Mosquito breeding site characterization (density, water quality, natural attractants) of Barangay Igang, Pototan, Philippines

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ABSTRACT

Mosquitoes are vectors that transmit pathogens that cause diseases such as dengue, malaria, and Zika fever. The range of the transmission of the pathogens is rapidly expanding in a geographic range which becomes a threat to public health. In the Philippines, dengue has the most reported cases which total to 100,000 cases in the first 6 months of the year 2019. In Iloilo Province, Pototan of Central Philippines has the most reported dengue cases with 1,452 cases. As a response, the mosquito habitats in one of the villages in Pototan were characterized based on the possible mosquito attractants as well as the mosquito larval density. This is to identify the causes of the frequent mosquito appearances in the area as well as to remove from the area and properly dispose of the mosquito larvae with the aim to remove the threat to public health caused by the presence of mosquitoes. The environmental variables, chemical and physical characteristics were measured with respect to larval habitats. The mosquito larval density and species were identified using the GLOBE Mosquito Protocol while the mosquito habitats were characterized based on the water quality and the plant species within the vicinity of the habitat. A total of seven sites were identified within the area that was positive for mosquito larval presence during the dry parts of the year. The site with the highest larval density had 113 larvae, 7 pupae, and 2 eggs. The oxygen concentrations in the water samples decreased as the number of larvae in the habitat increased. The dominant plant species that were within the proximity of the site was *Annona muricata* and *Dracaena reflexa.* Both the *Annona muricata* and *Dracaena reflexa* contain high phenolic content which is considered as strong mosquito attractants.

Keywords: Pototan, GLOBE, site characterization, larval density, dengue

RESEARCH QUESTIONS

This study aims to answer the following questions:

1. Where are mosquito breeding sites located in Barangay Igang, Pototan?
2. Between the two general types of breeding sites (natural or anthropogenic), which site is more common in Barangay Igang, Pototan?
3. What is the mosquito larval density in Barangay Igang, Pototan? What mosquito species are abundant based on the larval density?
4. What is the water quality (pH level, salinity, electrical conductivity) of larval breeding sites in Barangay Igang, Pototan?
5. What plant species can be associated with the abundance of mosquito larvae in Barangay Igang, Pototan?
6. What are the attractant compounds present in the plant species within the proximity of the larval breeding sites?

With the increasing abundance of cases caused by mosquito-borne diseases within the country, the causes of such abundance must be sought in order to lessen its threat to public health. Thus, the non-climatic factors which affect mosquito abundance, namely water quality and presence of attractant-releasing plant species in breeding sites, must be assessed in order to develop strategic measures for vector control within the area.

INTRODUCTION & REVIEW OF LITERATURE

Mosquitoes transmit a significant number of pathogens that cause life-threatening diseases (World Health Organization 2020). In the Philippines, dengue has been a serious threat to public health throughout the years. A national dengue epidemic was declared in 2019 due to the 146,062 reported cases of dengue and 622 deaths that occurred from January to July (Department of Health 2019). Within the same period in the province of Iloilo, the municipality of Pototan had the most reported dengue cases, and among the barangays in Pototan, Barangay Igang is recorded to have the most cases (Iloilo Provincial Health Office 2019; Figure 4).

One method to lessen cases of dengue is the prevention of mosquito breeding, as mosquito abundance often occurs before epidemics and increases vectorial capacity and reproductive rate (Reiter 2001; Lafferty 2009; as cited by Ruiz et al. 2014).

Aside from the more commonly known factors that affect mosquito breeding, natural attractants released by flora and fauna are known to attract mosquitoes. According to Davis et al. (2016), natural attractants released by flowering plants promote oviposition in nearby potential breeding sites. Emitted volatile organic compounds allow mosquitoes to distinguish suitable host plants and evade non-host plants (Visser 1986). Furthermore, mosquito genera such as Aedes, Anopheles, and Culex regularly feed on plant tissue (Muller and Schlein 2005). Preferably, mosquitoes feed on plants with high sugar content due to their reliance on carbohydrates for energy (Van Oppen et al. 2015). Although plant-insect relationships are used to combat agricultural pests (Nyesembe and Torto 2014), their implication on mosquito habitat preference lacks investigation.

