

Ecological Survey of *Aedes* Mosquito Larvae in Selected Dengue Outbreak Villages in Cagayan de Oro City

LESLEY CASAS LUBOS

lesleyclubos@liceo.edu.ph

ORCID No. 0000-0002-8761-3680

Vice President for Research, Publication, and Extension

Liceo de Cagayan University

Cagayan de Oro City, Philippines

ABSTRACT

In the recent years, dengue fever has become an international global public health concern. Dengue, a disease common in tropical and sub-tropical countries in the world, is transmitted through the bite of an *Aedes* mosquito. Dengue fever is potentially fatal and mainly affects children. The Philippines is one of the four countries in the Western Pacific which reported the highest dengue incidence in recent years. The study was done in June - October 2014 (Wet Season) and December 2014- April 2015 (Dry Season). Ten villages in Cagayan de Oro City were selected for the survey to identify the species and determine the abundance of mosquito larvae. A larval survey was conducted in each study area to determine the main breeding sites of dengue vector mosquitos and to examine the relationship between the ecological conditions surrounding the houses and the variation in larval density among houses. In this study the researcher uses oviposition traps designed to collect mosquito larvae. Of the mosquito larvae collected in the ten villages, the dominant identified species is *Aedes aegypti*. Data shows there are more *Aedes* larvae during the dry season. The study suggested that larval survey programme could serve as a tool not only to monitor the local dengue vector distribution but also to provide objective information for taking

appropriate action by the community against dengue vectors.

Keywords: Mosquito, *Aedes*, Ecological Survey, Wet and Dry Season, Villages

INTRODUCTION

In the recent years, dengue fever has become an international global public health concern as there has a dramatic increase in cases of dengue in tropical and subtropical regions around the world, predominantly in urban and semi-urban areas (Yboa and Labrague, 2013). Dengue and dengue hemorrhagic fever have become a serious threat to public health throughout Southeast Asia (Gubler, 1997).

According to WHO (2014), there are about 50 million cases of dengue fever incidence annually worldwide in which the highest is in tropical and subtropical regions where there is increase in disease activity recently.

The National Epidemiology Center of the Philippines' Department of Health reports a total of 59,943 dengue cases from January 1 to September 6, 2014. The majority of the infected patients were 5 to 14-year old children (38.91% of the total cases), and more than half were males (52.77%). A total of 242 deaths (CFR 0.40%) were recorded since January 2014, and most of them were children (WHO, 2014).

In Northern Mindanao, Cagayan de Oro City has the highest incidence with 2,208 recorded in September 2014 and increases to 2,813 as of November 2014 (Kumilat, 2014).

Locally, dengue cases are recorded as one of the major problem causing an increasing number of hospitalizations among children. Presently, with new technologies and profound findings of our brightest scientists such as virologist, it has been a challenge for all of to discover a vaccine, treatment and alternative solutions to lessen the number of dengue cases.

The rationale for choosing this experimental research was to discover new agents to stop the life cycle of the mosquito, thus preventing the development of larvae to reach the final adult stage. Furthermore, the effectiveness of 3% Iodized and Sea salt will be able to provide preventive management and to decrease the risk of dengue cases through these evidence-based findings.

The Ovitrap were used throughout the experiment because according to research, it can imitate a breeding ground for mosquitos in terms of color, texture, shape and size of the container (Kumiwat et al., 2014; Barrera et al., 2013; Ho et al., 2011; Ritchie et al., 2008; Tilak et al., 2005).

Classification of *Aedes*

Aedes is a genus of mosquitos that is originally found in tropical and subtropical zones which are widely spread around the world. It was Meigen during 1818 who gave the name and description. The generic name comes from the Ancient Greek word which means unpleasant or odious (Wikipedia, 2013).

