THE KEY BREEDING SITES BY PUPAL SURVEY FOR DENGUE MOSQUITO VECTORS, *AEDES AEGYPTI* (LINNAEUS) AND *AEDES ALBOPICTUS* (SKUSE), IN GUBA, CEBU CITY, PHILIPPINES

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Abstract. We conducted this study to assess how well a pupal survey of dengue mosquito vectors, *Aedes aegypti* and *Aedes albopictus*, is able to target the most productive breeding sites. The study was carried out monthly during the rainy season (8 months) in 2008 in Guba, Cebu City, Philippines. The hypotheses tested were: 1) most pupae of *Ae. aegypti* or *Ae. albopictus* were produced in a few types of breeding sites and 2) the most productive types of breeding sites for each species were the most abundant. Approximately 2,500 pupae were collected from 554 breeding sites in 279 houses. Thirty-eight point four percent of ten types of breeding sites were positive for *Ae. aegypti*, and 11.9% of nine types of sites were positive for *Ae. albopictus*. Plastic drums (40.2%), metal drums (29.6%), and plastic containers (10.5%) were the key sites for *Ae. aegypti* pupae, whereas bamboo stumps (28.5%), plastic drums (21.1%), and rubber tires (19.1%) were the key sites for *Ae. albopictus.* The most productive breeding sites for *Ae. aegypti* were common but not the most common for *Ae. albopictus.* These results are relevant for dengue vector control programs.

Keywords: *Aedes aegypti*, *Aedes albopictus*, pupal survey, productive breeding sites, dengue mosquitoes

INTRODUCTION

The elimination of the most productive breeding sites for the primary dengue mosquito vector, *Aedes aegypti* (Linnaeus), has been broadly adopted worldwide to keep vector population density below a critical threshold (Focks, 2003; Barrera *et al*, 2006; Seng *et al*, 2009; Tun-Lin *et al*, 2009; de Freitas and de Oliveira, 2011), however, pupal surveys of the secondary dengue vector, *Aedes albopictus (*Skuse), have, to our knowledge, not been reported. Since the pupae of dengue vectors emerge to become adults, controlling the key breeding sites that produce the most pupae could have the greatest impact on the adult population (Lenhart *et al*, 2006). Observations from several urban areas suggested most pupae of *Ae. aegypti* reproduce in only a few types of containers (Focks and Chadee, 1997). Pending an effective and affordable vaccine, dengue prevention methods rely on control of these mosquito vectors to reduce transmission (Bangs

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et al, 2007). The pupal survey technique generates an estimate of pupal density of vectors as a proxy for the number of adults (Barrera *et al,* 2006). The main thrust for prevention should be the control of breeding sites of these vectors by eliminating or treating the most productive ones to reduce mosquito density below a target threshold. Further surveillance will only require assessing the abundance of each type of breeding sites; counting pupae will not be needed each time in the same area surveyed.

Pupal surveys measuring *Ae. aegypti* productivity have not been conducted in the Philippines and surveys of *Ae. albopictus* have not been conducted in many countries. Only a few studies of dengue vectors and their control have been conducted in Cebu City (Schoenig, 1971, 1977, 1978; Edillo *et al,* 1995, 1996; Tomayao, 2000; Mahilum *et al,* 2005). Cebu City has the highest incidence of dengue and case fatality rate among the municipalities and cities in Cebu Province (Edillo *et al,* 2009). Identifying the epidemiologically important breeding sites of *Ae. aegypti* had been done in Brazil (De Freitas and De Oliveira, 2011), Cambodia (Seng *et al,* 2009), Colombia (Romero-Vivas *et al,* 2006), Cuba (Bisset *et al,* 2006), Mexico (Arredondo-Jimenez and Valdez-Delgado, 2006), Puerto Rico (Barrera *et al,* 2006), Trinidad (Focks and Chadee, 1997) and Venezuela (Lenhart *et al,* 2006), to mention a few. These studies reveal the different types of containers for *Ae. aegypti* to oviposit. Pupal surveys of *Ae. aegypti* are based on the assumption that pupal mosquitoes (pupae per person per unit of time) is a better proxy for the number of adult mosquitoes than traditional indices (house, container and Breteau) or larval counts (Focks 2003). In short, pupal surveys are more appropriate for assessing risk and directing dengue control programs because traditional larval indices correspond poorly with the actual number of pupae per person (Focks and Chadee, 1997).