Barangay Igang, Pototan is a wide rural area characterized by abundant vegetation and farmland, which may potentially contain natural attractants and feeding sources that invite mosquitoes to oviposit in bodies of water nearby. Due to the frequency of Dengue cases within the barangay, there is a need to assess the barangay's potential breeding sites and larval density, as well as the mosquito species.

This study aimed to characterize the water quality and plants near mosquito breeding sites in Barangay Igang, Pototan, in order to determine the habitat conditions that favor mosquito abundance within the area. This study will allow local residents to determine the larvae habitats and attractants that allow mosquitoes to thrive and spread diseases such as dengue. By identifying the factors that promote oviposition, the mosquito population can be controlled and hence, vector disease transmission can be mitigated.

METHODOLOGY

A. Overview of the Methodology

All the possible mosquito habitats in Barangay Igang, Pototan of Central Philippines were assessed. When the mosquito habitat is confirmed to have mosquito larvae, the coordinates of the different mosquito sites were marked with the use of the Global Positioning System tool in the GLOBE Mosquito Protocol. Water samples from the confirmed mosquito habitats were placed in polyethylene terephthalate bottles for larval counting and water quality assessment. Afterward, the habitats were described based on plant characterization. The samples were brought back to the laboratory and the larvae were counted for each habitat and then the larval species were identified using the mosquito protocol. The water quality of each of the habitats was tested using a TANITA salinity meter and a PASCO advanced water quality sensor.

B. Flow Chart of the Data Gathering Process

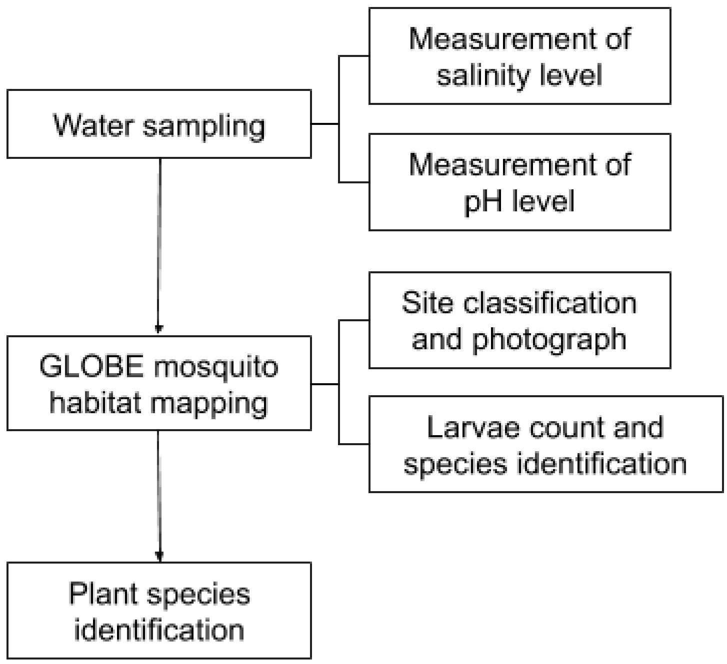


Figure 1. Flowchart of the Data Gathering Process

C. Research and/or Sampling Sites

The potential mosquito habitats in Brgy. Igang, Pototan, Iloilo was assessed for larval density, water quality, and plant characterization. The area was located at 10°54'45.5"N 122°38'10.2"E and had an elevation of 18 m above sea level. The Köppen-Geiger climate classification is tropical monsoon or Am since most months of the year are marked by significant rainfall (Climate-data.org). According to the Iloilo Provincial Health Office (2019), this barangay had the most reported cases of Dengue in the entire Iloilo Province last 2019 with 98 reported cases out of 3,732 people in the population (2015 Census).

Figure 2. Screenshot taken of Brgy Igang, Pototan, Iloilo City from Google Maps.

C.1 Sampling Design

A two-day assessment was conducted in Brgy. Igang, Pototan, Iloilo of Central Philippines. All the stagnant water or any potential mosquito habitats were assessed for larval presence. On the first assessment, the different potential sites for mosquito habitats were identified and visually assessed for mosquito larvae and eggs. Out of all the sites that were assessed, seven sites were positive for the presence of mosquito larvae and eggs. Water samples were taken from the six sites and then placed into polyethylene terephthalate bottles for larval counting and water quality assessment (pH, turbidity, salinity). The plants that were within the proximity of the mosquito habitat were taken pictures of and later identified for chemical characterization.