The mosquito especially *Ae. aegypti* originated in Africa, and it is a vector for transmitting various diseases, especially dengue fever which is the most common disease here in the Philippines. This mosquito was first named as *Culex aegypti* by Fredric Hasselquist in 1757 but was revised to honor his mentor Carl Linnaeus. Only the female mosquito sucks for blood in order to mature their eggs. Mosquitos chose to breed in any areas in the house which has stagnant water and wet spaces. Chemicals produced by mammals like ammonia, carbon dioxide, lactic acid and octenol are a good host for mosquitoes. Mosquitoes typically bite at dusk in outdoor, indoor, and shady areas and even during cloudy weather which is capable of the entire year of spreading infection (Control Disease Center, 2015).

Also known as Asian tiger mosquito, it is active throughout the year in tropical and subtropical location. It is a secondary vector capable of transmitting dengue. Frederick A. Askew Skuse put a name on the species based on its characteristic. It is small and dark with a dorsal stripe and banded legs. It is attracted to bite humans but will feed on cats, dogs, squirrels, deer, and other mammals like birds. It bites during daytime on the exposed skin either outdoors or indoors. After feeding on blood, the female mosquito lays its eggs above the surface water of natural habitats and artificial containers. When eggs are exposed to rain, they hatch and become larvae. The larvae feed on aquatic organisms, algae and, any particle of plants and animal material. The aquatic cycle happens from seven to nine days, while an adult mosquito lived around three weeks. The eggs are capable of living during winter with regards to temperature and climates (Control Disease Center, 2015).

Mosquito Life Cycle of *Ae. aegypti*

The *Ae. aegypti* has a complex life cycle that consists of four stages: egg, larva, pupa, and adult. The mosquitoes are attracted to any container that stores water suitable to become a nursery. Examples of containers are bowls, cups, tires, barrels, vases or even fountains. An adult female mosquito lays at least 100 eggs on habitats, which begins the first stage of the cycle. The eggs stick to container walls and can survive during dry out or winter. The second stage occurs when the water level rises and covers the mosquito eggs, then eventually they hatch and become larvae. Furthermore, the presence of floodwater and rainwater can also trigger the hatching ability. The larvae feed on microorganisms in the water in order to survive. Metamorphosis, triggered

after molting, takes place from which the larva develops into a pupa. The third stage occurs when the Pupa, develops its newly formed body into an adult flying mosquito then leaves the water. Finally, once adult mosquitoes emerge, they take their first meal. Male mosquitoes feed on nectar from flowers while the female mosquitoes feed on humans and animals for food needed to produce eggs. After feeding, female mosquitoes will look for water sources to lay more eggs. The mosquitoes can fly 1 to 1.5 miles per hour (Control Disease Center, 2009).

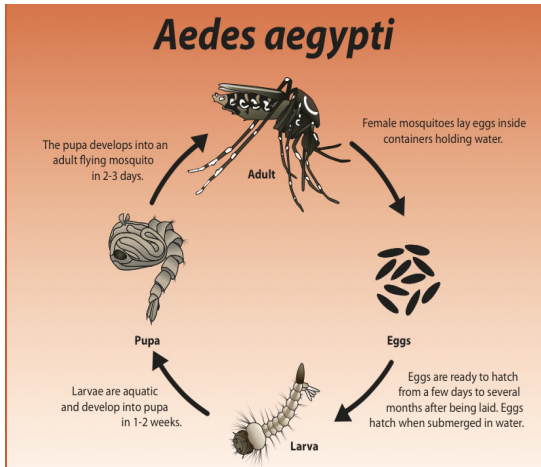


Figure 1. Mosquito life cycle of *Ae. Aegypti* (Control Disease Center, 2009).

Mosquito Larvae Behavior

The larvae are the most active aquatic form of the mosquito. They are a large part of the life cycle of mosquitoes and are therefore well adapted to their role. This stage can be considered just as important, in a way, as the adult stage. Mosquito larvae are usually filter feeders. They have fan-like mouth where they filter out small particles of decaying material and microorganisms which they then eat. Some larger larvae are known to eat species and even members of their own species. As these do not bite people when they turn into adults, and they control populations of biting mosquitoes, the predatory larvae have been called allies to humans in the fight against mosquitoes. Larvae swim by wiggling their bodies back and forth. They do so to move up and down the water column to feed and to avoid danger. When mosquito larvae sense a sudden change in light intensity, they immediately swim toward the bottom of the water container they are in to avoid being eaten from up above (Chmielewski, 2007).

