

## NIH Public Access

**Author Manuscript**

*Acta Trop*. Author manuscript; available in PMC 2014 June 25.

Published in final edited form as: *Acta Trop*. 2014 June ; 134: 33–42. doi:10.1016/j.actatropica.2014.01.011.

### **Stormwater Drains and Catch Basins as Sources for Production of Aedes aegypti and Culex quinquefasciatus**

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#### **Abstract**

We present data showing that structures serving as drains and catch basins for stormwater are important sources for production of the mosquito arbovirus vectors *Aedes aegypti* and *Culex quinquefasciatus* in Mérida City, México. We examined 1,761 stormwater drains – located in 45 different neighborhoods spread across the city – over dry and wet seasons from March 2012– March 2013. Of the examined stormwater drains, 262 (14.9%) held water at the time they were examined and 123 yielded mosquito immatures. In total, we collected 64,560 immatures representing nine species. The most commonly encountered species were *Cx. quinquefasciatus* (n=39,269) and *Ae. aegypti* (n=23,313). *Ae. aegypti* and *Cx. quinquefasciatus* were collected during all 11 months when we found water-filled stormwater drains, and both were found in stormwater drains located throughout Mérida City. We also present data for associations between structural characteristics of stormwater drains or water-related characteristics and the abundance of mosquito immatures. In conclusion, stormwater drains produce massive numbers of *Ae. aegypti* and *Cx. quinquefasciatus* across Mérida City, both in the wet and dry seasons, and represent nonresidential development sites that should be strongly considered for inclusion in the local mosquito surveillance and control program.

#### **Keywords**

*Aedes aegypti*; *Culex quinquefasciatus*; Catch basin; Drain; Stormwater

#### **1. Introduction**

A wide range of water-holding containers are exploited by the dengue virus vector *Aedes (Stegomyia) aegypti* as sites for oviposition of eggs and development of immatures (Focks and Alexander 2006, WHO 2009). The most important container types for production of this mosquito differ among geographic locations but often include water storage tanks or jars,

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barrels/drums, buckets, tires, and small trash items (Tun-Lin et al. 2009, Arunachalam et al. 2010). There also is an increasing recognition that atypical development sites may be important contributors to the production of *Ae. aegypti* immatures, especially after the container types traditionally perceived as being most productive in the local environment have been controlled. Moreover, contrary to early field surveys indicating that *Ae. aegypti* is absent from water containing sewage (James et al. 1914), there is increasing evidence for production of immatures in water containing a high concentration of decomposing organic matter (Murrell et al. 2011, Nguyen et al. 2012).

A variety of atypical development sites have been incriminated in the production of *Ae. aegypti*. Some of these are structures that hold relatively clean water, such as drains on residential premises (Morrison et al. 2004, Maciel-de-Freitas et al. 2007, David et al. 2009, Glasser et al. 2011), stormwater drains/catch basins/drain sumps on streets or sidewalks or in other non-residential settings (Tinker 1974; Gonzalez and Suárez 1995; Montgomery et al. 2004; Suárez-Rubio and Suárez 2004; Marquetti et al. 2005; Morrison et al. 2006; Rey et al. 2006; Giraldo-Calderón et al. 2008; Manrique-Saide et al. 2012, 2013), service manholes/ pits (Kay et al. 2000a, b), wells (Panicker et al. 1982, Lardeux 1992, Jennings et al. 1995, Tun-Lin et al. 1995, Russell et al. 1996, Nam et al. 1998, Gionar et al. 1999, Kay et al. 2002, Tsuzuki et al. 2009, Surendran et al. 2012), roof gutters (Tinker 1974; Montgomery and Ritchie 2002; Morrison et al. 2004, 2006; Glasser et al. 2011; Pilger et al. 2011) and roofs (Pilger et al. 2011). Other atypical development sites for *Ae. aegypti* hold water containing a high concentration of decomposing organic matter, such as septic tanks (Chinery 1970, Babu et al. 1983, Hribar et al. 2004, Barrera et al. 2008, MacKay et al. 2009, Burke et al. 2010, Somers et al. 2011) and cesspits or pit latrines (Curtis 1980, Hribar et al. 2004).

