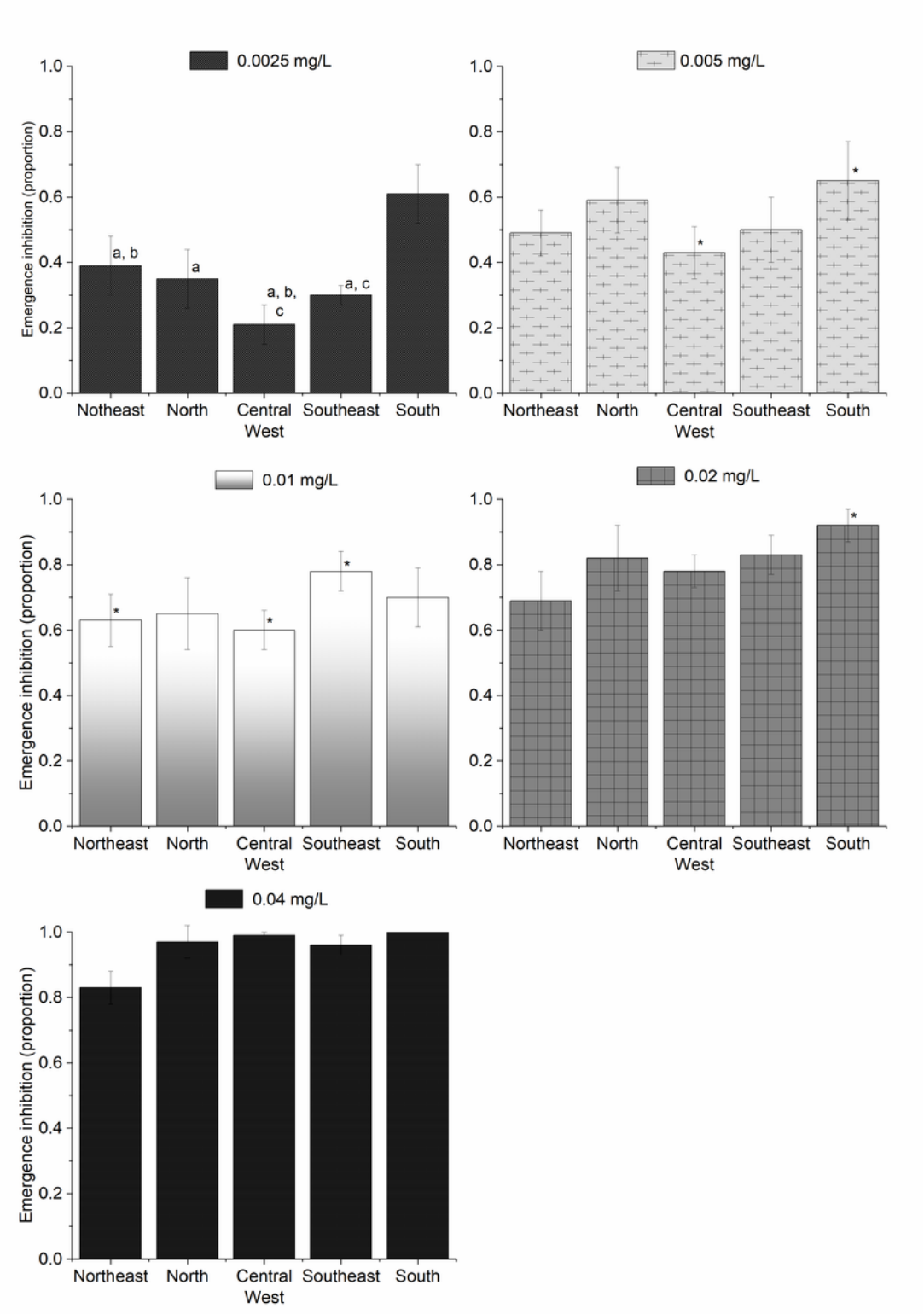
# Figures




## Figure 1

Proportion of emergence inhibition of *Aedes aegypti* to Pyriproxyfen in different thermal conditions to five Brazilian regions simulated in the laboratory.

NB: Bars with the same letter are not significantly different from each other.




## Figure 2

Multiple comparison results of different concentrations of Pyriproxyfen in emergence inhibition of *Aedes aegypti* among thermal simulations of five Brazilian regions

NB: Asterisk denotes treatments that differ significantly from each other.