This consideration caused us to explore the hypotheses: 1) most pupae of *Ae. aegypti* or *Ae. albopictus* produce in a few types of breeding sites, and 2) the most productive of those breeding sites are found abundantly in Guba, Cebu City, Philippines. Our intent is to obtain information for vector control programs which will focus on eliminating or treating the key breeding sites for dengue vectors to reduce their density below a target threshold and prevent dengue outbreaks.

MATERIALS AND METHODS

Study site

Our study was conducted in Guba, Cebu City, Philippines represented by seven localities (*sitios*) (Fig 1). These localities were: Agnawe, Balisong, Bangkal, Guba Proper, Kabulihan, Kambilog, and Katives 1 (Table 1). Guba is a rural mountainous area (350-496 m elevation) located approximately 15 km from Cebu City proper. Vegetation is mainly agricultural and dry forest. Population data from our study site were obtained from the Guba Health Center. We surveyed 279 houses. The water supply for Guba is a combination of: 1) artesian wells, 2) water supply developed from a reservoir which comes from a spring and 3) a commercial source. Water from the Metropolitan Cebu Water District (MCWD), the official supplier of piped water in Metropolitan Cebu, does not reach there.

Mosquito survey

Artificial and natural breeding sites for *Ae. aegypti* and *Ae. albopictus* in the houses of the residents of Guba were in-

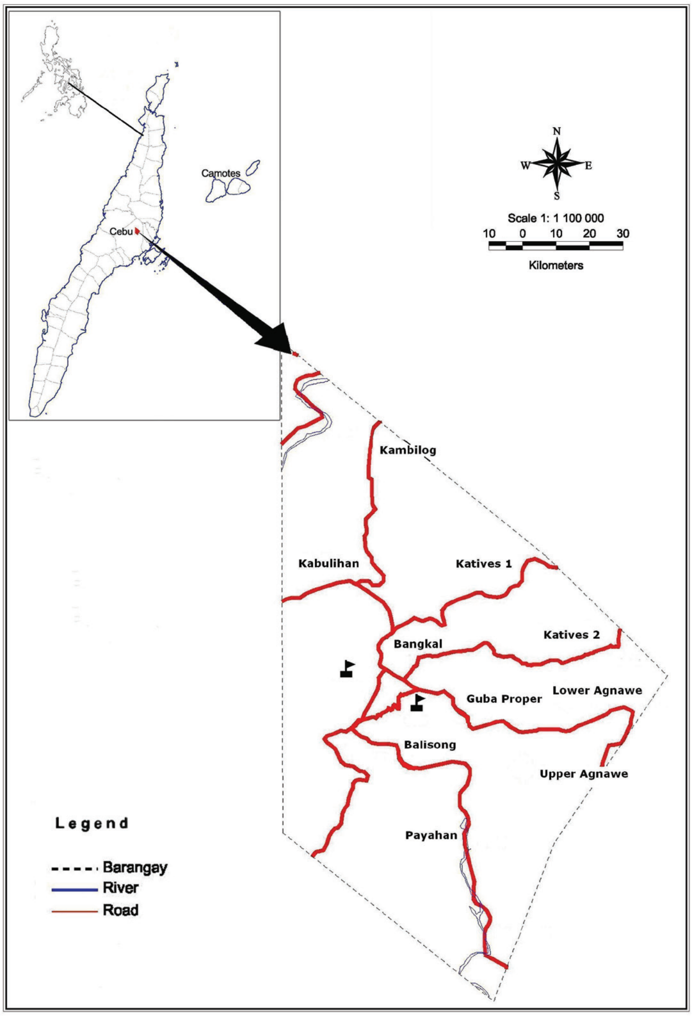

Fig 1–Map of Guba, Cebu City, Philippines showing seven localities (or *sitios*).

spected monthly for pupae and older larvae (third and fourth instar larvae) during the 2008 rainy season (January-February, and July-December). Pupae and larvae collection occurred between 10:00 AM and 5:00 PM. The numbers of pupae and larvae and the type, use and material of artificial containers (plastic, metal, porcelain, glass, cement, clay, rubber, plant material) for each breeding site was recorded.