D. Materials and Equipment

The materials needed for the data gathering process are as follows: polyethylene terephthalate bottles and glass beakers for water sampling, a clip-on microscope, dropper, and modified petri dish for larvae identification, and probes for water quality assessment. The pH level, dissolved oxygen concentration, dissolved oxygen saturation, oxygen gas concentration, and the electrical conductivity of the water samples was measured using the PASCO advanced water quality sensor pH probe. The salinity levels were measured using the TANITA salinity meter.

E. Water Sampling

For the water sampling process, water samples from mosquito habitats were collected from the mosquito habitats using a beaker in order to sample both the larval density and larval species as well as the water quality. All the mosquito habitats were photographed and additional remarks shall be made regarding the external factors that can cause mosquitoes to be attracted to the mosquito habitat.

The bottles with larval specimen were labeled according to site number and then transported to the Philippine Science High School-Western Visayas Campus laboratory for counting and identification. The location and category of the breeding sites were uploaded on the GLOBE database.

F. GLOBE Mosquito Habitat Mapping

The GLOBE Mosquito Habitat mapper was used to locate all the mosquito habitats within the exclusive area of the study. The water sources were classified according to water flow (still or flowing) and container type (natural or artificial). The larval species were then identified as well as the larval density through capturing images and manual counting of larvae present in the water sample.

G. Water Quality Assessment

The water samples were individually placed in a 250 mL beaker. The samples were first assessed for their salt content. The TANITA salinity meter was calibrated by dipping it into distilled water and checked if the reading is at 0.0%. After calibration, it should be dabbed dry using a tissue paper before dipping the salinity meter to the water sample. After dipping, the salinity will vibrate after the measuring of the salt content of the water sample. The salinity meter tipped must be washed using distilled water in a wash bottle and dabbed dry before using it in a different water sample.

For the testing of pH level, dissolved oxygen concentration, oxygen gas concentration, dissolved oxygen saturation, and electrical conductivity of the water samples, the PASCO advanced water quality sensor was used. The sensor was plugged into a laptop with the necessary probes needed to measure these parameters. The pH probe was dipped into the water sample and when the reading stabilized, the data was recorded as the pH level of the sample. The electrical conductivity probe (10x) was dipped into the water sample and when the reading stabilized, the data for the electrical conductivity of the water sample was recorded. Finally, the dissolved oxygen probe was dipped into the water sample and then when the readings stabilized, the data was recorded. The probes should be washed using distilled water in a wash bottle and dabbed dry using tissue paper in order to remove the residue liquid from the previous readings.

H. Identification of the Plant Species

The plant species within the area were identified in order to identify the possible attractants that are present within an area. Images of the plants within the barangay were taken, and plant species were identified with the aid of Google Images. The plant species that are identified to have attractants to mosquitoes were documented.

a. The species of plants that will be identified must be within the set area of influence. The area of influence will be dependent on the longest widthwise diameter of the water source.
b. The species of the plants will be identified and then possible attractants will be based on a database of plants' chemical characterization.

I. Identification of Attractant Compounds in Plant Species

The attractant compounds in the plants at the sites were determined based on the results of previous studies. Additionally, pictures of the different flowering plants around the barangay were taken because these flowering plants may contain attractants that cannot be activated due to the presence of dry season during the period of data gathering.

List of possible mosquito attractants:

Ammonia was attractive in concentrations from 17 ppb to 17 ppm in air when presented together with lactic acid. Aqueous solutions of ammonia salts in concentrations comparable to those found in human sweat also increased the attractiveness of lactic acid (Geier et al. 1999).

Octenol is an Environmental Protection Agency registered secondary mosquito attractant that mimics human breath. Octenol attracts most mosquito species, including salt marsh mosquitoes, as well as no-see-ums and black flies (Mosquito Magnet).

L-Lactic acid was the major component in material isolated from humans that were active as an attractant for female yellow fever mosquitoes, Aedes aegypti (L.) (Acree et al. 1968) **Lactic acid** alone attracted approximately the same numbers as CO2 alone (Kline et al. 1990).

Various combinations of mosquito attractants were tested against natural populations of mosquitoes using unlighted CDC-baited traps. **Phenol** (2.7 x) alone attracted larger numbers (Kline et al. 1990).

