

Intelligent monitoring of *Aedes aegypti* in a rural area of Rio de Janeiro State, Brazil

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ABSTRACT

The aim of this research was to monitor the presence of females of *Aedes aegypti* (Linnaeus, 1762) on the *Seropédica* municipality, Rio de Janeiro State, from 2010 to 2013. For this purpose, the Intelligent Dengue Monitoring (IM-Dengue) and Intelligent Virus Monitoring (IM-Virus) developed by Universidade Federal de Minas Gerais (Ecovec – Minas Gerais, Brazil), were used. IM-Dengue is a tool that allows achieving a weekly overview of *A. aegypti* infestation, while IM-Virus is another tool that allows detecting dengue virus directly from the mosquito, by Real Time-PCR. Both tools were developed for diagnosis in a prepathogenesis period of the disease, before infection occurrence. Traps were distributed in 19 locations inside the municipality and the bugs were collected weekly during the years of the research. As a result, the presence of 163 females of *A. aegypti* was recorded over the period; there was no circulation of the virus in the municipality. In one of the 19 study sites, a high degree of disease transmission risk was verified. The study concluded that the municipality, as a whole, showed no risk of disease transmission throughout the field research period.

KEYWORDS: Dengue. Virus. Epidemiology. Neglected diseases.

INTRODUCTION

Dengue is an acute febrile disease, whose etiologic agent is an arbovirus of the *Flavivirus* genus, belonging to the Flaviviridae Family. As vectors responsible for the transmission, it has mosquitos from the genus *Aedes* (Meigen, 1818), and more precisely in Americas, the *Aedes aegypti* (Linnaeus, 1762) species. There are currently four known different virus serotypes: DEN-1, DEN-2, DEN-3 e DEN-4^{1,2}.

Considered as a serious public health problem worldwide, especially in tropical and subtropical countries, in its severe form, it is a potentially lethal complication due to plasma leakage, fluid accumulation, breathing difficulties, drop in blood pressure, severe hemorrhage and organ damage³.

According to the World Health Organization, the disease incidence increased 30 times in the last five decades, with 390 million annual infections in more than 100 countries, meaning that nearly half of the world's population is at risk. An estimated number of 500,000 people suffering from the severe form of the disease requires hospitalization each year, and about 2.5% of them die³.

In Brazil, until the month of May 2015, 1,021,004 cases were reported, with 378 deaths. Data report that 340 cities are at risk and 877 on alert. The Southeast region had the highest number of cases (659,900; 64.63%), followed by the Northeast

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(162,053; 15.87%), Central-West (119,912; 11.74%), South (54,473; 5.33%) and North (24,666; 2.4%). The country allocated around 0.4 billion dollars to health surveillance actions, as well as additional resources, specific for each state and city⁴.

The Rio de Janeiro State, in the year of 2015, compared to the same period of the year of 2014 (first semester), showed an increase of 85.59% in the number of cases, with eight confirmed deaths. In the year of 2016, government agencies warned of the risk of epidemics in the cities of the metropolitan areas and in the state capital, due the circulation of serotypes DEN-2 and DEN-3^{4,5}.

In the *Seropédica* municipality, in the Rio de Janeiro State, 2,052 cases of dengue were recorded in the last ten years, from 2006 to 2015. In 2015, in the city, 92 cases of the disease were recorded. In 2016, until April, 41 cases were recorded, an increase by of 30.15%, compared to the same period of 2015⁶. The municipality uses the methodology “*Aedes aegypti* Infestation Index Rapid Survey” (LIRAA), with its tools, to diagnose and establish strategies for epidemiological surveillance in all municipality area, including the campus of the Universidade Federal Rural do Rio de Janeiro.

The disease evolution, in its severe form, is hyperacute and can kill the patient within 24 hours after the first symptoms. There is no vaccine yet to prevent the disease, being the main strategies related to actions to control the vector and to early diagnosis of the disease. Therefore, establishing technologies that optimize these measures is crucial for more effective preventive actions and, consequently, to reduce the risk to which populations are exposed.

Thus, the aim of this research was to monitor the presence of the female *A. aegypti* in the *Seropédica* municipality, Rio de Janeiro State, in the period from 2010 to 2013, using the Intelligent Dengue Monitoring (IM-Dengue) and Intelligent Monitoring virus (IM-Virus) methodologies, as tools for early diagnosis of dengue.

