

## Biological control of *Anopheles darlingi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae using shrimps

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### ABSTRACT

Mosquitoes can act as vectors of important diseases such as malaria, dengue, Zika virus, yellow fever, Chikungunya and Mayaro fever, in addition to filariasis. The use of insecticides, larvicides, bed nets and repellents, besides the use of drugs as chemoprevention and the treatment of the sick are currently the pillars of the control of these vectors. We studied the biological control against of *Anopheles darlingi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae using shrimps of the species *M. pantanalense*, *M. amazonicum*, *M. brasiliense* and *M. jelskii*. Larvae of mosquitoes were collected from the breeding environment and placed in a 500 and 1000 l tank containing 60 shrimps/m<sup>2</sup>. The predatory activity was evaluated for 30 days and, in all groups it was observed that 100% of the larvae were consumed in few minutes. In the environment, these same species of crustaceans were released in water bodies with the presence of larvae of these insects. In just 72 h there was a marked reduction of the larvae in the release sites of shrimps. Similarly, there was a reduction in the number of adult mosquitoes caught near the breeding sites, allowing to infer that, in places where the crustaceans were released, the predatory activity on the larvae of mosquitoes was sufficient to reduce the number of adult mosquitoes  $p \leq 0,05$ . This is the first description of the predatory activity of *M. pantanalense*, *M. amazonicum*, *M. brasiliense* and *M. jelskii* on *An. darlingi*, *A. aegypti* and *C. quinquefasciatus* larvae, constituting an important tool of biological control of these parasites-vectors.

### 1. Introduction

Malaria is the most important parasitic disease in the world and approximately 3.2 billion people live in risk areas for this disease. According to the World Health Organization, a total of 214 million of malaria cases occurred in 2015 alone, resulting in the death of 438.000 people. Of this total, 306.000 were children under the age of five (World Health Organization, 2016). In Brazil, malaria outbreaks have occurred in different states, especially in the northern region. The vector *Anopheles darlingi* is the main transmitter of malaria in Brazil, having as main breeding areas of natural vegetation with lakes (Póvoa et al., 2003).

The *Aedes aegypti* are important anthropophilic parasite, being considered as dengue vector (Tauli, 2001), yellow fever (Costa Vasconcelos, 2003), Zika virus (Costa Vasconcelos, 2015) and Chikungunya fever (Donalisio and Freitas, 2015). In addition to the high virulence attributed to pathogens transmitted by *A. aegypti*, recently, the association between the occurrence of fetal

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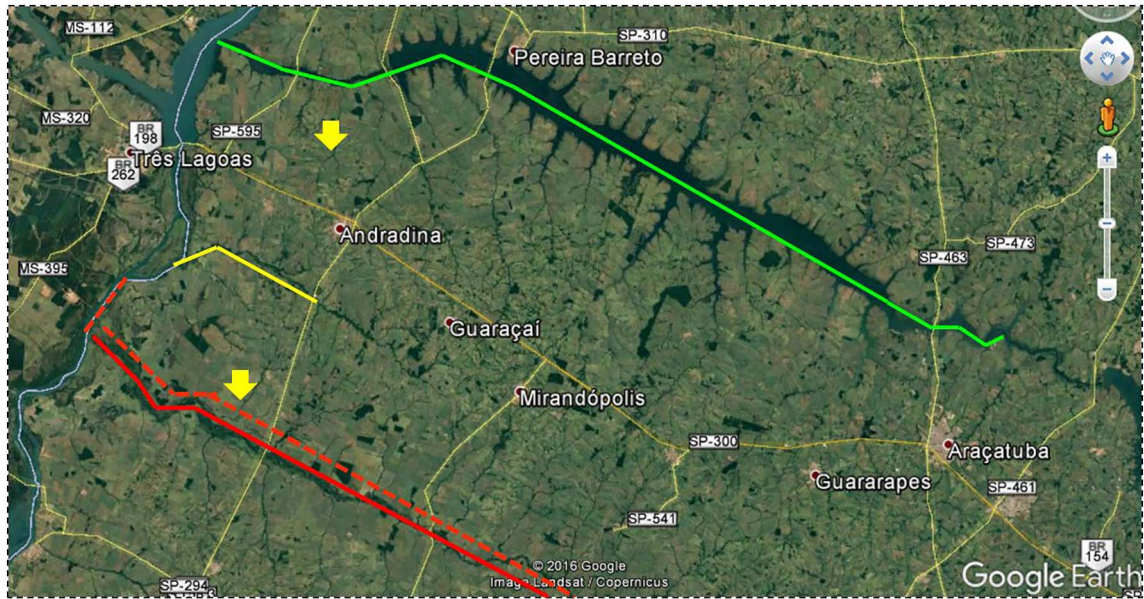


