BREEDING SITES OF DENGUE VECTORS, *Aedes aegypti* and *Aedes albopictus* IN DUMAGUETE CITY, NEGROS ORIENTAL, PHILIPPINES

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ABSTRACT

Aedes aegypti and Aedes albopictus are dominant mosquito vectors that transmit life-threatening arboviral diseases like dengue, yellow fever and chikungunya all over the world. Water holding containers produced by humans are main important habitats for the preimaginal development of these mosquitoes. The elimination of their breeding sites is among the ways to control dengue and other arboviral diseases. Thus, this study was conducted to assess the abundance and relative importance of each breeding site of Aedes aegypti and Aedes albopictus in order to minimize or eradicate dengue cases in Dumaguete City. In this study, all water holding containers in nine sampling sites were visually inspected for the presence of mosquito larvae and pupae. Mosquito larvae and pupae were then collected from the containers and identified. The relative importance of the breeding site per species was calculated. Results of this study showed that plastic container had the highest relative importance for *Aedes aegypti* larvae while drainage canal had the highest relative importance for *Aedes albopictus*. However, drainage canal showed the highest relative importance for the pupae of both species. This may show that plastic container is more preferred for oviposition by gravid *Ae aegypti* but drainage canal provides favorable pupal development. *Moderately turbid water and high organic content from fallen leaves and other debris in these breeding sites may serve as suitable artificial breeding habitat for Aedes mosquitoes.*

Keywords: ***breeding site, Aedes, Dumaguete, mosquito, Philippines***

INTRODUCTION

Humans are plagued by a prevalent mosquito-borne viral illness caused by dengue virus (DENV). According to WHO, dengue incidents has grown 30 time in the last 50 years. Annually, about 50-100 million infections occur in over 100 countries, thereby risking more than half of the world's population. Dengue virus belongs to the Family Flaviviridae and has four serotypes. These are transmitted by the domestic, daytime-biting mosquitoes, *Aedes aegypti (*Linnaeus, 1762*)* and *Aedes albopictus* (Skuse, 1894). *Aedes aegypti* is the principal vector while *Aedes albopictus* is the secondary vector of dengue. These species are also vectors of very important virus that lead to a disease called yellow fever, chikunggunya and Zika. It has been proven that these two Aedes species are now found in many urbanized and suburbanized areas around the world breeding primarily in artificial water containers and their lifecycles are closely associated with human activities (Vezzani and Schweigmann, 2002; Saleeza et al., 2011; Dieng et al., 2012, Edillo et al., 2012).

Though dengue vaccine was available but its wide spectrum efficacy has faced a lot of controversies(http://cnnphilippines.com/news/2017/12/09/The-Dengvaxia-controversy.html). Targeting its mosquito vector through insecticides and elimination of potential breeding sites are among the ways to control dengue (Kuan et al., 2009). However, the use of synthetic chemicals to kill the vector mosquito is not so effective because it is highly domesticated and many adults are found indoors (Ciccia et al., 2000). These chemicals are also harmful to the environment and mosquitoes will develop resistance on it. Eliminating or larviciding the freshwater habitats of the vector are important strategies for controlling the arboviral transmission worldwide. However, such measure failed to eradicate such diseases because *Aedes aegypti* and *Aedes albopictus* were observed to oviposit and undergo pre-imaginal development in brackish water (Ramasamy et al., 2011; Surendran et al., 2012; Surendran, 2018. Previous studies suggested that the main thrust of prevention is elimination of the most productive breeding sites to keep the vector density below a critical threshold (Maciel-de-Freitas and Lourenço-de-Oliveira, 2011; Edillo et al., 2012). In order to enhance prevention and vector control activities, there is a crucial need to identify the key breeding sites. Thus, this study was conducted to identify the breeding sites of *Ae. aegypti* and *Ae. albopictus* in Dumaguete, assess the abundance of breeding sites and determine the relative importance of each breeding site on *Ae. aegypti* and *Ae. albopictus* larvae and pupae. Results of this study will serve as baseline information on the main breeding containers of *Aedes* mosquitoes in Dumaguete City as a reference for further vector control activities and community engagements.