MATERIAL AND METHODS

The research was conducted in the *Seropédica* municipality, metropolitan region of the Rio de Janeiro State, in the period from December 2010 to April 2013. The municipality has an area of 283.76 square kilometers, geographic coordinates Latitude 22.48° South and Longitude 43.41° West, and altitude of 33 meters above sea level^{7,8}. The climate is semi-humid with abundant rains in the summer and dry winters, mean annual temperature of 24 °C and average rainfall of 1,250 mm¹. The municipality has an ecological area of Atlantic Forest, with small forest fragments inside and around the municipality⁹.

The research collected mosquitoes weekly, during the mentioned period, and then sent the insects and the related information to the biotechnology and bioinformatics company Ecovec® (Minas Gerais, Brazil), to formulate a database and laboratory diagnosis. The following methods were used for this purpose: Intelligent Dengue Monitoring (IM-Dengue) and Intelligent Monitoring of Virus (IM-Virus), both developed by the Universidade Federal de Minas Gerais.

Intelligent Dengue Monitoring (IM-Dengue)

It is an adult vector monitoring service, which allows the researchers weekly to obtain a picture of the *A. aegypti* infestation. It consists of a kit with a dark color trap (MosquiTRAP), composed of a vessel with water, an attractive synthetic oviposition (AtrAedes), an cylindrical adhesive card made for fixation of insects and a central lid with a funnel to avoid evasion; a mobile device (cell phone) to submit data; and a website that receives the data and made analytical information available to researchers in real time^{10,11}.

Traps were deployed in 19 strategic locations of the municipality, near edifications in order to cover areas with a greater traffic of people; each place was marked with a number of identification (ID), defined as follows: Inside the Universidade Federal Rural do Rio de Janeiro (UFRRJ): ID1 – Veterinary Institute, ID2 – Agronomy Institute, ID3 – Experimental Station for Parasitological Research W.O. Neitz, ID4 – Main Building, ID5 – Medical Center, ID6 – Geology Department, ID7 – Biology Institute, ID8 – Chemistry Institute, ID9 – Institute of Human and Social Sciences, ID10 – Guardhouse, ID16 – University Hall, ID17 – Biology Institute, ID18 – Student Accommodation, ID19 – Botanical Garden; outside the UFRRJ campus: ID11 – Brazilian Agricultural Research Company (Animal’s Health); ID12 – Technical College of *Seropédica*; ID13 – Animal Reproduction; ID14 – Pesagro-Rio (Organic Agriculture) and ID15 – *Seropédica*’s Maternity Hospital (Figure 1). They were installed in visible places with water and plants, protected from sun and rain. Each collection point was georeferenced using the Global Positioning System (GPS), setting each latitude and longitude. The AtrAedes and the adhesive card were replaced every 45 to 60 days.

Each inspection consisted in counting the insects fixed to the card and distinguishing them according to their species and sex^{12,13}. The inspection was done by UFRRJ’s undergraduate students who were trained to identify the insects. To distinguish the sex of the genus *Aedes*, a differentiation of the male’s feathery antennae and female’s hairy antennae was carried out. The collected data were sent