Fig. 1. Places of capture of culicids and shrimps. Tietê river (green line), Paraná river (blue line), Mill stream (yellow line), Aguapei river (red line). Places of release of shrimps (yellow arrows). Presence of *An. darlingi* (red dashes).

malformations has been associated with Zika virus infection. Similarly, the *Culex quinquefasciatus* has been incriminated as a vector of important filariasis (Medeiros et al., 2003) and viral infections (Diaz et al., 2006; Elizondo-Quiroga et al., 2005; Nitatpattana et al., 2005).

In order to combat the proliferation of these insects, many studies have been conducted, including biological control, such as the introduction of fish (Cavalcanti et al., 2004; Cavalcanti et al., 2007) and crustaceans (Collins, 1998; Rojas-Sahagún et al., 2012).

We studied the biological control against of *Anopheles darlingi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae using shrimps of the species *M. pantanalense*, *M. amazonicum*, *M. brasiliense* and *M. jelskii*.

## 2. Methods

### 2.1. Predatory activity

Larvae of the *An. darlingi*, *A. aegypti* and *C. quinquefasciatus* and other culicids were collected in the environment in the northwest region of São Paulo State, Brazil, between September 2015 and October 2016 (Fig. 1). The 4-stage larvae were identified according to the taxonomic keys. First, the larvae were aliquoted into 10 groups of 50 each and placed in rectangular aquarium of 50 l with a total of 20 shrimps of the species *M. pantanalense*, *M. amazonicum*, *M. brasiliense* and *M. jelskii*. The predatory activity was evaluated by visual inspection, every 2 h. Before the introduction of the larvae in tanks, was made 12-hour fast with the shrimp species. The crustaceans are from the Tiete river in the northwest region of São Paulo (Fig. 1).

Posteriorly, other 10 group with 100 larvae was aliquoted and placed in a 500 and 1000 l of water tank containing 60 individuals/m<sup>2</sup> of shrimps of the species *M. pantanalense*, *M. amazonicum*, *M. brasiliense* and *M. jelskii*. Similarly, other five groups formed with thousands of larvae were placed in tanks with shrimps being monitored twice a day, for 30 days in an attempt to view the emergence of adult mosquitoes. The tanks were covered by anti-mosquito devices for assessing the number of larvae reached the adult stage (Fig. 2). In tanks and aquarium was reproduced the habitat of shrimps containing water, substrate and vegetation from the places of origin. In all the stages a control group was created reproducing the same conditions of the tested groups, except the presence of shrimps. The main methods of observation were the visual verification every 12 h of the predatory activity and the quantification of the parasitic evolutionary forms captured in the traps present in the tanks when compared to the control groups.

In addition to the capture of the larvae, CDC-type traps were installed near the bodies of water to trace a statistical correlation between the presence of shrimps and the occurrence of the adult phase of the genus *Anopheles*, making a mapping of the distribution of this vector.

### 2.2. Environmental control

Two groups were initially formed in water tanks with capacity of 500 l. The group 1 (tank containing 60 shrimps/m<sup>2</sup>) and group 2 (with only water and aquatic vegetation) to investigate the occurrence of mosquito larvae.