We were particularly interested in structures serving as drains and catch basins for stormwater (hereinafter referred to as stormwater drains) because they can be important habitats for *Ae. aegypti* (Montgomery et al. 2004, Manrique-Saide et al. 2012, 2013), especially during drier parts of the year when they provide sheltered micro-environments where standing water may persist for extended time periods. Moreover, stormwater drains may be overlooked in mosquito control campaigns that focus on residential premises. A field survey in Cairns, Australia suggested that stormwater drains contributed nearly 15% of the standing crop of *Ae. aegypti* pupae during the dry season (Montgomery et al. 2004), and stormwater drains are considered to be among the most important development sites for *Ae. aegypti* in Cali, Colombia (González and Suárez 1995, Suárez-Rubio and Suárez 2004, Giraldo-Calderón et al. 2008). It also has been determined in the laboratory that *Ae. aegypti* females readily oviposit in water from stormwater drains (Chen et al. 2007).

Most recently, the importance of stormwater drains for production of *Ae. aegypti* was highlighted in two studies focusing on single neighborhoods in Mérida City, México during the rainy and dry seasons in October/November 2011 and March 2012, respectively (Manrique-Saide et al. 2012, 2013). Herein, we expand on these studies by reporting on mosquito collections from stormwater drains located geographically more broadly throughout Mérida City and sampled across dry and rainy seasons from March 2012 - March 2013. Additionally, we examine associations between water characteristics, shading, and

physical structure of the stormwater drains with the abundance of immatures of the two most commonly encountered species: *Ae. aegypti* and *Culex quinquefasciatus*.

#### **2. Materials and methods**

#### **2.1. Area and timing of study**

Studies were conducted within Mérida City (population  $\sim$  800,000) in the Yucatán Peninsula of southeastern México. Mean monthly maximum temperatures in Mérida range from 29 °C in December to 34 °C in July, and peak rainfall occurs from June to September (García-Rejón et al. 2008). Collections of mosquito immatures were undertaken from March of 2012 to March of 2013, and included sampling of 1,761 individual stormwater drains located in different parts of Mérida City: I-Southwest, II-Northwest, III-Northeast, and IV-Southeast (Figure 1). Two of these stormwater drains were sampled on two different dates, resulting in a total of 1,763 sampling occasions. Examples of locations for stormwater drains are shown in Figure 2. Based on monthly totals for rainfall during the study period (Table 1), November to March were classified as dry months (range, 1 to 47 mm) and April to October as wet months (range, 52 to 235 mm).

#### **2.2. Characteristics of stormwater drains**

Stormwater drains in Mérida City come in a variety of shapes and sizes. The most common type is a rectangular structure,  $\sim 2.0$  m long  $\times 0.5$  m wide  $\times 0.7$  m deep, covered with a metal grate and often equipped with a drainage pipe that can be connected to a well. Examples of different types of stormwater drains encountered in the study are shown in Figure 3.

The following data were recorded in the field (between 0900 and 1400 hours) for each occasion of a stormwater drain being examined: 1) date of examination; 2) nearest adjacent street address; 3) number of larvae and pupae collected; 4) size, classified as small (~ 1.0 m length  $\times$  0.5 m width  $\times$  0.7 m depth), medium ( $\sim$  2.0 m length  $\times$  0.5 m width  $\times$  0.7 m depth) or large ( $\sim$  4.0 m length  $\times$  0.5 m width  $\times$  0.7 m depth); 5) presence or absence and orientation of drainage pipe (lacking, vertical, or horizontal); 6) presence or absence of a well connected to the drainage pipe; 7) status of the walls, classified as finished and impermeable or unfinished and permeable; and 8) presence versus absence of trash items.

For the stormwater drains that contained water, we also noted: 9) water volume (L); 10) water quality (clear versus turbid); 11) water odor (present or absent); 12) water pH ( $\sqrt{7}$ versus > 7); 13) water temperature ( $25$ , 26–27, 28–29, or  $30^{\circ}$ C); 14) percentage of shade, classified as 0, 25, 50 or 100%; 15) types of organic matter present (primarily wood, grass, leaves, fruits, or flowers); and 16) status of the organic matter present (primarily intact versus decomposed). The locations of the examined stormwater drains were recorded using a global positioning system receiver (Garmin, Olathe, KS).

#### **2.3. Collection of mosquito immatures and species identification**

To collect mosquito immatures from stormwater drains, we used a long-handled (1.5 m) zooplankton net (35 × 25 cm, 100 μm mesh). A plastic dipper (capacity 0.5 liter) was used to determine the water volume (Strickman and Kittayapong 2003). In this study, we were not able to extract the entire water volume for all examined stormwater drains, leading to potential underestimation of the production of mosquito immatures from stormwater drains holding standing water in excess of 10 L.