MATERIALS AND METHODS

Study Site

This study was conducted in Dumaguete City, Philippines (9.3068°N, 123.3054°E) with nine sampling sites. The sampling sites were: Bagacay, Balugo, Bajumpandan, Batinguel, Cadawinonan, Candau-ay, Mang-nao, Piapi and Taclobo. Located in these sites are mass housing sites, dump site, schools and cemetery; areas which reported in previous studies to harbor many mosquito breeding sites (Chen et al., 2006; Biswas et al., 2014; Edillo et al., 2012; O'Meara et al., 1992; Seng et al., 1994; Vezzani & Schweigmann, 2002; Walker et al., 1996; Wan-Norfikah et al., 2012). Located in Candau-ay are dumpsite and mass housing units such as Mother Rita Habitat for Humanity and Scandinavian Village. Mass housing sites were also located in Balugo, Batinguel, Bajumpandan and Cadawinonan. Elementary schools with 36-75 classes are located in Mangnao and Taclobo while Bagacay has public cemetery (Figure 1).

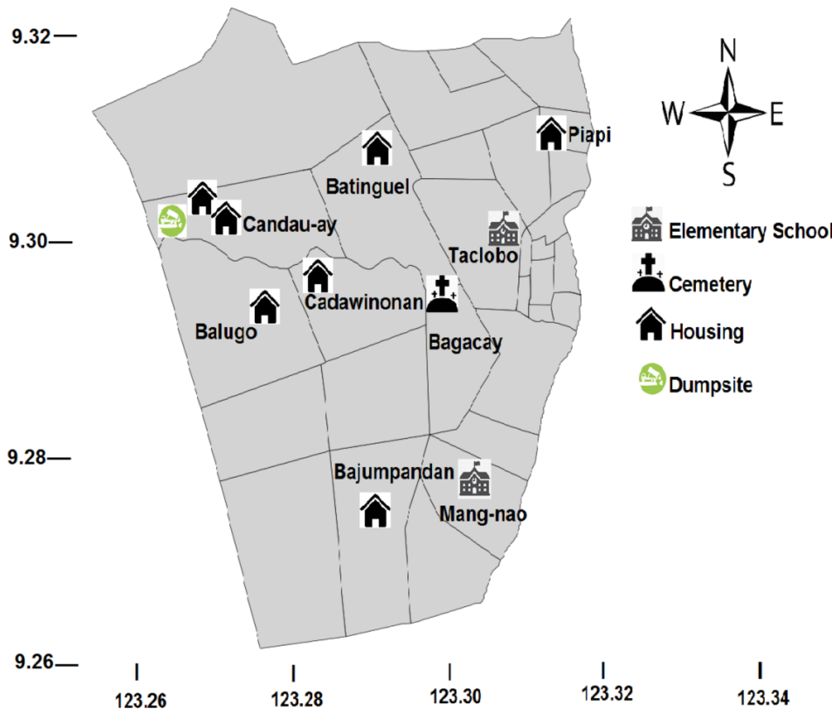

Figure 1. Map of Dumaguete City showing the sampling sites.

Mosquito Sampling and Identification

All artificial and natural containers holding water both indoor and outdoor were visually inspected for the presence of mosquito immature stages. Each type of container positive with mosquito larvae and pupae was classified as breeding site. Mosquito larvae and pupae were then collected from the containers following sampling strategy based on different water container volumes (Manrique-Saide et al., 2011). If the container has < 20L of water, the larvae/pupae were collected by either emptying the container or sieving the water directly through a mesh net or collecting the pupae with a pipette. If the container has > 20L of water but there is good visibility and low pupae density (<100 individuals), the larvae/pupae were collected by comprehensive netting. This was done by immersing the net carefully 7.5cm beneath the water surface of the container and move around the perimeter in a downwards spiral to create a funnel which concentrates the pupae at the bottom center of the container. If the container is large and has high larval/pupal density (>100 individuals), the sample was collected by sweeping the surface with a net. The total number of larvae/pupae was estimated from this sample using a calibration factor (CF) according to the amount of water in the container. The number of larvae/pupae collected was multiplied by a CF (1/3CF= 2.5; 2/3 CF= 3.0 and 3/3CF =3.5) (WHO, 2011). The mosquito samples were placed inside the labelled bottles and brought to the Biology Laboratory of Negros Oriental State University for identification and counting.