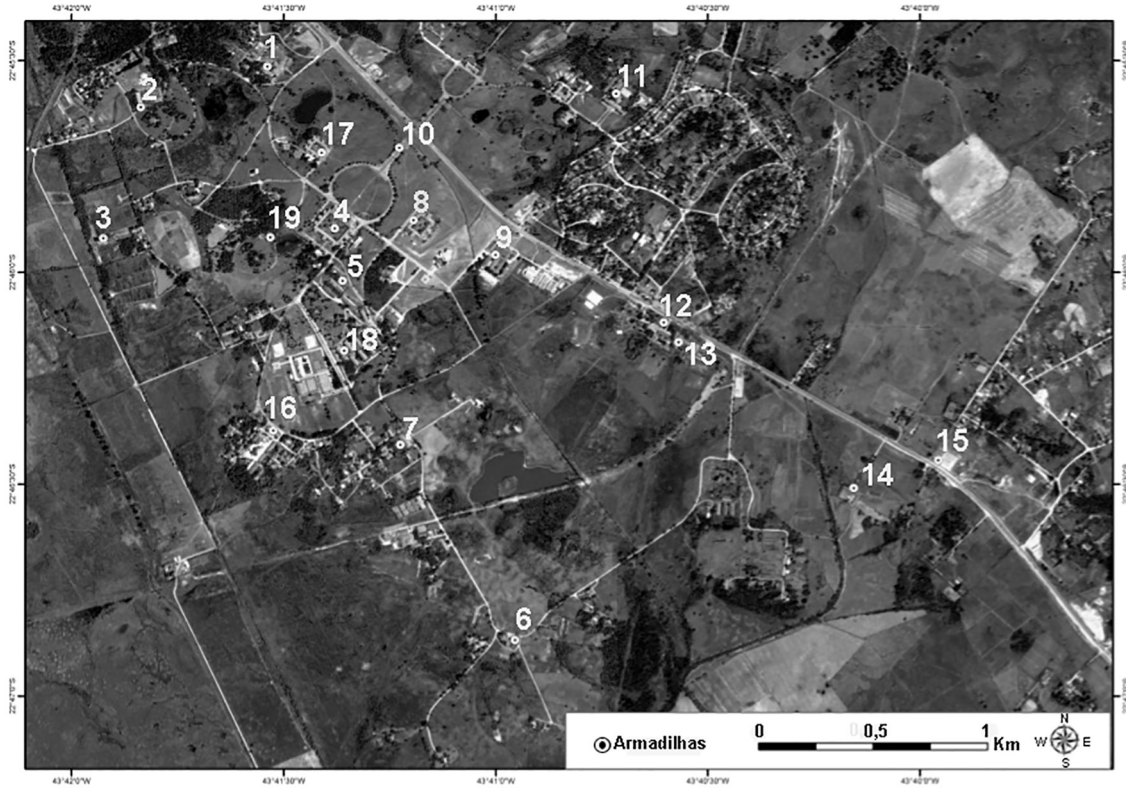


Figure 1 - Map showing the trap location and each trap ID number, *Seropédica* Municipality, Rio de Janeiro -State

in real time via a mobile device to the Ecovec website, to create the digital platform, chart and map projection.

To determine the disease's risk, the average index of the weekly collected females of *A. aegypti* (IMFA) was calculated by dividing the total number of females found by the total number of traps inspected during that week, ranking the levels in: satisfactory (< 0.15), moderate (0.15 to 0.30), alert (0.30 to 0.60) and critical (> 0.60), according to the Ecovec¹¹.

The average female *A. aegypti* index (IMFA) was used for estimation of the disease transmission risk level for each trap location during all research period, from 2010 to 2013, the total number of females found in a specific ID divided by the number of monitored traps in the same ID, over the period, was used.

Intelligent Monitoring Virus (IM-Virus)

IM-Virus allows the detection of the presence of viral infection in the collected mosquitoes. The trapped vectors of the genus *Aedes* are weekly collected and sent to the Ecovec laboratory. These vectors are analyzed by the RealTime-PCR technology, which is fast and has a high level of safety, and indicates the presence of RNA viral segments, specific of the Dengue Virus. The results are available as maps and tables¹¹.

The mosquitoes were separated for analysis with the aid of a disposable wooden toothpick, to avoid contamination between insects of other traps. Then, to be transported, they were placed in a tube containing Guanidine Thiocyanate, and, finally, sent to Ecovec to search for the presence or absence of the Dengue Virus.

The research has also analyzed the association between temperature and rainfall with the number of females of *A. aegypti* collected in the four seasons of the studied years, in order to know if the climate factors influenced a higher density of the vectors on campus. The average monthly rainfall data and temperature were obtained from the Instituto Nacional de Meteorologia, *Estação Automática Rio de Janeiro – Seropédica*¹⁴. The statistical analysis used was the Pearson linear correlation method provided by the SPSS Statistics 19.0 software¹⁵, and the interpretation of correlation coefficients was performed according to Callegari-Jacques¹⁶.

RESULTS

The 2,197 IDs inspections were made during all the research period. The number of mosquitoes collected was 2,243, *Aedes* and *Culex* (Linnaeus, 1758) genus and *A. aegypti* e *Aedes albopictus* (Skuse, 1894) species. *Culex* was the predominant genus found with the number of

1,302 mosquitoes. The number of collected females of *A. aegypti* was 163, the year of 2011 was one with the highest appearance of the species (73) (Tables 1 and 2).

Regarding the epidemiological weeks with most appearance of the *A. aegypti* species in the selected IDs, the 50th week of the year of 2011 (from 11 to 17 December) was the week with the highest number of mosquitoes (nine),

followed by week 13 of 2013 (from 24 to 30 March), with six mosquitoes. The weeks with appearance of the species in the given IDs were: from week 28 to 34 of 2011 (from July to August) and from week 19 to 30 of 2012 (from May to July) (Figure 2).