2-butanone can serve as a good replacement for CO2 in synthetic blends of attractants designed to attract host-seeking *An. gambiae s.l.* and *An. funestus* mosquitoes (Mburu et al. 2017).

L-amino acids were tested in the free-flight cage and 16 showed significantly positive stimulation. **Lysine** was the most attractive, representing a group of 11 which carried CO_2 in carbaminoyl or adsorbed form or in both; the other five, of which **tyrosine** was the most attractive, carried no CO_2 (Roessler and Brown 2009).

Of the single volatile compounds tested, **acetophenone** was attractive and **1-octanol** caused a flight aversive response (Oppen et al. 2015).

Anisaldehyde was effective in attracting *Ae. albopictus* when used alone but could also remarkably inhibit the host-seeking ability at a concentration of 96% (Hao et al. 2013)

J. Disposal

The proper disposal of mosquito larvae was followed by dumping the effluent into the dry ground since the larva cannot thrive in the ground. Proper disposal of the mosquito larvae was observed so that they cannot thrive in the new environment and continue breeding.

K. Health and Safety

To ensure that health and safety measures were met during the conduct of the data gathering, first aid kits were brought to the sampling site. The use of proper personal protective equipment was followed while collecting water samples and identifying and counting the larvae.

L. Ethics

No plants and animals, with the exception of mosquitoes as vectors of diseases, were severely harmed during the conduct of the data gathering process.

RESULTS

Out of the seven (7) sites that had mosquito larvae, the site with the highest larval density was site 2 with coordinates 10.91756° N 122.6315° E. Its density consisted of 113 larvae, 7 pupae, and 2 eggs. The plant species within the proximity of the site were *Annona muricata* and *Dracaena reflexa*. These plants contain high phenolic content which acts as strong mosquito attractants. The site with the least number of larvae was site 4 with 0 larvae and 3 eggs which has the coordinates of 10.915669 ° N 122.638945° E. The plant species within the proximity of the site was *Mikania micrantha.* The dominant species of mosquito larvae that were in the area was *Aedes aegypti* which was present at 4 sites with a total larval count of 127 larvae. The other species that was present in the area was *Aedes albopictus* which was present at 2 sites with a total larval count of 7 larvae.

Table 1. Mosquito density in samples obtained from breeding sites.

Site no.	Larval Density	Pupa Density	No. of Adult Mosquitoes	No. of Eggs
1	10	0	0	4
2	113	7	0	2
3	1	0	0	0
4	0	0	0	3
5	2	1	0	0
6	5	1	0	12
7	3	0	0	0

Table 2. Mosquito Larvae Species (Sites 1-4)

Species	Site no.			
	1	2	3	4
Aedes aegypti	10	113	1	0
Aedes albopictus	0	0	0	0

Table 3. Mosquito Larvae Species (Sites 5-7)

Species	Site no.		
	5	6	7
Aedes aegypti	0	0	3
Aedes albopictus	2	5	0

The only site that had a salinity content of 0.1% was site 6. The site with the least O_2 gas concentration and dissolved O_2 concentration was site 2 with an O_2 gas concentration of 5.31% and dissolved O_2 concentration of 1.91 mg/L. Site 6 was also the most acidic water sample with a pH level of 4.78, while the most basic water sample was site 7 with a pH level of 8.80. The site with the most O_2 gas concentration and dissolved O_2 concentration was site 7 with an O_2 gas concentration of 17.75% and dissolved O_2 concentration of 6.36 mg/L. The site with the highest electrical conductivity was site 1 with 2677.63 μS/cm while the site with the least electrical conductivity was site 3 with 129.22 μS/cm.

Table 4. Water quality assessment

Site no.	Salinity (%)	O_2 gas concentration (%)	DO_2 saturation (%)	DO_2 concentration (mg/L)	Electrical conductivity (µS/cm)	pH
1	0.00	17.32	83	6.24	2677.63	7.43
2	0.00	5.31	25	1.91	1239.87	4.78
3	0.00	16.90	81	6.07	129.22	7.60
4	0.00	15.78	75	5.67	735.38	7.34
5	0.00	15.20	73	5.48	972.08	7.21
6	0.10	17.40	83	6.25	1750.28	7.60
7	0.00	17.75	85	6.36	666.62	8.80