The larval stages of *Ae. aegypti* and *Ae. Albopictus* can be differentiated by the shape of the comb scales on the eighth segment of the abdomen, shape of the pecten teeth on the siphon and the number of setae at the ventral brush (4-X). The comb scales of *Ae. aegypti* larvae have well developed lateral denticles but the pecten teeth have less defined denticles. Whereas, the comb scales *of Ae. albopictus* larvae have no lateral denticles but the pecten teeth have three well defined pointed denticles (Cheong,1986; WHO, 1995 as cited by Sivanathan, 2006).The ventral brush (4-X) of *Ae. aegypti* has five pairs of setae while *Ae. albopictus* has only four pairs of setae (Harrison & Rattanarithikul, 1973; Rueda, 2004). The pupae of Aedes were distinguished from other mosquito genera by the absence of tracheiod portion in the trumpet meatus/respiratory trumpet. *Ae. albopictus* pupae have midrib of paddle reaching apex and the margin has visible setae. On the other hand, the midrib of paddle of *Ae. aegypti* does not reach the apex with submarginal serrations.

Data Analysis

The relative importance of each breeding site to the total count of larvae/pupae was calculated by taking the total number of larvae/pupae in a category of container and dividing it by the number of pupae in all containers. The most abundant breeding site was determined by dividing the number of containers per type by the total number of containers in the area being studied multiplied with 100%. T-test was used to determine the significant difference between the number of *Ae a.egypti* and *Ae. albopictus.*

RESULTS

There were twelve (12) types of breeding sites with a total of fifty three (53) containers identified in this study; glass aquarium, concrete aquarium, can, drainage canal, flower vase, flower pot, jar, motor part, plastic container, coconut shell, bucket and discarded tire. Out of 12, only coconut shell is a natural breeding site. Among the artificial breeding sites, discarded tire was observed to be the most abundant at 30% while motor part, jar and aquarium were the least (Fig 2.).

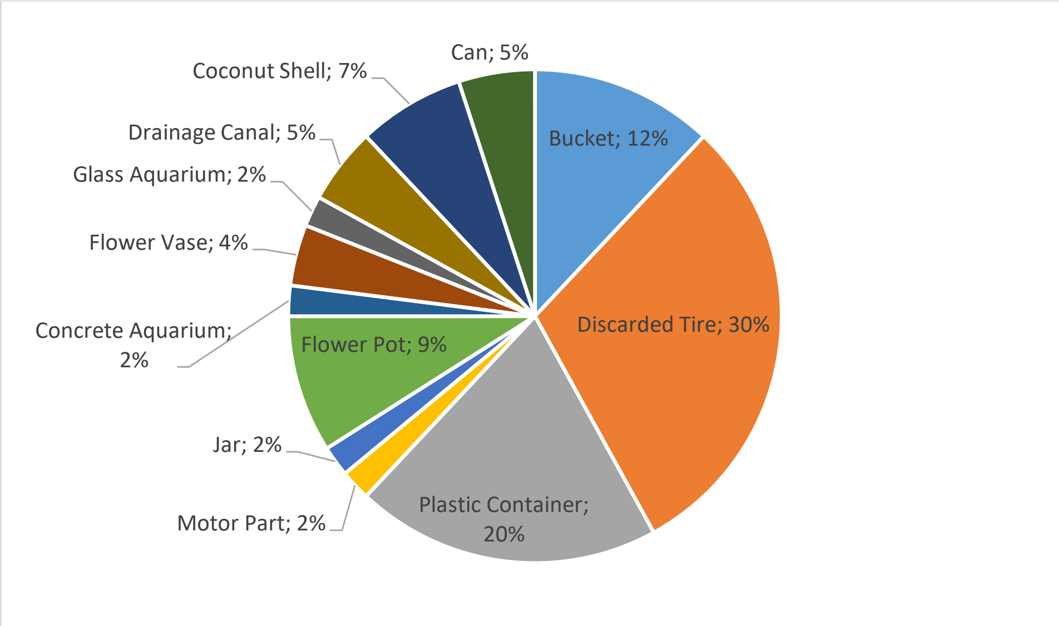

Figure 2. Percent abundance of each breeding site found in all sampling sites.