Dengue

A mosquito-borne-disease such as dengue is caused by one of the four related dengue viruses (DENV-1,-2,-3, and -4). One of these types of infection gives immunity on that serotype for life but provides no long-term immunity to other serotypes. With each serotype, a person can be infected four times. *Aedes* mosquitoes (most often *Ae. aegypti*) like dengue viruses are transmitted from person to person in the domestic environment (Control Disease Center, 2015).

Surveillance of Dengue Incidence in Cagayan de Oro City

Surveillance of dengue vectors is an important part in the control and prevention of dengue fever epidemics. Surveillance is essential in dengue fever prone areas as it is the only available means of preventing or limiting a dengue epidemic. A baseline of data needs to be established and of which vector control staff can be referred to when determining when and where preventative measures can be implemented. There are two general methods of monitoring dengue vector numbers; the first is via adult numbers and the second via immature numbers (Long, 2013).

In 2014, Cagayan de Oro City had the highest dengue cases in Northern Mindanao with 2,208 recorders in September and 2,813 as of November 8. The Regional Epidemiology Surveillance and Health Emergency Management region data showed 9,309 dengue cases from January this year until November 8 and recorded 45 deaths (Kumilat, 2014).

A clean-up drive was conducted over the weekend to combat the threat of the potentially deadly disease dengue. The residents took part in a barangay-to-barangay cleanup of areas where dengue-carrying mosquitoes are likely to breed. The city health office urged residents to make sure their surroundings are clean and dry, so that dengue-carrying mosquitoes will have little chance of breeding. The health office said there had been more than 1,600 cases of dengue in the first eight months of 2014, compared to 1,359 in the same period last year in Cagayan de Oro City (Locsin, 2014).

OBJECTIVES OF THE STUDY

The general objectives of the study aimed to determine the abundance of mosquito larvae during wet and dry seasons in 10 selected villages of Cagayan de Oro City.

Specifically, it aimed to:

1. identify the species of mosquito larvae from the Ovitrap; and
2. compare the number of mosquito larvae found in the Ovitrap during wet and dry season in 10 villages.

MATERIALS AND METHODS

Research Protocol

The researcher strictly followed the Ethical standards in conducting the study in the select community. As an entry protocol, the researcher did a courtesy call to the community chief to explain the nature and scope of the study and asked for permission to conduct study in their area. Moreover, the community leader was also consulted on the areas where the ovitrap can be placed during the duration of the study. The researcher carried out all procedures in accordance with ethical principles and considerations.

Study area

The study was conducted in Cagayan de Oro City (8° 28'56" N, 124° 38'50" E), with approximately 602,088 population, located in the northern Mindanao region (NSO Census 2010). The timeframe of the experiment was from September 2015 to February 2016.

Ten barangays were selected for the survey of dengue vector mosquito larvae: The 10 Barangays chosen for the study were Carmen, Kauswagan, Balulang, Bulua, Lapan, Bugo, Camaman-an, Gusa, Iponan, and Patag. The reason for choosing these locations was that it has been noted by the Department of Health (DOH) that these Barangays belong to the top ten highest dengue incidences in Cagayan de Oro city.

The location belongs to the top-ten barangays with high incident dengue incidence as of 2014 namely: Brgy. Kauswagan, Brgy. Carmen, Brgy. Balulang, and Brgy. Bulua (DOH, 2014). The selections of the areas were based on criteria that are known to support the production of *Ae. aegypti* mosquito such as the presence of human habitats reported dengue cases, field size, geographical isolation, economic stability, and control site (Mckemey et al., 2008).

A total of 5,400 containers from 300 areas in the selected ten villages in Cagayan de Oro City were examined, and 9140 mosquito larvae were collected.