Collected larvae and pupae were placed in plastic containers labeled with date of collection and address for the stormwater drain, and transported to the Laboratorio de Arbovirología at Universidad Autónoma de Yucatán. Larval specimens were counted, and then reared in the laboratory (28  $\pm$  1 °C water temperature and a photoperiod of 12 hr light and 12 hr dark) to fourth instar for more accurate species identification. Pupae were allowed to emerge as adults, and the adults were then identified to species. Species identification was done using stereomicroscopes and published keys (Carpenter and LaCasse 1955, Ibañez-Bernal and Martinez-Campos 1994, Darsie and Ward 2005).

#### **2.4. Data presentation and statistical analyses**

Summary data for collection of mosquito immatures are shown in Tables 1–3. Statistical analyses were performed using IBM SPSS Statistics version 19 (IBM Corporation, Armonk, NY) and results were considered significant when *P* < 0.05. We first compared the abundance of mosquito immatures, separately for *Ae. aegypti* and *Cx. quinquefasciatus*, in infested stormwater drains between dry months (November–March) and wet months (April– October); because the data did not meet the assumptions of normality and homogeneity of variances, a Mann-Whitney U-test was used. Further univariate tests were based on combined data from wet and dry months, and similarly compared the abundance of mosquito immatures, separately for *Ae. aegypti* and *Cx. quinquefasciatus*, for water-filled and infested stormwater drains with different characteristics as listed in Table 3 (using the Mann-Whitney U-test or Kruskal-Wallis test, as appropriate). Finally, we used a principal component analysis to determine associations between the numbers of *Ae. aegypti* or *Cx. quinquefasciatus* immatures collected per infested stormwater drain and potentially explanatory independent variables. These included variables related to the stormwater drain itself (size, presence/absence and orientation of the drainage pipe, presence versus absence of a well, status of the walls, and shade percentage class), the water contained in the stormwater drain (volume, quality, odor, pH, and temperature), or trash or organic matter present in the water (presence/absence of trash and primary type and status of the organic matter present). Based on the outcomes, the results of the principal component analysis are presented only for *Ae. aegypti*.

#### **3. Results**

#### **3.1. Summary of mosquito collections**

We examined 1,761 individual stormwater drains, of which 262 (14.9%) held water at the time they were examined and 123 yielded mosquito immatures (7.0% of all examined stormwater drains; 46.9 % of the ones holding water) (Table 1). In total, we collected 64,560 immatures representing nine species. The most commonly encountered species were *Cx. quinquefasciatus* (n=39,269 specimens) and *Ae. aegypti* (n=23,313) (Tables 1–2). Other species collectively accounted for only 3.1% of all immatures collected: they included *Culex*

*coronator* (n=963), *Culex lactator* (n=898), *Culex thriambus* (n=58), *Culex interrogator* (n=42), *Culex salinarius* (n=11), *Culex tarsalis* (n=5), and *Aedes* (*Ochlerotatus*) *taeniorhynchus* (n=1).

Both *Ae. aegypti* and *Cx. quinquefasciatus* were collected during all 11 months when we found water-filled stormwater drains (Table 1). Based on data for nine months during which at least 10 water-filled stormwater drains were examined in each month, the average number of immatures collected per examined stormwater drain that contained water ranged from 9.8 (February 2013) to 256.4 (November 2012) for *Ae. aegypti*, and from 6.4 (February 2013) to 372.8 (April 2012) for *Cx. quinquefasciatus*. The corresponding averages for only those stormwater drains that had mosquito immatures present ranged from 59.0 (February 2013) to 286.9 (October 2012) for *Ae. aegypti*, and from 38.3 (February 2013) to 548.2 (April 2012) for *Cx. quinquefasciatus* (Table 1). Over the full study period (March 2012 to March 2013), the average numbers of immatures collected per water-filled, and water-filled and infested, stormwater drain were 89.0 and 189.5, respectively, for *Ae. aegypti*, and 149.9 and 319.3, respectively, for *Cx. quinquefasciatus* (Table 1).