Laboratory evaluation

The pupae and larvae were collected and placed in bottles and transported to the laboratory where they were reared until adult emergence. The adults were killed in a covered Petri dish containing cotton soaked in ethyl acetate. The adult mosquitoes were then mounted and examined under a stereo microscope for species identification following Belkin (1962) and Basio (1971).

Statistical analyses

Statistical analyses were performed using SPSS (version 15) statistical software (SPSS, Chicago, IL). The null hypotheses that the numbers of *Ae. aegypti* and *Ae. albopictus* pupae did not differ by the type of breeding site and among the seven localities in Guba were tested using analysis of variance (ANOVA). A Spearman correlation between the rank orders of the number of types of breeding sites and the number of pupae per species was used. A two-tailed Student's *t*-test was used to determine whether the numbers of *Ae. aegypti* or *Ae. albopictus* differed.

RESULTS

We collected approximately 2,500 pupae and 5,500 larvae from 554 containers in 279 houses during the survey (January-February and June-December, 2008) in Guba, Cebu City, Philippines.

Table 1
Coordinates, elevation, 2008 population and type of water supply (a=deep well, b=faucet, c=commercial or purchase of mineral water) for household use at the study localities (*sitios*) in Guba, Cebu City, Philippines.

Localities (*Sitios*)	Coordinates	Elevation (m)	Population	Type of water supply
Agnawe	10° 25.46′N 123° 54.200′E	350-376	664	a=103, b=42, c=92
Balisong	10° 25.348′N 123° 53.127′E	475	484	a=17, b=71, c=27
Bangkal	10° 25.921′N 123° 53.319′E	432	454	b=63, c=25
Guba Proper	10° 25.713′N 123° 53.324′E	427	739	a=51, b=81, c=40
Kabulihan	10° 25.889′N 123° 52.867′E	417	605	b=90, c=39
Kambilog	10° 25.522′N 123° 52.353′E	496	261	b=43, c=14
Katives 1	10° 26.065′N 123° 53.286′E	400	676	a=57, b=81, c=5

There were more *Ae. aegypti* (959=38.4%) than *Ae. albopictus* (298= 11.9%) collected (p<0.05). Other mosquito species collected were *Ae. poicilius* Theobald (0.2%), *Aedes* sp (0.2%), *Armigeres* sp (12%), *Culex quinquefasciatus* Say (10.0 %), *Culex* sp (2.0%), *Toxorhynchites* sp (0.6%), and *Uranotaenia* sp (0.4%).

Aedes aegypti pupae by type of breeding sites

A total of 554 breeding sites for *Aedes* mosquitoes were found in Guba during the 2008 rainy season. We classified the containers into three types of natural breeding sites and seven artificial ones (Fig 2). The natural breeding sites were: 1) plant parts such as leaf axils of banana plants (*Musa* sp), taro plants [*Colocasia esculenta* (Linnaeus) Schott and Endl], bromeliads (Bromeliaceae) and banana plant stumps, 2) discarded coconut shells (*Cocos nucifera* Linnaeus) and 3) bamboo (*Bambusa* sp, Poaceae) stumps or fences. The artificial breeding sites were: 1) cement water tanks, 2) discarded containers (bottles, cans, broken glasses, cooking pots, clay jars), 3) metal drums, 4) plastic drums, 5) small plastic containers (basins and buckets or pails), 6) rubber tanks and 7) discarded rubber tires. Thirty-eight point four percent of these ten types of breeding sites were positive for *Ae. aegypti* pupae (Fig 3). Average container index in Guba was 38.2%, and the Breteau Index was 82.2% indicating a high risk for dengue transmission.