House survey

A larval survey was conducted in each study area to determine the main breeding sites of dengue vector mosquitos and to examine the relationship between the ecological conditions surrounding the houses and the variation in larval density among houses. Larvae were collected using a pipette and small hand net (20 cm diameter) and taken to the laboratory for identification. The kind, location (indoor/outdoor) and size of each container and the presence/absence of larvae were recorded. There were seven kinds of container: cement water tanks; large water jars (>60 cm in diameter); medium-sized water jars (35 - 55 cm); small water jars (<30 cm); used tyres; drums; and other small or discarded containers (ant-traps, tin cans, bottles, coconut shells etc).

Ovitrap survey

A dark plastic container (15 cm diameter; 12 cm depth) was used as an ovitrap. In each study area, eight houses were selected at random; four traps were set for each house: two indoors and two outdoors. The inside wall of the ovitrap was covered with a piece of cloth (8 x 28 cm); the cloth was changed weekly, and the number of eggs was recorded. The larvae that hatched were reared to adulthood and identified to estimate the proportion of *Ae. aegypti* and *Ae. albopictus* in the eggs.

Preparation of the Ovitrap

The Ovitrap used in the present study was made up of black, cylindrical, plastic container that can fill 1-liter capacity. The cover called wire mesh with a very small hole to prevent the mature mosquito from flying. A 2-inches hole serves drainage and avoids overflowing of water after rainfall (Kumawat et al., 2014; Ho et al., 2005).

Materials needed for making an Ovitrap:

- Black plastic dipper – used as the main Ovitrap container.
- Metal saw – used to remove the handles of the dipper.
- Drill – used to punch holes in the dipper.
- Ruler – used to measure the depth of holes from the base of the dipper.

Materials needed for making of wire mesh:

- Black Dragonfly screen with small holes – used to trap mosquito.
- Black 3mm LISO – used as rubber stopper to prevent the mesh from sinking.
- Measuring tape – used to measure the size of materials needed.
- Scissor – used for cutting LISO and dragonfly screen.
- Sewing machine – used to attach the dragonfly screen and LISO together.
- Black thread – used to bind the dragonfly screen and LISO together.

Procedure:

1. Assembled the materials needed to save time and effort.
2. Using a metal saw, carefully cut the handles of the dippers.
3. Using an electric drill, made two-10cm diameter holes opposite to each other at least 1 inch below the opening of the dipper. This was to serve as the overflow hole.
4. To make a wire mesh, cut portion of the dragonfly screen in a circular form. This was to serve as a trap preventing the ability of an adult mosquito to fly.
5. Cut two pieces of 3mm LISO forming a circle shape with a hole in the center. The wire mesh was sewed in between the two LISO to serve as the stopper and at the same time floatation device.



Plate 1. The Ovitrap

Preparation of the Brace

The braces were made up of wood and aluminum screen used to protect the Ovitrap from spilling. It was designed to fit perfectly and accommodate eighteen (18) Ovitrap. Extra space was used for labeling to identify each row properly.

Materials needed for making Brace:

- 1x2 inch wooden sticks – used to form square frames.
- 1-inch nails – used to hold the wooden sticks and aluminum screen.
- Aluminum screen – used to support the base of the Ovitrap.
- Hammer – needed for punching some nails.
- Measuring tape – to get the appropriate sizes of the materials needed.
- Saw – used for cutting the wooden sticks.
- Screen cutter – specifically for cutting the screen.

- Black paint – for the wooden sticks look the same color as the dipper and attracts the mosquito.
- Pencil – used in marking the wooden sticks.

Procedure:

1. Assembled materials needed.
2. Measured the wooden sticks to its appropriate sizes by using a measuring tape and marked with a pencil.
3. Carefully cut the marked sticks using a saw.
4. Formed six rows with 3 column square frames using the 3ft. wooden sticks enough to fit 18 Ovitrap.
5. Used hammer and nails to form braces.
6. By using a screen cutter, cut the aluminum screen in the same size as the brace, and nailed it under to support the Ovitrap and prevent from spilling.
7. Painted the braces in color black and let it dry.