The IMFA, calculating for each epidemiologic week, evidenced that, in the years 2011 and 2013, the alert level was

Table 1 - Number of inspected traps, collected females of *Aedes aegypti* (IFMA), by deployed locations (ID), Seropédica municipality, RJ, from 2010 to 2013

| Years | IDs | Number of traps inspected | Number of <i>A. aegypti</i> females | IFMA | |
|-------|------|---------------------------|-------------------------------------|-------|------|
| 2010 | 1 | 2 | 0 | 0.00 | |
| | 2 | 2 | 0 | 0.00 | |
| | 3 | 2 | 0 | 0.00 | |
| | 4 | 1 | 0 | 0.00 | |
| | 5 | 2 | 0 | 0.00 | |
| | 6 | 2 | 0 | 0.00 | |
| | 7 | 2 | 0 | 0.50 | |
| | 8 | 2 | 0 | 0.00 | |
| | 9 | 2 | 0 | 0.00 | |
| | 10 | 1 | 0 | 0.00 | |
| | 11 | 1 | 2 | 2.00 | |
| | 12 | 2 | 0 | 0.00 | |
| | 13 | 1 | 0 | 0.00 | |
| | 14 | 2 | 0 | 0.00 | |
| | 15 | 2 | 0 | 0.00 | |
| | 16 | 2 | 0 | 0.00 | |
| | 17 | 2 | 0 | 0.00 | |
| | 18 | 2 | 0 | 0.00 | |
| | 19 | 0 | 0 | 0.00 | |
| 2011 | 1 | 50 | 47 | 0.94 | |
| | 2 | 50 | 3 | 0.06 | |
| | 3 | 50 | 0 | 0.00 | |
| | 4 | 50 | 0 | 0.00 | |
| | 5 | 50 | 2 | 0.04 | |
| | 6 | 50 | 2 | 0.04 | |
| | 7 | 50 | 2 | 0.04 | |
| | 8 | 50 | 1 | 0.02 | |
| | 9 | 50 | 0 | 0.00 | |
| | 10 | 50 | 4 | 0.08 | |
| | 11 | 50 | 2 | 0.04 | |
| | 12 | 50 | 2 | 0.04 | |
| | 13 | 50 | 0 | 0.00 | |
| | 14 | 50 | 0 | 0.00 | |
| | 15 | 50 | 3 | 0.06 | |
| | 16 | 50 | 0 | 0.00 | |
| | 17 | 50 | 1 | 0.02 | |
| | 18 | 50 | 3 | 0.06 | |
| | 19 | 37 | 1 | 0.02 | |
| 2012 | 1 | 49 | 43 | 0.87 | |
| | 2 | 49 | 4 | 0.08 | |
| 2012 | 3 | 48 | 0 | 0.00 | |
| | 4 | 49 | 3 | 0.06 | |
| | 5 | 49 | 2 | 0.04 | |
| | 6 | 49 | 2 | 0.04 | |
| | 7 | 49 | 0 | 0.00 | |
| | 8 | 49 | 3 | 0.06 | |
| | 9 | 48 | 1 | 0.02 | |
| | 10 | 49 | 3 | 0.06 | |
| | 11 | 48 | 2 | 0.04 | |
| | 12 | 47 | 1 | 0.02 | |
| | 13 | 49 | 0 | 0.00 | |
| | 14 | 49 | 0 | 0.00 | |
| | 15 | 49 | 1 | 0.02 | |
| | 16 | 48 | 0 | 0.00 | |
| | 17 | 49 | 2 | 0.04 | |
| | 18 | 48 | 1 | 0.02 | |
| | 19 | 49 | 2 | 0.04 | |
| | 2013 | 1 | 16 | 15 | 0.93 |
| | | 2 | 16 | 1 | 0.06 |
| 3 | | 16 | 0 | 0.00 | |
| 4 | | 16 | 0 | 0.00 | |
| 5 | | 16 | 0 | 0.00 | |
| 6 | | 16 | 1 | 0.06 | |
| 7 | | 16 | 0 | 0.00 | |
| 8 | | 16 | 0 | 0.00 | |
| 9 | | 16 | 1 | 0.06 | |
| 10 | | 16 | 0 | 0.00 | |
| 11 | | 16 | 0 | 0.00 | |
| 12 | | 16 | 0 | 0.00 | |
| 13 | | 16 | 0 | 0.00 | |
| 14 | | 16 | 0 | 0.00 | |
| 15 | | 16 | 0 | 0.00 | |
| 16 | | 16 | 0 | 0.00 | |
| 17 | | 16 | 0 | 0.00 | |
| 18 | | 16 | 0 | 0.00 | |
| 19 | | 16 | 0 | 0.00 | |
| Total | | 2,197 | 163 | 0.07* | |

* IMFA average total of the municipality of Seropédica during the years 2010 to 2013.