In this study, Batinguel had the most number of containers positive with Aedes larvae and pupae (Table 1). Most of these containers were discarded tires. However, Taclobo had the most number of breeding type both indoor and outdoor. Flower vase and bucket were found indoor while discarded tire, plastic container, flower pot and drainage canals were outdoor. Plastic containers were found in almost all sites but Candau-ay had the most number. Natural breeding site positive with mosquito larvae and pupae were found only in Balugo. Bagacay, Bajumpandan, Cadawinonan, Mangnao and Piapi had few breeding sites observed with one to three containers, most were outdoor.

Table 1. Type and number of breeding sites found in nine sampling sites of Dumaguete City.

Sampling Site	Breeding Site												
	Concrete Aquarium	Glass Aquarium	Bucket	Can	Drainage canal	Coconut Shell	Flower Pot	Flower Vase	Jar	Motor Part	Plastic Container	Discarded Tire	Total
Bagacay									1				1
Bajumpandan					1						1		2
Balugo		1		2		7					2		12
Batinguel							1				1	11	13
Cadawinonan												1	1
Candau-ay	1									1	5	3	10
Mangnao					2							1	3
Piapi			1	1							1		3
Taclobo			2		1		4	2			1	1	11
Total	1	1	3	3	4	7	5	2	1	1	11	16	56

A total of 2954 Aedes larvae and pupae were collected from 56 breeding sites. Between the two species of Aedes mosquitoes, *Ae. aegypti* is more abundant than *Ae. albopictus* both in larval and pupal stages. Out of 2954, 1359 were *Ae. aegypti* larvae while 620 were *Ae. albopictus*. Eight hundred sixty seven (867) were *Ae. aegypti* pupae while 108 were *Ae. albopictus* (Table 2). Overall, there was a significant difference between the number of *Ae. aegypti* and *Ae.albopictus* (p=0.02 CI=95%). Among the nine sites, Taclobo showed the highest total number of Aedes larvae and pupae for both species. This is followed by Candau-ay with a total of 751 Aedes mosquitoes. However, it is interesting to note that even if Candau-ay ranks 2nd in the number of Aedes and 4rth in terms of number of containers, it harbours the highest number of *Ae. aegypti* larvae with a total of 458 and next to Taclobo in terms of pupae with 208 individuals.. Balugo ranks next with a total number of 302 individuals, 230 of which was *Ae. aegypti* larvae. In case of *Ae.albopictus*, Piapi has the 2nd highest number of larvae with 101 individuals. Batinguel having the highest number of breeding sites has only 159 individuals of

Ae. aegypti and *Ae.albopictus* larvae and pupae. The lowest number of Aedes was observed in Bagacay and followed by Mangnao.

Table 2. Total number of Aedes mosquito larvae and pupae collected per site of Dumaguete City. (p=0.02; CI=95%)

Sampling Site	Number of Breeding Sites Found	Larvae		Pupae		Total
		Aedes aegpti	*Aedes albopictus*	*Aedes aegpti*	*Aedes albopictus*	
Candau-ay	10	458	82	208	3	751
Piapi	3	75	101	24	2	202
Taclobo	11	325	299	536	93	1253
Batinguel	13	96	50	12	1	159
Balugo	12	230	41	22	9	302
Mangnao	3	10	2	31	0	43
Bajumpandan	2	50	14	31	0	95
Cadawinonan	1	89	25	1	0	115
Bagacay	1	26	6	2	0	34
Total	56	1359	620	867	108	2954

Table 3 showed that plastic container contained the highest number of *Ae. aegypti* larvae at 528 individuals even if it is 2nd most abundant breeding site. Discarded tire being the most abundant breeding site contain only 229 individuals of *Ae. aegypti* larvae. In case *of Ae. albopictus* larvae, drainage canal contained the highest number of individuals (266 individuals) followed by plastic container (101 individuals) then bucket (95 individuals). For pupa, drainage canal contain the most number of *Ae.aegypti* and *Ae. albopictus.* Among the breeding site with less number of larvae and pupae in both species are coconut shell, motor part and jar.

Table 3. Number of larva and pupa of *Aedes aegypti* and *Ae. albopictus* found in each breeding site.