Plate 2. The Ovitrap and Braces

Identification of the mosquito larvae

The mosquito larvae were visualized under the dissecting microscope. The species of the larvae were identified using the guide image from Control Diseases Center (CDC); one of a governmental health agency in the United States.

The researcher compared the taxonomic characteristic of the mosquito larvae *Ae. aegypti*, *Ae. albopictus*, *Culex annulirostris* identified by Control Disease Center to the actual larvae found on the Ovitrap.

RESULTS AND DISCUSSION

Objective 1. Identify the species of mosquito larvae from the Ovitrap.

A total of 5,400 containers from 300 areas in the selected ten villages in Cagayan de Oro City were examined, and 9140 mosquito larvae were collected.

Three mosquito species such as *Ae. aegypti*, *Ae. albopictus* and *Culex annulirostris* were identified in the ten villages. The most dominant species was *Ae. aegypti*. Overall, about 95% of the larvae caught in the ovttraps were *Aedes* dengue vector mosquitoes and only 5 % comprised of non-dengue vector mosquitoes, such as *Culex* and other *Aedes* mosquitoes and occasional non-mosquito larvae. Laboratory examination of larvae showed that 75% of the mosquito caught by the ovttraps in Cagayan de Oro City composed of *Ae. aegypti*, only 20% *Ae. albopictus*, 4% in *Culex annulirostris*, and 1% in occasional non-mosquito larvae.

Objective 2. Compare the number of mosquito larvae found in the Ovitrap during wet and dry season in 10 Villages.

Table 1 presents the total number of larvae during wet and dry seasons in the 10 Selected Villages. Based on the number of larvae found in the Ovitrap among the ten villages, Carmen village ranked 1 because of the significantly higher in number of mosquito larvae (1016), followed by Kauswagan (984), then Gusa (959), Bulua (925 larvae), Iponan (907), Lapasan (899), Balulang (884), Camaman-an (872), Patag (861), and Bugo (833).

Table 1. Total Number of Larvae during wet and dry seasons
in the 10 Selected Villages

Barangay	WET June- October 2014	DRY December 2014- April 2015	Overall Total	Rank
2011-2014 Top 10 Dengue	Total no. of larvae	Total no. of larvae		
1. Carmen	266	750	1016	1
2. Kauswagan	242	742	984	2
3. Balulang	196	688	884	7
4. Bulua	232	693	925	4
5. Lapasan	265	634	899	6
6. Bugo	227	606	833	10
7. Camaman-an	240	632	872	8
8. Gusa	253	706	959	3
9. Iponan	248	659	907	5
10. Patag	230	631	861	9
Average total No. of mosquito larvae	2399	6741	9140	

As shown in Table 1, dry season has more mosquito larvae (6741) than wet season (2399). The result of the study support also the findings of Lubos (2015); and Picardal and Elnar (2012) that the occurrence of mosquito particularly the *Aedes* could attribute to near constant fluctuations of temperature, considering that extreme temperature dictates survivorship.

Mosquito eggs hatch when the weather becomes warm again (Chmielewski, 2007). Vector-borne diseases, such as dengue, may be particularly sensitive to both periodic fluctuations and sustained changes in global and local climates because vector biology and viral replication are temperatures and moisture-dependent (Thai & Anders, 2011).

CONCLUSIONS

The presence of more larval mosquito during the dry season and dominance of *Aedes aegypti* larvae could attribute due to the rising of temperature. The study suggested that larval survey programme could serve as a tool not only to monitor the local dengue vector distribution but also to provide objective information for taking appropriate action by the community against dengue vectors.

RECOMMENDATIONS

1. This information might be helpful for the public health authorities to prepare for the likely increase of dengue due to climate change.
2. Public health departments should adjust their dengue prevention and control strategies to account for changing temperatures, altered distribution ranges and different epidemic modes of dengue.

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