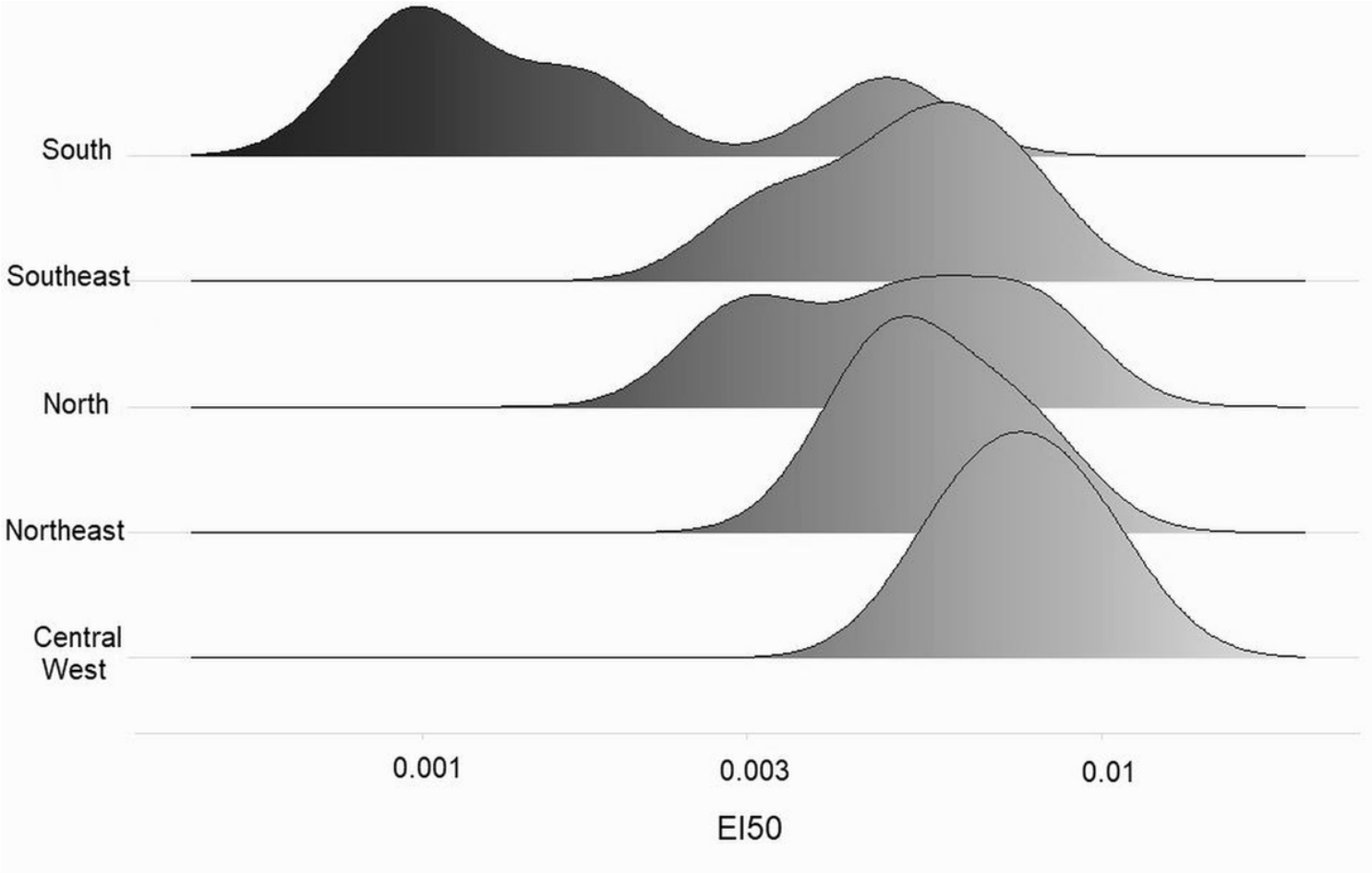



**Figure 3**

Distribution of the concentration of Pyriproxyfen that inhibits the emergence of 50% of the population of Aedes aegypti larvae in each region simulated in the laboratory.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFileCentralWestregioncities.jpeg
- SupplementalFileSouthregioncities.jpeg
- SupplementalFileSoutheastregioncities.jpeg
- SupplementalFileNorthregioncities.jpeg
- SupplementalFileNortheastregioncities.jpeg