Breeding Site	Number of Larva		Number of Pupa	
	Aedes aegypti	*Aedes albopictus*	*Aedes aegypti*	*Aedes albopictus*
Coconut Shell	0	1	0	8
Aquarium	28	0	11	0
Concrete Aquarium	64	11	74	2
Can	93	32	10	0
Bucket	43	95	11	0
Flower Pot	66	21	16	0
Flower Vase	95	7	12	1
Jar	31	6	2	0
Motor Part	13	5	0	0
Plastic Container	528	101	121	4
Tire	229	75	48	9
Drainage Canal	169	266	562	84
Total	1359	620	867	108

Plastic container, tire and water drainage had high relative importance for *Ae. aegypti* larvae in which plastic container was the highest at 38.9%. On the other hand, water drainage, bucket, plastic container and tire were among the breeding sites with high relative importance for *Ae. albopictus* larvae. Drainage canal had the highest relative importance for *Ae. albopictus* larvae at 42.9% (Table 4). In case of pupae, drainage canal, tire, coconut shell and aquarium showed high relative importance for *Ae. aegypti* and *Ae. albopictus*. Drainage canal showed the highest relative importance in terms of pupae for both species at 65% and 78%, respectively. This may show that though plastic container is more preferred than water drainage for oviposition by gravid *Ae aegypti* but drainage canal is favourable for pupal development.

Table 4. Relative importance (%) of each breeding site in terms of Aedes larvae and pupae.

Breeding Site	Relative Importance (%) in terms of larvae		Relative Importance (%) in terms of Pupae	
	Aedes aegypti	*Aedes albopictus*	*Aedes aegypti*	*Aedes albopictus*
Coconut Shell	0.0	0.2	0.0	7.4
Aquarium	2.1	0.0	1.3	0.0
Concrete Aquarium	4.7	1.8	8.5	1.9
Can	6.8	5.2	1.2	0.0
Bucket	3.2	15.3	1.3	0.0
Flower Pot	4.9	3.4	1.8	0.0
Flower Vase	7.0	1.1	1.4	0.9
Jar	2.3	1.0	0.2	0.0
Motor Part	1.0	0.8	0.0	0.0
Plastic Container	38.9	16.3	14.0	3.7
Discarded Tire	16.9	12.1	5.5	8.3
Drainage Canal	12.4	42.9	64.8	77.8

DISCUSSION

Aedes mosquito's main important larval habitats are man-made water-holding containers, which strongly influence the density of the resulting adult mosquito populations. In this study, the most preferred breeding sites of *Aedes aegypti* were drainage canal, plastic container, tire and bucket. Drainage canal is the most preferred breeding site for *Aedes albopictus*. This could be attributed to the water quality of the water drainage which make a suitable breeding site for *Aedes albopictus*. It has been found out in previous studies that water quality characteristics affect the productivity of a potential mosquito breeding habitat (Kenawy et al., 2013; Nazri et al., 2013; Nazri et al., 2016; Madzlan et al., 2016). Results of the study by Nazri et al. (2013) showed that female *Aedes albopictus* prefer to oviposit in a natural and outdoor manmade container that contain a high amount of organic debris. In this study, water drainage may serve as good artificial breeding habitat due to its stagnant moderately turbid water with high organic content from fallen leaves and other debris. This calls attention to the community in Bajumpandan and Taclobo. Attentiveness of the school administrators, residents and barangay officials towards cleanliness should be improved in order to avoid formation of stagnant water.

Furthermore, improperly disposed containers such as plastic containers and old tire that have accumulated rainwater also serve as productive habitat for Aedes mosquitoes in the study areas. Tires discarded and dumped haphazardly within the settlement provide a suitable habitat for *Aedes aegypti* due to unobservable water collected inside, invulnerable to human disturbance, humidity, cool temperature and reduced light inside. Tires have been also identified as efficient breeding sites in India, (Viiajyakumar et al., 2014) Dares Salaam, Tanzania (Ramachandra et al., 1973; Philbert and Ijumba, 2013) and Cameroon, Central Africa (Simard, 2005) and Malaysia (Chang and Nagum, 1995). Barrera et al. (2006) found out that productivity of immature mosquitoes in tires is linked to the number of trees in the area, with more leaf litter helping the growth of mosquito larvae.