Table 2 - Total number of collected mosquitoes during the period from December 2010 to 2013, in the studied locations of the *Seropédica* municipality, RJ, with the determination of species and sex

| Species | Sex | Years | | | | Total |
|-------------------------|--------|-------|------|-------|------|-------|
| | | 2010 | 2011 | 2012 | 2013 | |
| <i>Aedes</i> sp. | Male | 2 | 0 | 0 | 0 | 2 |
| | Female | 1 | 25 | 7 | 3 | 36 |
| <i>Aedes aegypti</i> | Male | 0 | 3 | 2 | 0 | 5 |
| | Female | 2 | 73 | 70 | 18 | 163 |
| <i>Aedes albopictus</i> | Male | 0 | 11 | 5 | 14 | 30 |
| | Female | 11 | 233 | 335 | 126 | 705 |
| <i>Culex</i> sp. | Male | 9 | 180 | 154 | 47 | 390 |
| | Female | 23 | 323 | 443 | 123 | 912 |
| Total | | 48 | 848 | 1,016 | 331 | 2,243 |

reached for transmission of the disease in the epidemiological weeks 50 (0.47) and 13 (0.32), respectively. The year of 2012 was that in which the moderate level was more present sometimes coming close to the alert level (Figure 3).

By the location where the traps were deployed, the ID1 (*Instituto de Veterinária* - UFRRJ) was the one which reached the critical level of the disease (0.90), with the sum of the number of females of *A. aegypti* found throughout the research period. All the other IDs remained within the range stipulated as satisfactory. The IDs 3, 13, 14 and 16 were those with negative indices of disease transmission. The IMFA calculated from the total number of female *A. aegypti* by the total number of traps inspected, during the entire research, found the value of 0.07, classified as satisfactory (Table 1, Figure 3).

The IM-Virus was conducted only in the epidemiological weeks of the year 2012 and did not detect the presence of dengue serotypes in mosquitoes collected by the municipality selected IDs.

The statistical analysis confirmed a strong association ($r=0.7160$) between the number of females of *A. aegypti* and average temperatures from weeks 14 to 26 corresponding to the months of April, May and June (autumn). Regarding the association between the number of females of *A. aegypti* and the average rainfall, a moderate association ($r=0.4043$) was found in weeks 40-52, for the months of October, November and December (spring) (Figure 4).

DISCUSSION

During the entire research, the ID1 (*Instituto de Veterinária* - UFRRJ) had the largest number of females of *A. aegypti*, reaching an IFMA considered as critical (0.90), considering the risk of disease transmission. The ID1 is the only one surrounded by a remaining forest with lakes.

This insect is primarily a forest species, occurring on its edges, moving into human households and with preference for humidity; its breeding place consists predominantly of places with standing water. Indeed, the environment itself makes ID1 a favorable area for proliferation of the mosquito, something that does not happen with the other IDs^{12,17-19}.

The IDs 3, 13 and 14 are animal breeding and reproduction areas, which may explain the negative ratio of mosquito presence in the research period, given the frequency in use of insecticides in the environment, using for baths and sprinkling^{20,21}. The ID16, the University Hall, is the starting point of all anti-vector and pathology campaign, thus becoming an excellent inspection place.

The low number of females of *A. aegypti* studied in epidemiological weeks 28 to 34, 2011 (July and August), and 19 to 30, 2012 (from May to July), can be explained by the beginning of a drop in temperature and rainfall occurred in the town, representing the autumn and winter seasons. Ribeiro *et al.*²², working on the association between dengue incidence and climate variables, noted that high temperature and rainfall contribute to a higher density of mosquitoes and disease occurrence. Costa *et al.*²³ and Souza *et al.*²⁴ evidenced a large incidence of the mosquito in high temperature and rainy seasons. All these studies, either by the value observed during the epidemiological weeks, either by the statistical analysis results, proved that there is a weak correlation between lower temperatures/rainfall and the number of *A. aegypti* in the environment, the opposite of what really happens in the warmer months of the year.