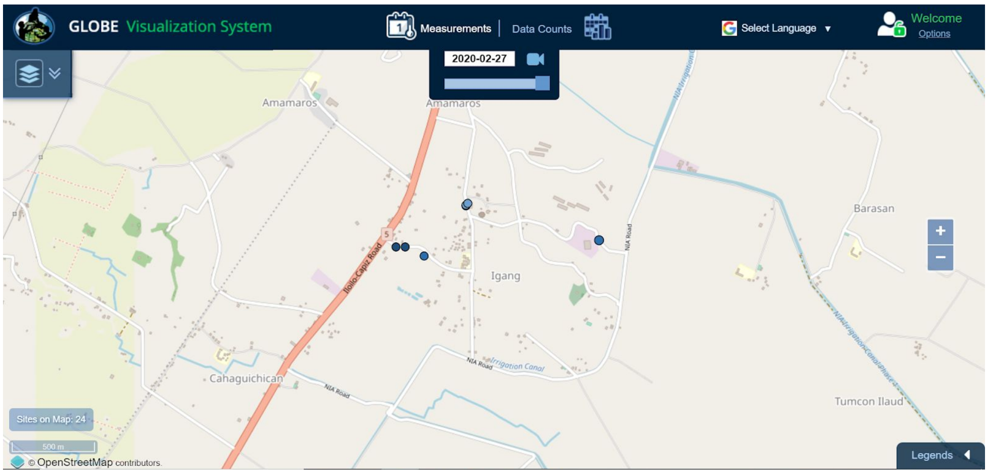
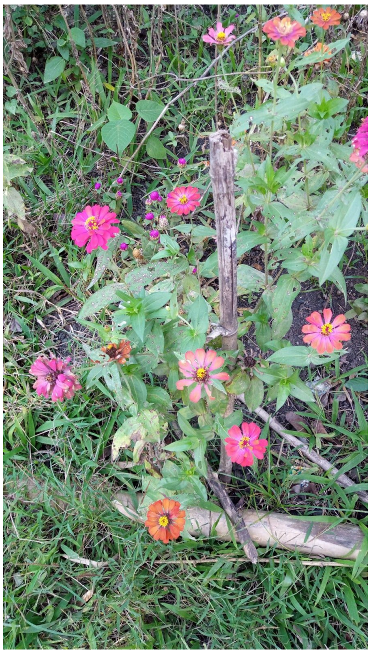


Figure 3. Screenshot of GLOBE Visualization page

Plants Identified:

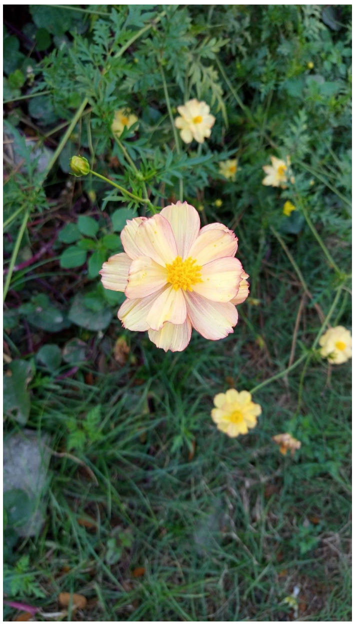


Zinnia elegans

Hibiscus rosa-sinensis

Zinnia elegans contains polyphenolic compounds in the structures of its active

Hibiscus rosa-sinensis contains phenolic content of 0.092 mg/g. This means that the ingredients which acts as attractants for mosquitoes (Burlec et al. 2019).



Cosmos bipinnatus

Cosmos bipinnatus contains phenolic compounds that act as a mosquito attractant (Saleem et al. 2017).

Bougainvillea glabra

Bougainvillea glabra contains 320 mg/g of phenolic compounds that are considered as

flowering plant can serve as an attractant for mosquitoes (Al-Snafi 2018).

Hibiscus rosa-sinensis var rubro-plenus

Hibiscus rosa-sinensis contains phenolic content of 0.092 mg/g. This means that the flowering plant can serve as an attractant for mosquitoes (Al-Snafi 2018).

Ipomoea aquatica

Ipomoea aquatica contains low concentrations of phenol that serves as a mosquito attractants (Guerrero et al. 2016).

mosquito attractant (Igwenyi et al.2014). This plant species can be found within the proximity of site 3.