The habit of storing water in the comfort rooms of some schools in Taclobo due to irregular supply make bucket as one of the preferred breeding sites of Aedes mosquitoes. In the urban parts of Singapore, domestic containers used as water storage, ornamentation or the prevention of pests constituted 95% of the total breeding habitats of both *Ae. aegypti* and *Ae. albopictus* (Chan et al.,1971). Containers or receptacles exposed to rain, even when treated, may still be infested by the breeding of mosquito larvae (Morato et al. 2005). On the other hand, the low prevalence of *Ae. aegypti* and *Ae. albopictus* in coconut shell could be attributed by the heavy organic content of the water as the epicarp or fragments of the nut decay as also observed in the Carribean Region (Chadee et al., 1998. The low relative importance of can or metal containers could be attributed to its metal content. Studies in Florida (O'Meara et al. 1992a, b, Walker et al. 1996), shwed that Aedes larvae were less found in bronze vessels probably due to toxicity. Also, overheating of water in metal containers is a factor.

The current study observed that breeding sites of Aedes mosquitoes were mostly outdoor. This could be a manifestation on the change of breeding habit of *Aedes*; from photophobia to photophiliy as observed by Biswaz et al. (2014). Gravid *Aedes aegypti* and *Ae. albopictus* females were also observed in Malaysia to prefer laying eggs outside even if the ovitraps were placed indoors. Though the water temperature in outdoor containers was much higher than in indoor containers, *Ae. aegypti* chose outdoor containers as its preferred spawning sites (Norafikah et al., 2012). According to Ramachandra (1973), the relatively low degree of infestation of indoor containers is due to low prevalence in the practice of storing water for long periods, thus the chances that *Ae. aegypti* will breed in water containers, are minimized. The prevalence of outdoor breeding sites observed in this study could be due to improper waste disposal and poor sanitation.

In has been also observed in this study that *Aedes aegypti* and *Aedes albopictus* share breeding sites except in coconut shell. According to Klowden (1993), mixed breeding between *Aedis aegypti* and *Ae. albopictus* are found because they are sympatric species and occupy similar ecological niche. This finding supports Braks et al. (2003),that their larvae are commonly located together in the same larval developmental site in regions where both species cohabit. According to Nazri et al. (2016), coexistence of *Aedes aegypti* and *Ae. albopictus* in breeding containers is likely attributed to the abundance of suitable and favourable containers and the availability of shade and sufficient organic material for larval feeding. Mixed breeding was also observed in Singapore (Chan et al., 1971), Sarawak (Chang & Jute, 1994) and Malaysia (Chen et al., 2006). Invasion and further spread of *Ae. albopictus* into areas where *Ae. aegypti* is endemic could have public health interest because of high vector competence demonstrated by *Ae. albopictus* for a number of highly pathogenic viruses such as dengue fever under experimental conditions (Shroyer, 1986, Mitchell 1995, Johnson et al. 2002). Its strong anthropophily combined with its ability to colonize both urban and sub-urban areas make *Ae. albopictus* a possible bridge vector that might increase the risk of introduction and further transmission of arboviruses. Moreover, the ability of *Ae. albopictus* to survive more successfully in high temperature and precipitation under laboratory conditions allows its dispersal and growth (Neto and Navarro-Silva, 2004).

CONCLUSION

In nine sampling sites, the abundant containers for breeding Aedes mosquitoes were discarded tires and plastic containers. Plastic container had the highest relative importance for *Aedes aegypti* larvae while drainage canal had the highest relative importance for *Aedes albopictus*. Water drainage showed the highest relative importance for the pupae for both species. This may show that though plastic container is more preferred for oviposition by gravid *Ae aegypti* but drainage canal gives better pupal development in both species.

RECOMMENDATION

Environmental management practices should be carried out routinely within the community members, school, cemetery and other public places to avoid the unnecessary mosquito breeding habitats. Health education involving the community should be actively conducted by the Rural Health Unit or Department of Health so that they will be conscious about the risks of the diseases spread by Aedes and their participations towards cleanliness could be ensured. Community gathering institutions such as school should strictly observed proper collection and disposal of their garbage, regular cleaning and supply of water to avoid water storage.

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