By calculating the average IMFA of the studied IDs (0.07), counting all the years, it can be said that the chosen locations did not pose a risk of disease transmission to local passers-by during the whole research. That goes against what was observed in the ID1 (*Instituto de Veterinária* - UFRRJ). The ID1 has a high rate of the occurrence of the

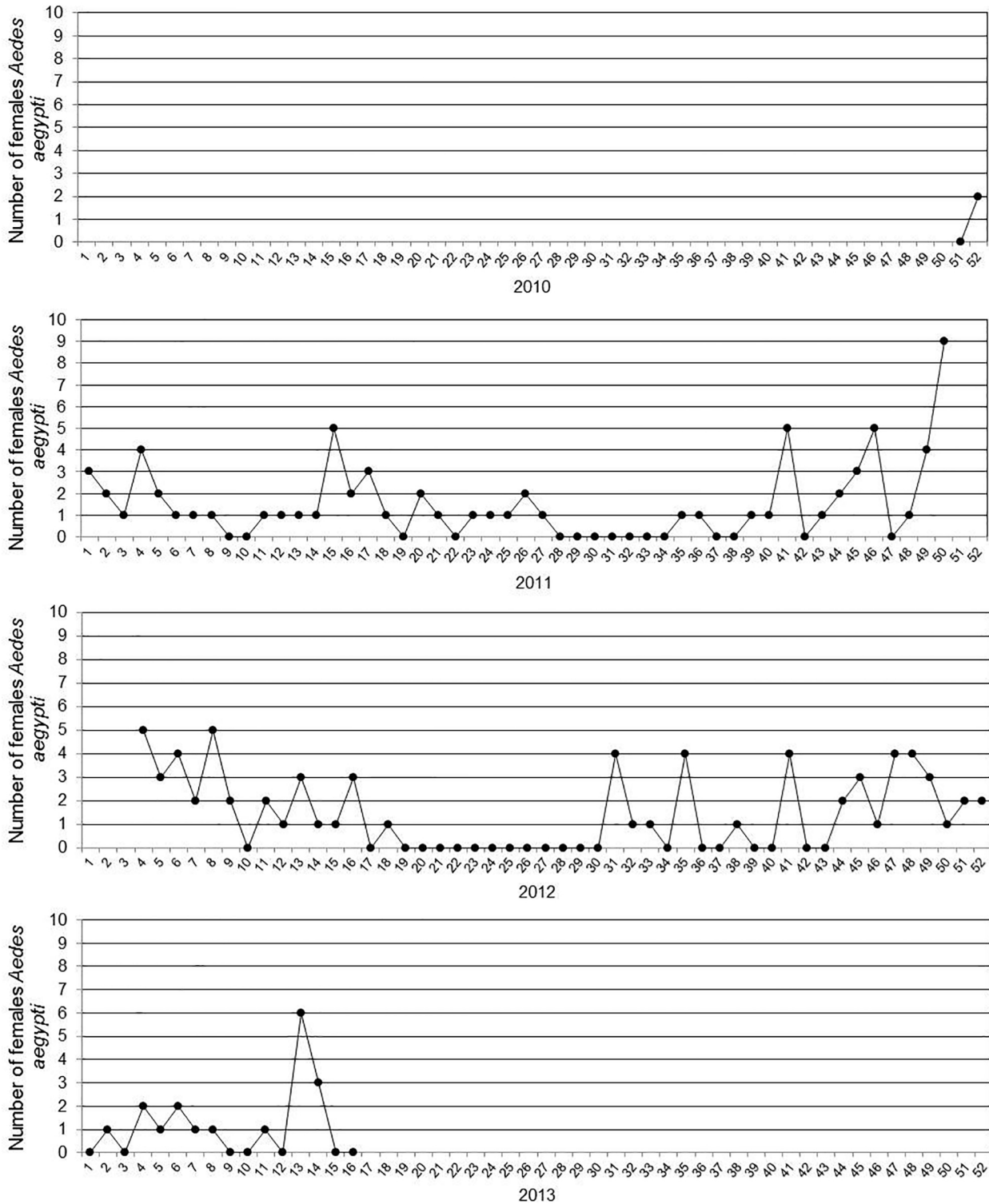


Figure 2 - Number of collected females of *Aedes aegypti*, by epidemiologic week, during the period from December 2010 to April 2013, in 19 locations from the *Seropédica* municipality, RJ

disease. This ID is considered an imminent danger zone, and authorities should take necessary measures to reverse this situation.

The data analysis does not show a significant increase in the number of females of *A. aegypti* during the entire

research, however, preventive measures are necessary in order to maintain or reduce this number. The data obtained from the research demonstrated the importance of the tools used in the city and, thus, can locate and target the necessary measures to prevent possible cases of the disease.