In the environment, two water bodies that serve as breeding grounds for mosquitoes were released large amount of shrimps (Fig. 3). First, the prevalence and quantification of larvae in breeding sites and their genus were determined. Simultaneously, traps



Fig. 2. Tank covered with anti-mosquito devices for assessing the number of larvae that reached the adult stage.

for adult culicids were installed at the site (day zero). After, the shrimps were released in the breeding sites, proceeding with the quantification of larvae and adult insects in these areas every 24 h for three days using the technique of entomological shell. In these areas, the capture of the adult forms of mosquitoes was carried out with the use of human bait and CDC trap (Fig. 4). At the end of 30 days a new collection was performed. The main method of observation of the impact of the release of shrimps on the environment was the quantification of the larvae and pupae of culicidae in the breeding grounds before and after the presence of crustaceans, as well as the population of mosquitoes caught in the traps installed in the environment.

A climate database was prepared, containing the annotations of the various climatic variables (meteorological elements) on a defined space-time scale, obtained daily online through the portal of the Brazilian National Institute of Meteorology. To obtain the results the study was replicated 10 times, reproducing in full the methodology initially proposed. During the experiment, the predatory activity was recorded in video by means of a high resolution underwater camera.

These variables were analyzed using the chi-square test ( $\chi^2$ ) or the Fisher's exact test (Zar, 1999), using the SAS program (SAS,



Fig. 3. Permanent breeding place for culicids (A). Population of shrimps release at the breeding ground (B).



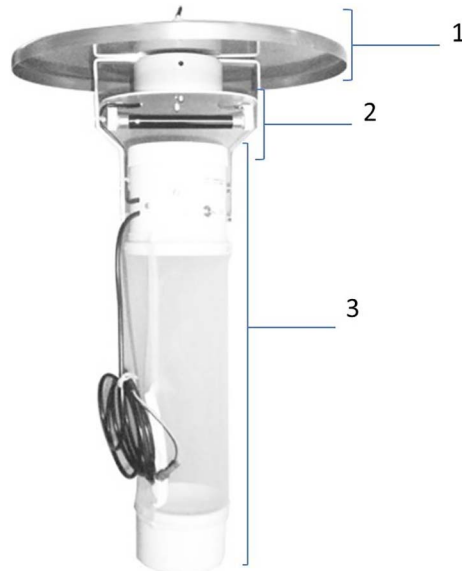


Fig. 4. CDC trap. 1-Piece of support and sustentation of kit. 2-Black light. 3-PVC body with thin mesh. 12-volt battery power.

1999), with a significance level of 5%.

### 3. Results

In the aquarium was observed that 100% of the larvae (L4) were consumed within 2 min. In the control group, 97% (47/50) of larvae evolved into adult phase. These aquariums also contained some crabs of species *T. petropolitanus* that were caught along with shrimps. Surprisingly, was observed that these crabs also fed the larvae that remained immobilized on the tank bottom or adhered to aquatic plants.

The shrimps showed predatory activity against mosquitoes regardless of larval stages, pupa and adult phase, including other species of small animals.

In the water tanks covered by anti-mosquito devices where the larvae were released, there were no observed adult insects that have emerged from the water for 30 days. In contrast, in the control group, 92% (92/100) of larvae reached at adult phase. There was a statistical association between the presence of shrimps and the reduction in the population of larvae, pupae and adult insects  $p \leq 0,05$ . Was observed that, in group 1, there was no occurrence of mosquito larvae until the end of the experiment and, in group 2, there was capture of large amount of larvae throughout the study period.

It was determined a significant statistical correlation between the major population of shrimps in the environment and the reduction on the presence of larvae and mosquitoes. As for the quantity of shrimps caught in the traps, the population of these crustaceans in the Tietê, Paraná and Aguapeí rivers is considered to be very high, moderate and absent, respectively.

In the bodies of water, in just 72 h there was a large reduction in the larval population in places where shrimps and crabs were released. Tables 1 and 2 show the reduction in the capture of adult stages and larvae of culicids in the environment. In places with little water and distant from major rivers were not collected larvae and adult forms of *Anopheles* sp. There was no statistical difference between the climatic variables and the predatory activity of the shrimps  $p \geq 0,05$ .

Table 1

Mean and absolute values of evolutionary forms of *A. aegypti* and *C. quinquefasciatus* captured in two breeding sites before and after the release of shrimps in the northwest region of São Paulo state, Brazil.