The examined stormwater drains were spread across 45 different neighborhoods (Colonias), and >240 (range, 242–792) stormwater drains were examined within each of the four zones of Mérida City (Table 2, Figure 1). Among the four zones, the percentages of water-filled stormwater drains out of those examined ranged from 11.3–21.9%, and the percentages of water-filled stormwater drains with immatures present ranged from 39.6–53.8% (Table 2). Out of 35 neighborhoods within each of which at least five stormwater drains were examined, *Ae. aegypti* immatures were collected from 32 of the neighborhoods (91.4%) and *Cx. quinquefasciatus* immatures from 30 of the neighborhoods (85.7%) (Table 2). Statistical analysis revealed no significant difference across zones for the abundance of immatures in infested stormwater drains for either *Ae. aegypti* ( $X^2 = 6.626$ ,  $df = 3$ ,  $P = 0.085$ ) or *Cx*. *quinquefasciatus* ( $X^2 = 3.823$ , *df* = 3, *P* = 0.281)

#### **3.2. Characteristics of stormwater drains**

Data for selected characteristics of examined stormwater drains, for all zones and months combined, in relation to the total number of *Ae. aegypti* and *Cx. quinquefasciatus* immatures collected are shown in Table 3. Of the examined stormwater drains, 42.0% (739/1,761) lacked any drainage pipe and only 23.8% (419/1,761) of those with a drainage pipe present had it connected to a well to facilitate water being removed from the catch basin. For those stormwater drains that contained water when they were examined, the majority (79.4%) held turbid water, the water volume most often (89.7%) exceeded 10 liters, and most (82.8%) had no shade. Water temperatures most commonly (58.4%) were in the 28–29 °C range. The water commonly held leaves (75.2%) or other organic matter, and this often (40.5%) was decomposed.

#### **3.3. Associations of mosquito abundance with rainfall and characteristics of stormwater drains**

We found no significant difference among dry and wet months for the abundance of immatures in infested stormwater drains, either for *Ae. aegypti* (Z = −0.318, *P* = 0.750) or

for *Cx. quinquefasciatus* ( $Z = -0.242$ ,  $P = 0.809$ ). Further analyses therefore were based on data for infested stormwater drains from dry and wet months combined.

Without regard to the number of the examined stormwater drains with a given specific characteristic, such as trash being present or absent, the majority of all recovered *Ae. aegypti* immatures came from those stormwater drains with the following specific characteristics: medium size (91.5%); lack of a drainage pipe (82.0%); absence of a well to aid in drainage  $(95.4\%)$ ; unfinished walls  $(63.6\%)$ ; trash items present  $(68.4\%)$ ; turbid water  $(76.4\%)$ ; water volume >10 liters (96.6%); water pH >7 (67.1%); water temperature in the 26–29 °C range (96.0%); no shade (79.8%); presence of organic matter in the form of leaves (90.9%); and primarily intact organic matter (92.9%) (Table 3). Trends were similar but often less pronounced for *Cx. quinquefasciatus* immatures (Table 3).

Notable discrepancies for the number of *Ae. aegypti* immatures collected, in relation to the number of stormwater drains with a given characteristic, include overrepresentation of immatures from stormwater drains that: 1) lacked a drainage pipe (accounting for 42.0% of the examined stormwater drains versus 82.0% of total immatures recovered); 2) had trash present (accounting for 22.8% of examined stormwater drains versus 68.4% of total immatures); and 3) contained primarily intact organic matter (accounting for 59.5% of the water-filled stormwater drains versus 92.9% of total immatures) (Table 3). The only discrepancy of a similar magnitude for *Cx. quinquefasciatus* immatures occurred for stormwater drains with trash present, which accounted for 22.8% of examined stormwater drains but produced 57.0% of the total immatures recovered (Table 3).

Univariate tests for the variables listed in Table 3 demonstrated significant differences for the abundance of *Ae. aegypti* immatures in infested stormwater drains for the following variables: 1) water temperature ( $X^2$ =22.852,  $df$  = 3,  $P$  < 0.001), with elevated abundance for the 26–27 °C range; 2) well ( $Z = -2.146$ ,  $P = 0.032$ ), with elevated abundance when a well was absent; and 3) status of organic matter  $(Z = -3.303, P = 0.001)$ , with elevated abundance for primarily intact organic matter. The corresponding tests for *Cx. quinquefasciatus* immatures showed significant differences in abundance in infested stormwater drains for the following variables: 1) water temperature ( $X^2$ =16.696,  $df = 3$ ,  $P =$ 0.001), again with elevated abundance for the 26–27 °C range; and 2) size ( $X^2$ =8.413, *df* = 2,  $P = 0.015$ , with elevated abundance for medium-sized stormwater drains.