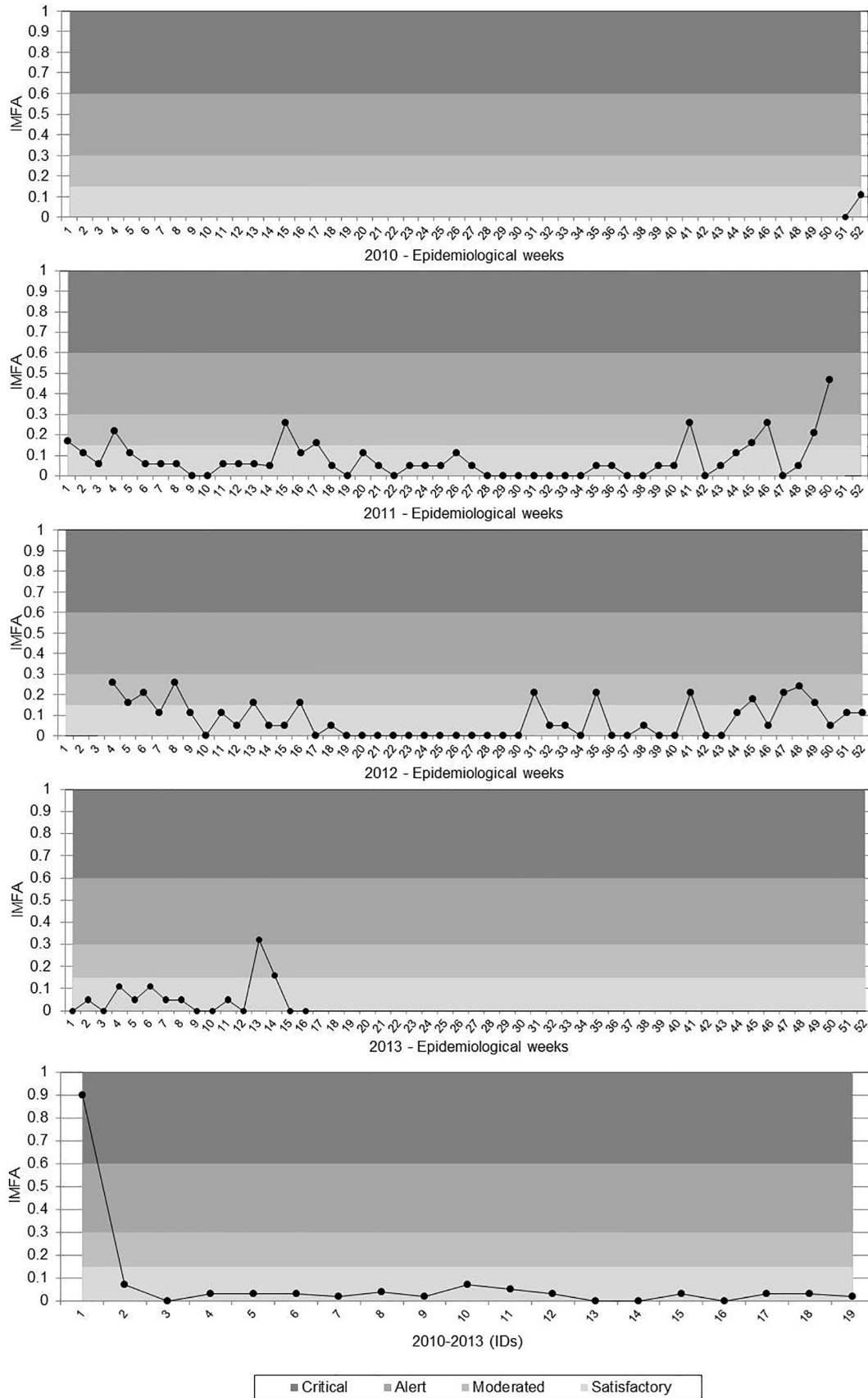


Figure 3 - Average index of females of *Aedes aegypti* (IFMA), calculated by epidemiological week and the inspected trap locations (IDs), from December 2010 to April 2013, in the *Seropédica* municipality, RJ, indicating the transmission risk levels of the disease