The null hypothesis stating *Ae. aegypti* pupae did not differ by type of breeding sites was rejected (F=15.128, df=9, p<0.05). The two most productive breeding sites for *Ae. aegypti* found consistently throughout the rainy season were plastic drums (40.2%) and metal drums (29.6%). Ten point five percent of plastic containers yielded *Ae. aegypti* during the rainy months except for January and February, 2008. Seven point nine percent of discarded rubber tires yielded *Ae. aegypti* during the rainy season except for October and November 2008. A Spearman correlation between the rank orders of the number of breeding sites and the number of *Ae. aegypti* pupae was significant (n=10, r=0.745, p<0.05) suggesting the most productive containers were abundant and common in Guba. Three types of natural breeding

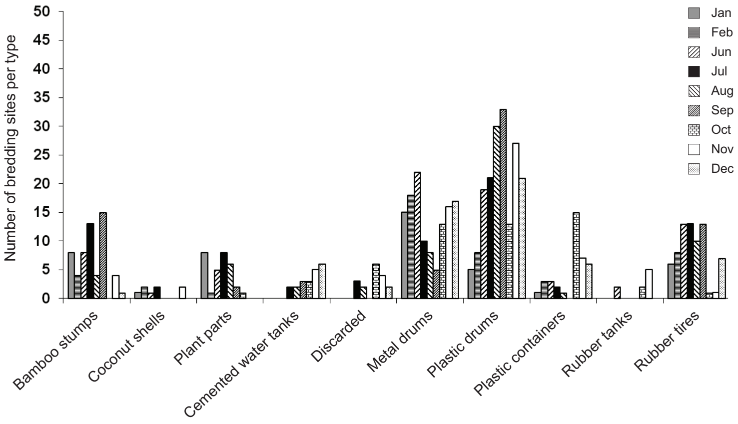

Fig 2–Types of breeding sites of *Aedes* mosquitoes in Guba, Cebu City, Philippines in January-February and June-December 2008.

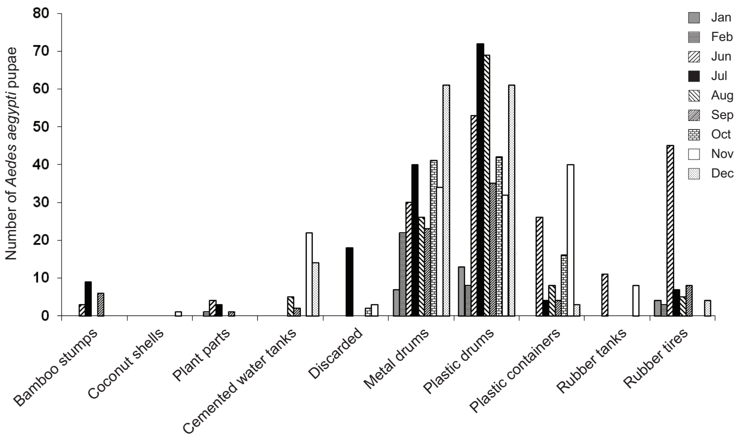

Fig 3–Monthly distribution of *Aedes aegypti* pupae among ten types of breeding sites in Guba, Cebu City, Philippines in January-February and June-December 2008.

sites had only few *Ae. aegypti* pupae: discarded coconut shells (0.1%), plant parts (0.9%) and bamboo stumps (1.9%).

Guba proper had the highest percent of *Ae. aegypti* (31.7%) found in plastic drums (13.4%), plastic containers (8.2%) and metal drums (6.5%) (Fig 4). Kambilog ranked next with 22.5% of containers having *Ae. aegypti*, followed by Kabulihan (13.1%). Metal drums were the most common container with *Ae. aegypti*. Katives 1 had the lowest percent of *Ae. aegypti* (1.1%).

