

## Aedes aegypti egg counting system

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**Abstract**— New monitoring methods of dengue vector and evaluation of public policies on dengue control are major concerns for several tropical countries. Drawback on monitoring methods base on oviposition surveys are the counting process of mosquito eggs, information store and analysis. Here we present a new automated egg counting system for remote *Aedes aegypti* population survey. The system is base on an optical scanning platform, a man-machine interface, and a software for mosquitoes eggs counting. Acquired information are sent over the internet and remotely analyzed. Prototypes of the device were installed and implement in two different cities.

### I. INTRODUCTION

Dengue is a disease caused by a virus that lodges in the *Aedes aegypti* mosquito and it is transmits to humans through the insect bite. The *Aedes aegypti* is originally from Africa (probably in the northeast region) and it was spread to Asia and Americas, mainly through the maritime traffic.

The monitoring of the dengue vector and effectiveness of public policies on dengue control are based on the determination of dengue vector presence, density and frequency of occurrence. Methods that allow such measurements are numerous, including the identification of eggs, larvae and adult mosquitoes. Monitoring methods base on oviposition surveys are well established and explored.

Nowadays a major drawback of these techniques is related to the applied counting process of mosquito eggs. The count of eggs is done manually with the aid of a magnifying lens or optical microscopes, which makes the process slow and exhausting. Even being performed by a trained professional, the counting method presents several error sources and variables to be controlled.

Here we describe an automated system for *Aedes aegypti* eggs counting deposited in traps (palettes), previously installed in several small cities in the northeast of Brazil. The system optically scans the reeds, generating a digital image of the analyzed palette. Imaging processing is also performed and a developed egg counting software assist the determination of egg population. The system has already been deployed and implemented at the city of Recife and at Santa Cruz do Capibaribe, both in the state of Pernambuco (Brazil).

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### II. DENGUE CONTROL

The *Aedes aegypti* (Fig. 1a) is an insect with behavior strictly urban, and it is rare to find samples of their eggs or larvae in water reservoirs in the forest. The dengue virus is carried only by the mosquito female, that becomes a permanent vector of the disease, and their offspring are born already infected.

The *Aedes aegypti* eggs are laid millimeters above water surface (Fig. 1b), in places such as empty cans, bottles, tires, gutters and pots of plants. The eggs of the mosquito can resist up to one year without contact with the water. When it rains, the water level rises, comes into contact with the eggs which hatch in just over 30 minutes. In five to seven days period, the larvae (Fig. 1c) go through four different growing stages giving rise to a new mosquito. The larvae life cycle depends on breeding containing standing water for their development [1].

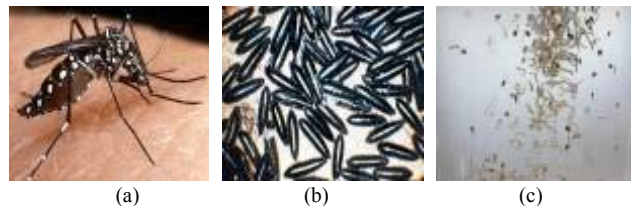


Fig. 1. The figures are (not real proportions): (a) *Aedes aegypti* mosquito, (b) the mosquito eggs and (c) its larval stage.

The eradication of *Aedes aegypti* is considered practically impossible on account to population growth, occupation of the environment and the lack of infrastructure in large cities [2], [3]. An effective program against the mosquito should be focused on social awareness for the elimination of breeding sites. The population should be targeted to cover its reservoirs, leaving wet areas inaccessible to mosquito. In fact, the dengue control should be done with attention to environmental hygiene, sanitation and education communities. Although education and sanitation plays an important rule on *Aedes aegypti* proliferation control also explore the application of insecticides in water and in air, placing the population at risk by their exposure to products that are known to be neurotoxic and allergenic, and, therefore, independent on dose to produce its toxic effect.

Ovitrap are being used on the statistical analysis of *Aedes aegypti* population grows. Ovitrap are special traps to collect mosquito eggs. As mosquito laid eggs preferably in containers with a dark color, clean water or little organic matter, each Ovitrap (Fig. 2a) consists of a small black

plastic bucket, with two palette of plywood, water, alfalfa (to attract the female mosquito) and biological larvicide (Bti-G) [4], [5]. Each Ovitrap can collect more than 1000 eggs, therefore Ovitrap can also contribute to the reduction of *Aedes aegypti* population.

Every four weeks, the palettes are replaced. The former palettes are collected by health agents and taken to an Egg Count laboratory installed in each municipality. With a slow counting technique, palettes accumulate and the mosquito eggs start to fall out the palettes, which affects subsequent counting.

### III. METHODS

The *Aedes aegypti* egg counting system (hardware and software), presented in Fig. 2b, is an equipment that performs optical scanning images of Ovitrap palettes, assist the counting of the trapped mosquito eggs and remotely analyzes the palettes images.

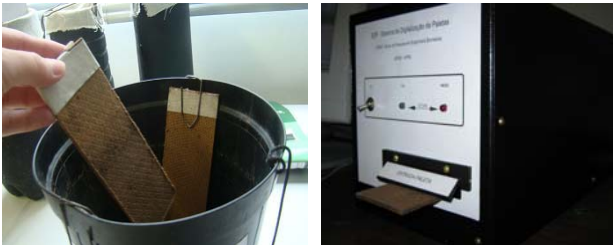


Fig. 2. (a) Photo of mounting ovi-trap with palettes and (b) System prototype.