The principal component analysis produced five factors that collectively explained 61.2% of the variation in abundance of *Ae. aegypti* immatures in infested stormwater drains, with a significant Bartlett sphericity ( $P < 0.001$ ) (Table 4). Factors included associations with water characteristics (PC1: water odor, pH, and quality, and presence/absence of trash in the water; PC4: water volume and temperature), organic matter (PC2: primary type and status of organic matter in the water), and the structural components of the stormwater drain (PC3: presence/absence and orientation of drainage pipe, presence/absence of a well; PC5: status of the walls).

#### **4. Discussion**

In agreement with other studies from Latin America (Tinker 1974; González and Suárez 1995; Suárez-Rubio and Suárez 2004; Marquetti et al. 2005; Morrison et al. 2006; Giraldo-Calderón et al. 2008; Manrique-Saide et al. 2012, 2013), our results indicate that stormwater drains are important sources for production of both *Ae. aegypti* and *Cx. quinquefasciatus* in Mérida City. Previous studies in Mérida City, limited geographically to single neighborhoods, reported collection from stormwater drains of substantial numbers of immatures of *Ae. aegypti* and *Cx. quinquefasciatus* during the rainy season (Manrique-Saide et al. 2012) and of emerging *Ae. aegypti* adults during the dry season (Manrique-Saide et al. 2013). We expand on these findings by demonstrating that stormwater drains are productive sources for both *Ae. aegypti* and *Cx. quinquefasciatus* throughout Mérida City during both wet and dry months (Tables 1–2, Figure 1). Moreover, it has been estimated that Mérida City harbors >20,000 stormwater drains (Manrique-Saide et al. 2012). Consequently, this type of non-residential development site should be strongly considered for inclusion in the local mosquito surveillance and control program.

We demonstrate that stormwater drains in Mérida City contain standing water not only in wet months – 23.7% of examined stormwater drains were found to hold water during April to October 2012 when monthly rainfall consistently exceeded 50 mm – but also in dry months as 10.5% of examined stormwater drains were found to hold water during March 2012 and November 2012 to March 2013 when monthly rainfall typically was <20 mm and never exceeded 50 mm. We speculate that the commonplace structures represented by stormwater drains may serve as particularly important development sites for *Ae. aegypti* immatures in Mérida City during the dry season, when this mosquito is least abundant and potentially can be severely impacted by interventions targeted to key development sites. Moreover, as suggested previously by Barrera et al. (2008), atypical development sites – such as septic tanks or stormwater drains – may contribute to keep *Ae. aegypti* populations at high enough levels for dengue virus transmission to persist even during dry months.

As noted above, the stormwater drains yielded large numbers of *Cx. quinquefasciatus*. This commonly bird-feeding mosquito also is a nuisance biter of humans (Elizondo-Quiroga et al. 2006; García-Rejón et al. 2008, 2010). It is capable of transmitting several arboviruses known to cause human disease, including West Nile virus (Turell et al. 2005). West Nile virus has been detected from *Cx. quinquefasciatus* in Nuevo Leon State in northern México (Elizondo-Quiroga et al. 2005) but extensive efforts in Mérida City and other locations in Yucatán State have failed to detect West Nile virus in this mosquito species (Farfan-Ale et al. 2009, 2010). However, recent studies from Yucatán State revealed the presence in *Cx. quinquefasciatus* of insect-only viruses as well as other viruses with unknown pathogenicity to humans, including the T'Ho virus (Farfan-Ale et al. 2009, 2010; Saiyasombat et al. 2010).

In addition to occurring in large numbers throughout Mérida City, stormwater drains have several features that make them potentially very productive sources for *Ae. aegypti* and *Cx. quinquefasciatus*. The drains and catch basins provide a subterranean microhabitat that is cooled by the ground itself and also provides partial protection from solar insolation, thus reducing the rate of evaporation of standing water. Some structural features also may

stormwater drains. Several of the aforementioned features (including the lack of a drainage pipe, lack of a well connected to the drainage pipe, and presence of trash or intact organic matter) were associated with elevated numbers of mosquito immatures in our study.