Mikania micrantha

Mikania micrantha contains volatile oil compounds that are considered as toxins to insects (Zhang et al. 2003). This plant species can be seen within the proximity of site 4.

Dracaena reflexa

Dracaena reflexa contains high amounts of phenolic content that acts as mosquito attractants (Shukla et al. 2015). This plant species can be found within the proximity of site 2.

Leucaena leucocephala

Leucaena leucocephala showed a good balance of amino acids specifically a large amount of methionine that can serve as mosquito attractants (Agbede 2004).

Annona muricata

Annona muricata contains high phenolic content that acts as a strong attractant for mosquitoes (Ragasa et al. 2014). This plant species can be found within the proximity of site 2.

Oryza sativa

Oryza sativa found within the proximity of site 5 and site 6 contains 7.84 umol/g amino acids as attractants as well as 0.18 umol/g phenol which are both considered as attractants for mosquitoes (Bacilio-Jiménez et al. 2003).

DISCUSSION

Site 1 which has the coordinates of 10.91775° N 122.63156° E was an artificial container, specifically a plastic drum inside one of the public buildings in the barangay. Site 2 which has the coordinates of 10.91756° N 122.6315° E was an artificial container, specifically a plastic drum located in one of the residences. Site 3 which has the coordinates of 10.920438° N 122.629160° E was a puddle near a creek. Site 4 which has the coordinates of 10.915669° N 122.638945° E was at the stagnant part of a creek. Site 5 which has the coordinates of 10.9152841° N 122.6281081° E was a ditch in a rice paddy. Site 6 which has the coordinates of 10.9153361° N 122.6275864° E was also a ditch in a rice paddy. Site 7 which has the coordinates of 10.9214646° N 122.6329964° E was an artificial container, specifically a plastic drum along the road. Only seven sites were present with larvae this is because of the time of data gathering which was within the dry season period of the area.

There is insufficient data to show which site, natural or anthropogenic, was more common in the barangay throughout the year but we can conclude that the anthropogenic sites are more common during dry seasons because the mosquitoes rely on artificial habitats.

Around 131 mosquito larvae were counted, in which 113 larvae were obtained from a single site. Most of the larvae were identified as Aedes aegypti or Aedes albopictus, which are major vectors of Dengue. This is the dominant genus within the area which is the reason why there are many reported dengue cases within the area.

Salinity levels of the water samples either reached 0.0% or 0.1%. As for the pH levels, most obtained samples maintained pH levels ranging from 7.20 to 8.80 except for one outlier that had a pH level of 4.78. This site also had the highest larval density and the least oxygen and dissolved oxygen concentration. Most of the oxygen content and dissolved oxygen content were utilized by the larvae since it is inversely proportional to the number of larvae that can be found in the specific mosquito habitat.

Due to the abundance of flowering plants such as *Ipomoeae aquatica* and other plant species such as *Annona muricata*, *Oryza sativa*, *Leucaena leucocephala, and Dracaena reflexa* in Barangay Igang, Pototan of Central Philippines, there is an abundance of mosquito larvae in the area. This is due to the fact that these plant species contain compounds that attract mosquitoes leading them to transform these places into their breeding grounds.

The attractant compounds commonly present in the proximity of the larval breeding sites are phenolic compounds and amino acids. These two compounds are strong attractants for mosquitoes and are contained by most of the flowers and plant species within the barangay. If the data gathering period was during the wet season, the number of sites should be significantly more and the larval density in the area would also increase since there is an abundance of plants that have attractive compounds within the area.

CONCLUSION

The area had an abundance of plants that contain attractive compounds which reflected the number of dengue cases reported during the wet season last year. The major attractive compounds that are found in the area are phenolic compounds and amino acids. The presence of these attractive compounds resulted in the high larval density of vectors of diseases.

Water quality assessment could be added as an additional factor to record using mosquito habitat mapper since the oxygen concentrations in the water samples decreased as the number of larvae in the habitat increased. The lower levels of oxygen and dissolved oxygen concentrations could be a sign of higher larval density in the mosquito habitat.

To properly assess an area for possible mosquito habitats, both the wet season and dry season mosquito habitats should be assessed and compared to each other so that the mosquito presence within an area could properly be assessed all throughout the year.