The developed system components are described in Fig. 3. The system is composed by a control unit, an image capture device, a LED lighting system and a mechanical support associated with a motorized linear translation stage. The control unit is based on a firmware/microcontroller (PIC 16F876), which activates the mechanical system and the image capture device, placed 3 cm above the palette. The image capture device used is a digital camera with 7.1 mega-pixel with "super macro" operation mode. The capture device chosen allows us to obtain images with 3072 pixels x 2304 pixels, and file size of 3.5 MBytes in JPEG format. The generated file size minimizes the computational cost of the image automatic analysis and allows a visual identification of the *Aedes aegypti* eggs.

The equipment was built and mounted in a metal box (30 cm x 16 cm x 18 cm), allowing user access only to the platform door, where the palettes are introduced into the system (Fig. 2b). The equipment layout was developed for easy handling by the user. In the rear part of the equipment, one can find a DB9 connector for serial communication (an USB/SERIAL cable converter is used on the high speed computer-equipment interface). The system is triggered by a software installed on a computer IBM-PC. The computer also receives the images captured by the system and sends the JPEG standard, to a server system, which performs the automatic counting of eggs.

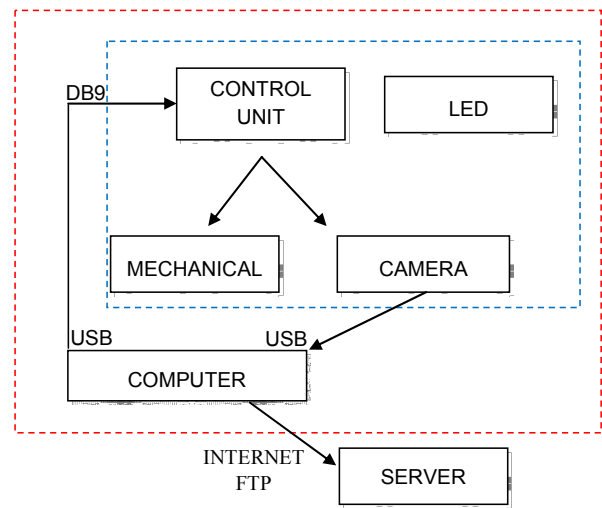


Fig. 3. Egg counting system description.

A human-machine interface was developed, providing a full control of the equipment. The entry screen of the developed software is present in Fig. 4a. Moreover the interface software provides to the user the option of performing a manual counting of the eggs number on the imaged captured. On every mouse click over the palette picture the egg location is registered and count by the software (Fig. 4b).



Fig. 4. Human-Machine Interface, (a) entry screen, (b) after image acquisition, with manual counting computer-assisted, where the eggs are marked with red dots and (c) eggs marked with red dots to score computer-assisted.

Image acquisition starts when the user enters the palette in the system, the software sends information through the USB port to the microcontroller, which drives the operation of a stepper motor that runs the displacement of the platform with the sample. The camera is powered on and is made to capture the images. Due to sample size (12 cm x 5 cm) and the need for high image quality, three areas are registered in the palette. After scanning of sections of the palette images are superimposed to form a single image of the full palette. The final image has generated 5071pixels x 2304pixels dimensions and file size of 4.5 MBytes. The scanning process takes about 40s.

The captured image and the manual counting result are send over the Internet via FTP (File Transfer Protocol) directly to a server at the Federal University of Pernambuco. The server automatically counts the eggs using pattern recognition software, base on segmentation and filtering procedures. The automatic counting algorithm is described in [6].

#### IV. RESULTS

The palette digitalized image displayed by the software (Fig. 4b) allows the user to perform computer-assisted counting. To verify the efficiency of the system on egg counting, the counting time of different palettes analyzed with magnifying glass and with the our device were determined and compared. These results are presented in TABLE 1, whose columns shows the number of eggs on 10 different pallets, the counting time with a magnifying glass and the time of count made with the proposed system.

TABLE 1  
TEMPORAL COMPARISON OF METHODS OF COUNTING.

Number of eggs	Magnifying glass (min)	Developed System (min)
2	10	2
7	15	4
17	10	2
61	20	7
126	25	9
197	30	10
218	40	13
458	30	10
512	30	9
816	70	25

As can be seen also in TABLE 1, on both methods the counting time was not determined only by the number of eggs on the pallets. The degree of dirt on the palettes and the superposition of the eggs are also important aspects on the system efficiency.

Validation of automatic algorithms was performed by comparing the results of automatic counting with those obtained by manual counting process assisted by computer.

On the valuation of 100 palettes 3297 eggs were identified by the manual procedure assisted by the computer and 3385 counts were performed by the pattern recognition automated software.

#### V. CONCLUSION AND PERSPECTIVES

The *Aedes aegypti* mosquito that causes dengue disease was rapidly spread across America, and its eradication is a major problem at several countries. The monitoring of possible outbreaks is extremely important in order to take a step toward dengue eradication. In Brazil, this monitoring is being done by analyzing Ovitrap palettes. Here we present a system that acquires images of Ovitrap palettes. The system optically scans the reeds, generating and storing a digital image of the analyzed palette. The software allows the user recognize *Aedes aegypti* eggs on the digitalized image and register the number of identified structures.

We verified that manual counting procedure assisted by our system allows palettes analyses faster (at least 2x) than the conventional method with a magnifying glass.

A full automated counting algorithm has developed and evaluated. The method applied in a experimental set of 100 images obtained an average error count of 2.67%.

Two prototypes of the developed system were implemented on two different dengue risk areas, and it results are being integrated into a geographic information system, showing the focus of the mosquito in a geo-referenced map. On this count, knowing where are the focuses of the dengue vector, public administration can act much faster, avoiding the development of the disease.

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