Aedes albopictus pupae by type of breeding sites

Seventy-nine of 554 breeding sites (11.8%) were positive for *Ae. albopictus* pupae in Guba during 2008 rainy season. *Ae. albopictus* bred in similar types of breeding sites as those of *Ae. aegypti* except for rubber tanks (Fig 5). The null hypothesis that *Ae. albopictus* pupae did not significantly differ by breeding site was accepted (F=1.676, df=9, p>0.05). The top three breeding sites for *Ae. albopictus* were bamboo stumps (28.5%), plastic barrels (21.1%) and discarded rubber tires (19.1%). Eleven point eight percent of discarded coconut shells yielded *Ae. albopictus* as did 9.4% of metal drums. A Spearman correlation between the rank orders on the number of types of breeding sites and the number of *Ae. albopictus* pupae was not significant (n=10, r=0.641, p=0.059) suggesting the most productive breeding sites were not the most common in Guba. The least productive breeding sites were cement water tanks (1.3%), plant parts and discarded containers (2.0% each).

Among the surveyed localities in Guba, Balisong had the highest number of containers positive for *Ae. albopictus* (50.7%), where bamboo stumps (21.3%), discarded coconut shells and rubber tires

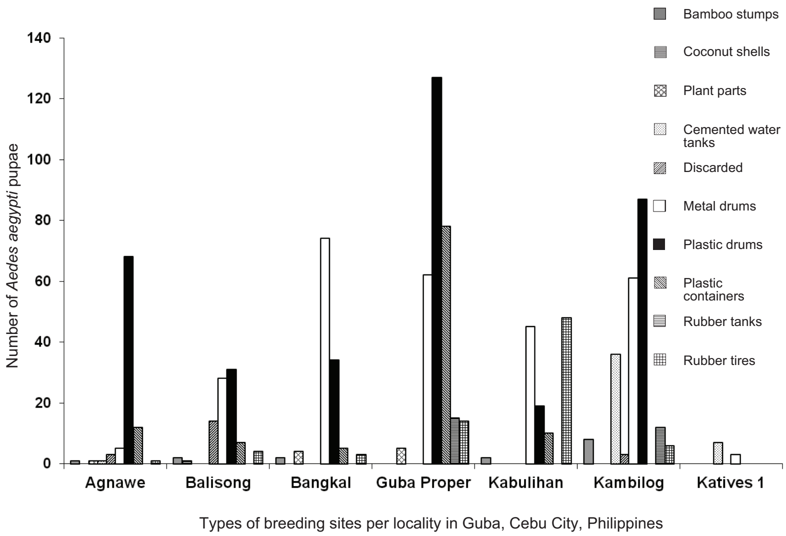

Fig 4–Distribution of *Aedes aegypti* pupae among ten types of breeding sites in seven localities (*sitios*) surveyed in Guba, Cebu City, Philippines during the rainy season of 2008.

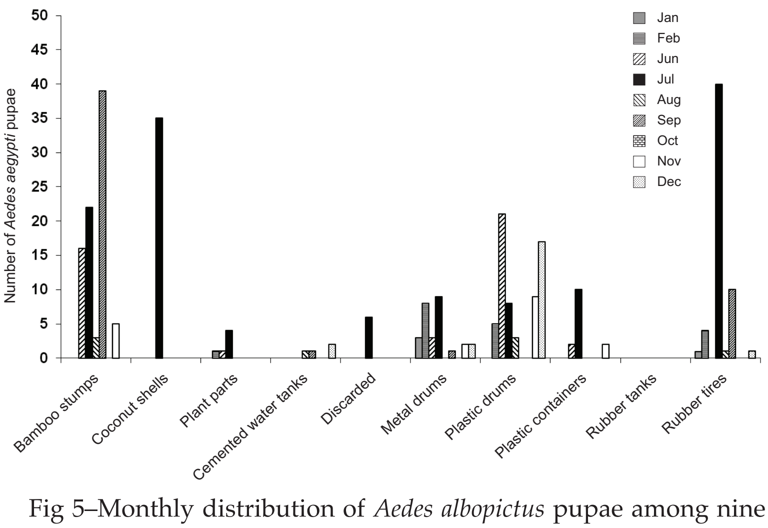

Fig 5–Monthly distribution of *Aedes albopictus* pupae among nine types of breeding sites in Guba, Cebu City, Philippines during January-February and June- December 2008.

(11.8%) were the most productive breeding sites (Fig 6). Kambilog ranked second in *Ae. albopictus* (12.5%), followed by Guba proper (10.5%). Plastic drums were the most common type of breeding sites. Katives 1 had the lowest number of *Ae. albopictus* pupae (3.4%).