	<i>A. aegypti</i> Mean (min–max) Larvae	Mean (min–max) Adult	<i>C. quinquefasciatus</i> Mean (min–max) Larvae	Mean (min–max) Adult
Before				
Day zero	47,5 (15–80)	25,5 (2–49)	394,5 (198–591)	157 (78–236)
After				
Day 1	41,5 (7–76)	5,5 (1 – 10)	326,5 (147–506)	101,5 (61–142)
Day 2	19 (4–34)	6 (3–9)	105,5 (75–136)	97 (44–150)
Day 3	3 (0–6)	5,5 (4–7)	72,5 (33 – 112)	84,5 (32–137)
Day 30	0 (0–0)	1,5 (0–3)	5,5 (4–7)	29,5 (12–47)

Legend: Minimum: min. Maximum: max.

**Table 2**

Mean and absolute values of evolutionary forms of *An. darling* captured close to the breeding before and after the release of shrimps in the northwest region of São Paulo state, Brazil.

	<i>An. darling</i> Mean (min–max) Larvae	Mean (min–max) Adult
Before		
Day zero	11 (5–17)	8 (2–14)
After		
Day 1	1 (0–2)	3,5 (1–6)
Day 2	0 (0–0)	2 (1–3)
Day 3	0,5 (0–1)	2,5 (0–5)
Day 30	0 (0–0)	1 (0–2)

Legend: Minimum: min. Maximum: max.

It was found that the shrimps used in this study also act as surface predators, capturing mosquitoes, flies and other invertebrates that land on water or are on vegetation.

#### 4. Discussion

In our study we determined the predatory activity of four different species of shrimps from the Tietê and Paraná Rivers in northwest region of São Paulo state, Brazil, in aquariums and tanks simulating the natural habitat of these crustaceans and also directly into the environment. Similarly, Rojas-Sahagún et al. (2012) found satisfactory predatory activity of *Macrobrachium tenellum* on larvae of *A. aegypti* in laboratory conditions, in México. These authors report that these animals are not aggressive and supports wide range of oxygen concentration. These data differ from ours because the species *Macrobrachium* found in this region are extremely aggressive, attacking people entering the water, fishes and animals injured, and do not support well water with low oxygen.

This is also the first description of predation of crabs *T. petropolitanus* on larvae of culicids. However, it is important that these animals only fed the larvae were temporarily immobilized in the tank bottom or adhered to aquatic vegetation.

The incidence of mosquitoes adults caught in traps have marked reduction, allowing to infer that, in places where the crustaceans were released, the predatory activity on the larvae of mosquitoes was sufficient to reduce the number of adult mosquitoes. An important observation to be made is that, in Tiete River, which have large populations of shrimps and crabs by m<sup>2</sup>, is not observed the presence of evolutionary forms of *Anopheles* spp., or these parasites exist in small quantities, with punctual distribution.

Cases of Zika virus and microcephaly are concentrated in the northeast of Brazil. It is important to remember that water scarcity in the region, the extensive use of water reservoirs, the precarious sanitation services and disability in storm water runoff make the favorable scenario for maintaining high density of *A. aegypti*. Our results demonstrate that the release of these shrimps can provide the elimination of these and other larvae in these reservoirs within hours, eliminating the necessity of use of pesticides in drinking water destined for human, whose impact on people's health have not been properly established.

The stagnant water bodies act as important breeding of *Anopheles* species which are natural vectors *Plasmodium* spp. (Póvoa et al., 2003). Our results show that in the same extent that it increases the number of the shrimps in the rivers occurs, on the same scale, reduction in *Anopheles* sp. population.

We believe that in addition to a balanced ecological environment, the strong correlation between the presence of these shrimps, their high numbers, voracity and the reduction in the culicids population, particularly the *Anopheles* sp. can help to reduce the cases of diseases transmitted by these vectors, especially the malaria.

We concluded that natural shrimps from of Tietê river, state of São Paulo, Brazil, are predators of *A. aegypti*, *An. darlingi* and *C. quinquefasciatus*, in their larval and adult forms, able to consume large quantities of larvae in a short time, constituting an important tool of biological control of these parasites-vectors in the environment.

#### Conflict of interest statement

There was no interest conflict during the execution of this study.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.parepi.2017.05.002>.

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