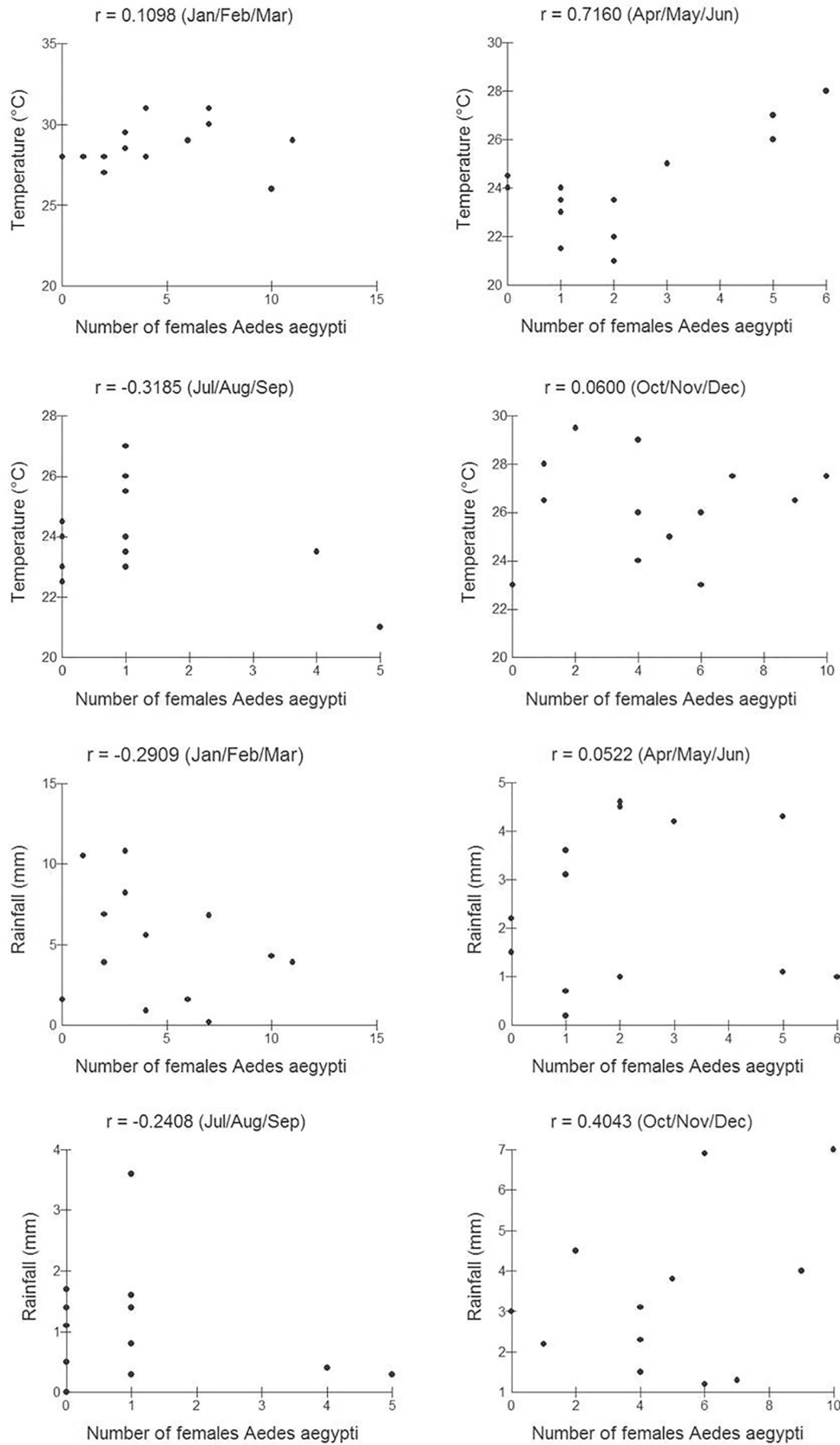


Figure 4 - Scatter plot of the number of females of *Aedes aegypti* with average environmental temperature and average rainfall, in the 52 weeks of the research period (2010-2013), in the *Seropédica* municipality, RJ: summer (Jan/Feb/Mar) - weeks 1 to 13, autumn (Apr/May/June) - weeks 14 to 26, winter (Jul/Aug/Sep) - weeks 27-39 and spring (Oct/Nov/Dec) - weeks 40-52

The absence of dengue virus in selected locations can be evaluated as a positive point; however, the presence of the mosquito does not rule out the possibility of future contamination of passers-by. The municipality is basically an university city, with much of its population coming from other municipalities of the Rio de Janeiro State, as well as from other states; this means that people infected with the virus in their city of origin, and still in the incubation period of the disease, can act as a carrier and source of the virus favoring its circulation in the city, and together with the vector, will cause other dengue cases. Prophylactic measures should always be taken against *A. aegypti* breeding locations thus preventing their proliferation and the spread of this disease.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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