Overall, there was a significant difference (F=6.963, df=1, p<0.05) in the number of *Ae. aegypti* and *Ae. albopictus* among the seven localities surveyed in Guba. There was no association between these localities and dengue mosquito species (F=1.594, df=6, p>0.05).

Temporal distribution of *Aedes aegypti* and *Aedes albopictus*

The monthly number of *Ae. aegypti* pupae during the 2008 rainy season did not differ (F=0.871, df=8, p=0.545). Fig 3 shows this species was found regularly, particularly in metal and plastic drums throughout the eight survey months. However, the number of *Ae. albopictus* pupae did differ by month (F=3.374, df=8, p<0.05) with July 2008 having the highest number (Fig 5).

DISCUSSION

Guba was a dengue hotspot in Cebu City, Philippines in 2007 when this study was conceived (Napallacan, 2007), which prompted an intervention strategy to suppress the dengue mosquito vectors, *Ae. aegypti* and *Ae. albopictus*. We hypothesized that most pupae of *Ae. aegypti,* the primary dengue vector, reproduced in only a few types of breeding sites; this was

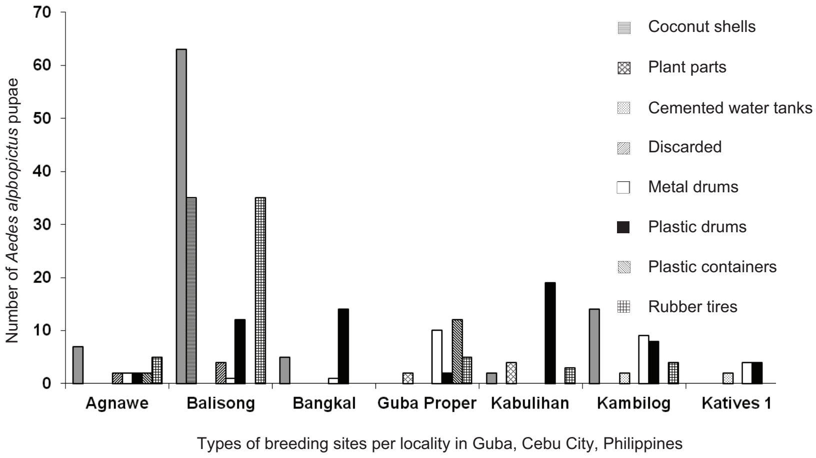

Fig 6–Distribution of *Aedes albopictus* pupae among nine types of breeding sites at seven localities (*sitios*) surveyed in Guba, Cebu City, Philippines during the rainy season of 2008.

supported by the data. Storing rainwater in 1,000-liter plastic and metal drums and plastic containers is obligatory in mountainous Guba because only 54.9% of householders use the local water supply from a reservoir distributed by a hose system (Table 1). The official supply of piped water system in Cebu City (MCWD) does not reach there. Sixty-nine point eight percent of plastic and metal drums contained mosquito pupae, whereas 10.5% of plastic containers contained pupae. These serve as the top three reproduction areas for *Ae. aegypti* pupae (80.3%) in Guba (Fig 3). In a similar study (Romero-Vivas *et al*, 2006), despite a reasonably reliable supply of piped water in Colombia, 75% of large storage containers for domestic water, such as cement ground tanks, plastic and metal drums were positive for *Ae. aegypti* pupae during the wet season, and 80.4% during the dry season. In Malindi District, Kenya (Midega *et al*, 2006), 70% of metal drums contained *Ae. aegypti* pupae indoors during the rainy season and 84% of outdoor plastic drums contained larvae during the dry season. In Venezuela, large water drums contained the greatest number of pupae all year round (Lenhart *et al*, 2006). In Brazil (de-Freitas and de-Oliveira 2011; Pilger *et al*, 2011) and Cuba (Bisset *et al*, 2006), water tanks were the most productive breeding sites for *Ae. aegypti*.