We observed accumulations of organic matter in 87% of examined stormwater drains that held water. Decomposing organic matter can provide nutrients for mosquito larvae and also may provide cues for females searching for suitable oviposition sites (Barrera et al. 2006, Murrell et al. 2011). Previous studies also have shown that gravid females follow visual and olfactory cues when choosing oviposition substrates, and are guided by chemical cues in and physical properties of the water when deciding on whether or not to deposit eggs (Gjullin et al. 1965, Muir 1988, Bentley and Day 1989, Millar et al. 1994, Torres- Estrada et al. 2001, Chen et al. 2007). Such considerations may help to explain why some stormwater drains may be more attractive oviposition sites than others.

The study had some notable weaknesses. Firstly, we were not able to sample the entire water volume for all examined stormwater drains, leading to underestimation of the production of mosquito immatures from stormwater drains holding standing water in excess of 10 L. Secondly, although all four zones were sampled in both dry and wet months, we were not able to conduct sampling in a fully temporally synchronized scheme across the zones. Thirdly, because of unknown mortality of immatures in stormwater drains, collection of emerging adults, rather than immatures, would have provided data of more direct relevance to assess the risk for human-biting. Further studies are needed to quantify the relative production of *Ae. aegypti* from stormwater drains and other non-residential mosquito production sites – such as vacant lots – versus residential premises in Mérida City, including data not only for average mosquito production in containers found in these respective environments (based on repeated sampling of individual sites across wet and dry seasons) but also for the numbers of stormwater drains, vacant lots and residential premises present within the study area.

#### **Acknowledgments**

We thank Maria Puc-Tinal, Victor Rivero-Osorno, Mildred López-Uribe, Genny López-Uribe, and Carlos Coba-Tun of Universidad Autónoma de Yucatán for technical assistance. The study was supported by the National Institutes of Health/National Institute of Allergy and Infectious Diseases (International Collaborations in Infectious Disease Research Program U01-AI-088647). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIAID or NIH.

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**Figure 1.**



**Figure 2.**



**Figure 3.**



Collections of mosquito immatures from stormwater drains and catch basins (SWDCBs) in Mérida City by month from March 2012 to March 2013. Collections of mosquito immatures from stormwater drains and catch basins (SWDCBs) in Mérida City by month from March 2012 to March 2013.



ZU12. <sup>*a*</sup>No SWDCBs were sampled in August 2012.  ${\tt Augu}_{{\tt M}}$ ≣ WUID NO SWIJLISS

Based on data from a weather station at the Mérida airport operated by Comisión Nacional del Agua. *b*Based on data from a weather station at the Mérida airport operated by Comisión Nacional del Agua.

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Collections of immatures of Ae. aegypti and Cx. quinquefasciatus from stormwater drains and catch basins (SWDCBs) in Mérida City, by zone and Collections of immatures of *Ae. aegypti* and *Cx. quinquefasciatus* from stormwater drains and catch basins (SWDCBs) in Mérida City, by zone and neighborhood, from March 2012 to March 2013. neighborhood, from March 2012 to March 2013.





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**No. immatures collected**

No. immatures collected



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 $a<sup>a</sup>$ Including Vergel, I, II, III, and IV.

 $a_{\mbox{Including\,Vergel, I, II, III, and IV.}}$ 

#### **Table 3**

Selected characteristics of examined stormwater drains and catch basins in Mérida City, for all zones and months combined, in relation to the total number of *Ae. aegypti* and *Cx. quinquefasciatus* immatures collected.





*a*<br>
Small: ∼1.0 m length × 0.5 m width × 0.7 m depth; Medium: ∼ 2.0 m length × 0.5 m width × 0.7 m depth; Large: ∼ 4.0 m length × 0.5 m width × 0.7 m depth.

*b* Based on 262 stormwater drains and catch basins that held water at the time they were examined.

# **Table 4**

Rotated factor pattern scores from 13 principal components relating to characteristics of stormwater drains and catch basins to explain the number of Ae. Rotated factor pattern scores from 13 principal components relating to characteristics of stormwater drains and catch basins to explain the number of *Ae.* aegypti immatures in infested stormwater drains and catch basins. *aegypti* immatures in infested stormwater drains and catch basins.



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), PC4 (9.1%), and PC5 (8.1%); *a*The percentage variation for each component is: PC1 (17.3%), PC2 (14.4%), PC3 (12.2%), PC4 (9.1%), and PC5 (8.1%); P,  $\frac{1}{2}$ ż  $\ddot{\cdot}$ ₹  $\tilde{\mathfrak{g}}_r$ 

 $b$ Scores >0.5.