To avoid the increase in reported cases, regular inspections for mosquito larvae should be carried out in wastewater ponds and their effluents to determine whether breeding is occurring and to determine the necessity for weed control or chemical control. All stagnant water must be checked regularly for the presence of larvae and must be disposed of properly to prevent further problems caused by these vectors of diseases.

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APPENDICES

Provincial Health Office
Epidemiology and Surveillance Unit
Municipality of Pototan
Dengue Cases 2019

BARANGAY	ALIVE	DEATH	TOTAL
ABANGAY	38	0	38
AMAMAROS	49	0	49
BAGACAY	70	0	70
BARASAN	34	0	34
BATUAN	76	1	77
BONGCO	11	1	12
CAHAGUICHICAN	59	0	59
CALLAN	4	0	4
CANSILAYAN	5	0	5
CASALSAGAN	11	0	11
CATO-OGAN	34	0	34
CAU-AYAN	40	0	40
CULOB	1	0	1
DANAO	4	0	4
DAPITAN	22	0	22
DAWIS	14	0	14
DONGSOL	4	0	4
FERNANDO PARCON WARD (POB.)	15	0	15
FUNDACION	5	0	5
GUIBUANGAN	23	0	23
GUINACAS	29	0	29
IGANG	97	1	98
INTALUAN	11	0	11
IWA ILAUD	29	0	29
IWA ILAYA	29	0	29
JAMABALUD	21	0	21
JEBIOC	6	0	6
LAY-AHAN	17	0	17
LOPEZ JAENA WARD (POB.)	25	1	26
LUMBO	27	1	28
MACATOL	20	0	20
MALUSGOD	65	0	65
NABITASAN	10	0	10
NAGA	8	0	8
NANGA	9	0	9
NASL	1	0	1
NASLO	52	1	53
PAJO	7	0	7
PALANGUIA	47	0	47
PITOGO	4	0	4
POLOT-AN	31	0	31
PRIMITIVO LEDESMA WARD (POB.)	36	0	36
PUROG	42	0	42
RUMBANG	81	0	81
SAN JOSE WARD (POB.)	63	4	67
SINUAGAN	4	0	4
TUBURAN	40	0	40
TUMCON ILAUD	33	1	34
TUMCON ILAYA	13	1	14
UBANG	20	0	20
ZARRAGUE	35	0	35
(blank)	21	0	21
	1452	**12**	**1464**

Figure 4. Data for the reported cases in each barangay of the province of Pototan in Central Philippines

BADGES SELECTED

- **Be a Collaborator**

Raymond T. Borres is one of the two members and he was assigned to help during the data gathering to collect the water samples from the mosquito habitats. He was also tasked to assess the water quality of the samples based on the pH, dissolved oxygen concentration and saturation, oxygen gas concentration, and electrical conductivity. For the analysis of data, he was the one who researched the different attractive compounds that the different identified plants contain. For the making of the report, he was tasked to help finish the abstract, the methodology, the results, and the discussion. He also helped copyread the different parts of the report.

Francine T. Tarrazona is one of the two members and she was assigned to help during the data gathering process by taking note of the coordinates of the different sample sites, documenting the different sample sites, and labeling the different sample sites. She was the one who was assigned to assess the salinity content of the different water samples. She also contributed to the larval density counting and taking pictures of the different larvae gathered. For the analysis of data, she was the one who identified the different plant species that were taken pictures of during the data gathering process. For the creation of the report, she was tasked to help finish the abstract, the methodology, the results, and the discussion. For the process of making the report, she was tasked to do the introduction, the conclusion, and the references. She also helped review the different information that was included in the study.

- **Be a Data Scientist**

Our report earned this badge by inferring the number of sites that were gathered during the data gathering process. We inferred that the small number of sites is due to the dry season that is currently affecting the study site. The different data points gathered through the water samples gathered from different mosquito habitats were analyzed. We considered the data given by the government regarding the reported cases of dengue within the province of Iloilo in Central Philippines as the basis for the problem of our study.

- **Make an Impact**

One of the major global issues are diseases caused by vectors such as mosquitoes. Locally, dengue is the major local problem regarding vector-borne diseases. In this study, we gathered past data from a government agency and used it as the basis for our study site. Due to the numerous cases reported last year, we concluded that regular check-ups of potential mosquito habitats must be done in order to reduce the cases of dengue within the area.