The lack of an adequate solid waste management system in Guba, Cebu City also creates numerous small artificial breeding sites, such as discarded rubber tires, 7.9% of which had *Ae. aegypti* and discarded artificial containers (2.4%). Discarded rubber tires made up the largest category of *Ae. aegypti* infested containers along the Texas-Mexican border (Ramos *et al*, 2008) and in Puerto Rico (Barrera *et al*, 2006).

We explored whether the most productive breeding sites for *Ae. aegypti* were the most abundant breeding sites because it is more practical to target to eliminate the most common type of breeding sites in Guba. This hypothesis was supported by the data. The most productive breeding sites for *Ae. aegypti* were plastic and metal drums and plastic containers, mainly used for water storage.

We extended the above hypothesis to *Ae. albopictus*, the second most common dengue vector. The hypothesis that most pupae of *Ae. albopictus* are found in a few types of breeding sites in Guba was not supported by the data. The top four sites for *Ae. albopictus* pupae were bamboo stumps, plastic drums, discarded rubber tires and coconut shells, in that order. In other studies (Sucharit *et al*, 1978; Chen *et al*, 2006), *Ae. albopictus* preferred to oviposit in natural breeding sites, whereas *Ae. aegypti* bred in artificial containers.

The hypothesis that the most productive breeding sites for *Ae. albopictus* corresponded with the most abundant breeding sites was not supported by the data, suggesting some commonly found breeding sites had few *Ae. albopictus* pupae and a few uncommonly found breeding sites had large numbers of pupae. Bamboo stumps comprised 10.3% of the total breeding sites (Fig 2) but 28.5% had *Ae. albopictus* (Fig 5), whereas plastic drums comprised 32.0% of breeding sites yet only 21.1% had *Ae. albopictus.* Discarded coconut shells comprised only 1.5% of containers but 11.8% had *Ae. albopictus* pupae. Thus, a reduction in *Ae. albopictus* pupae by 60-80% would require controlling the most abundant containers (*ie*, plastic drums) but this involves a larger number of plastic drums. Proper disposal of bamboo stumps, coconut shells and used rubber tires could reduce *Ae. albopictus* by up to 59.1%.

Katives 1 had the fewest *Ae. aegypti* and *Ae. albopictus* of the seven localities in Guba, primarily because there were few breeding sites there; namely, cement water tanks near a barn where cows drank water and, metal and plastic drums (Figs 4 and 6). Katives 1 is located at a lower elevation, most households used faucets connected to a hose from a nearby spring, or wells for their water supply (Table 1), minimizing the use of artificial containers for water storage.

While conducting the study we explained to residents in Guba the importance of covering their water storage containers to prevent reproduction of *Ae. aegypti* and *Ae. albopictus*. This improved awareness among residents, which might had contributed to the reduced dengue incidence rate in 2008 compared to 2007 (0.5 cases per 1,000 population in 2008 versus 5.7 cases per 1,000 population in 2007) (DOH-RESU 7, 2010). We recommend piped water should reach to higher elevated areas to reduce the risk for dengue vectors. Potable water must be delivered in sufficient quantities and quality to reduce the use of water storage containers that serve as larval habitats for dengue mosquitoes (WHO, 2012).

This study supports the recommendations of the National Dengue Prevention and Control Programs in the Philippines (DOH, Republic of the Philippines, 2011): 1) integrated vector control, 2) early case detection and management, 3) surveillance, 4) health education to multiple sectors, and 5) implementation of the 4S *Kontra* (Filipino word which means against) dengue and the “4 o’clock habit”. The 4S *Kontra* dengue includes: 1) search and destroy mosquito breeding sites, 2) self-protection measures, 3) seek early treatment, and 4) say “no” to indiscriminate fogging. The “4 o’clock habit” involves cleaning of surroundings and draining water containers to prevent spread of mosquitoes.

In conclusion, this pupal survey found the most productive breeding sites for *Ae. aegypti* and *Ae. albopictus* in order to reduce dengue mosquito populations in Guba, Cebu City, Philippines. This is relevant for both the National Dengue Prevention and Control Program and the Special Program for Research and Training in Tropical Diseases of the WHO (Focks, 2003). This can serve as a model for dengue prevention in the Philippines and in other countries as well.

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