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The hidden world of Asian tiger mosquitoes: immature *Aedes albopictus* (Skuse) dominate in rainwater corrugated extension spouts

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Background: The primary sources of *Aedes albopictus* (Skuse) in its exotic range in North America are artificial containers in backyards, which vary widely in accessibility. In this study we examined their prevalence in two container types that are difficult to inspect: catch basins and corrugated extension spouts (CES), the latter used to divert rainwater in downspouts away from house foundations.

Methods: We conducted larval and pupal surveys in catch basins, CES and open containers such as buckets and plant saucers in three urban locations in Mercer County, New Jersey, USA.

Results: We found that *Ae. albopictus* were rare in catch basins but prevalent in CES, and were often the only species collected in CES. Specific characteristics of the CES were not significantly associated with the presence or number of *Ae. albopictus* in them, but those longer and closer to the ground were significantly more likely to contain water, and therefore mosquitoes. During peak season (July-August), the abundance of immature *Ae. albopictus* was significantly higher in CES than open containers.

Conclusions: We found that CES are an important source of *Ae. albopictus* in our region and propose that effective control strategies should be implemented to minimize mosquito populations from these cryptic habitats.

Keywords: *Aedes albopictus*, Container-inhabiting mosquito, Cryptic habitat, Larval survey, New Jersey

Introduction

Locally-acquired dengue and chikungunya cases associated with exotic mosquitoes reported recently in Europe and the United States[1,2] remind us that exotic pathogens and vectors can be transported to new areas anywhere in the world; an unwelcome consequence of today's interconnected and rapidly changing environment.[3] *Aedes albopictus* (Skuse), the Asian tiger mosquito, was incriminated as the sole vector of the dengue outbreak in Hawaii, USA during 2001–2002[4] and of chikungunya virus infections in Ravenna, Italy in 2007.[1] This species is also an important nuisance that reduces residents' activities and enjoyment of the outdoors.[5,6] These impacts to public health underscore the importance of pre-emptive surveillance and control of mosquitoes and disease vectors.

In its exotic range *Ae. albopictus* is found significantly more often in artificial rather than natural containers.[7] Commonly used container habitats in residential backyards include tires, buckets, bird baths, plant saucers, recycle bins, etc.[7–10] This variety of potential sources creates a formidable challenge for mosquito control programs that struggle to find and treat all containers positive with larvae and pupae.[11] Because source reduction is an important component of *Ae. albopictus* suppression programs, targeting the most preferred and productive containers should lessen the work load and increase efficacy.[7,11]

While performing surveillance for larval and pupal *Ae. albopictus* in several locations in New Jersey, an Atlantic coastal state in temperate North America,[7] we have rarely detected this species in urban catch basins, unlike studies in suburban Italy and Japan that found *Ae. albopictus* in this environment.[12,13] Instead, we found evidence of the presence of *Ae. albopictus* in corrugated extension spouts (CES), which are plastic or PVC (polyvinyl chloride) corrugated pipes attached to the lower opening of downspouts of rain gutters. They are used to divert rainwater away from house foundations and sensitive landscapes in residential backyards (Figure 1A). The objectives of the present study were to develop qualitative assessments of the occurrence of *Ae. albopictus* in catch basins and CES. To further investigate the importance of CES as a habitat for immature mosquitoes we set to: 1. Identify and quantify mosquito larvae present in CES; 2. Determine which environmental variables may contribute to the presence, absence or number of larvae and pupae; and 3. Ascertain the relative abundance of immature *Ae. albopictus* in CES and in nearby open containers such as tires and buckets.

Figure 1. Examples of corrugated extension spouts (CES) within residential backyards in the New Jersey, USA. All CES within this figure were holding water and contained immature *Aedes albopictus*. (A) Brown CES with a square opening and incorrect pitch. (B) Example of a catch basin located at the curb. (C) and (D) Elongated black CES displaying excessive length (>10 m) and flat pitch that allows water accumulation in the folds. (E) Elongated green CES with flat pitch. (F) View of the inside of a CES viewed from the side originally attached to the rain gutter downspout. The small pockets of water in the folds are a cryptic habitat for immature *A. albopictus*.

Methods

Study sites

We carried out surveys first in two study sites in the City of Trenton, New Jersey, USA during 2012. One site (40°22′N, 74°73′W) called South Olden in previous publications since South Olden Avenue is the largest nearby road, is a densely populated urban area.[14] The site covers approximately 48.6 ha with 1251 parcels that include a house or a commercial structure with surrounding garden or yard, often with vegetation. Houses were primarily two-story residential row homes with median parcel sizes of 210 m^2, and in most cases contained a front and a back yard with a surrounding fence.[15] A second site (40°20′N, 74°72′W, referred to as South Clinton) covered approximately 62.4 ha with 1064 parcels.[15] Houses were mostly single homes with median parcel sizes of 246 m^2 with a front and back yard. In 2013, we selected a third site in Ewing Township (40°14′N, 74°46′W, Ewing). This site was approximately 297 ha with 3716 parcels and most houses were single homes with median parcel sizes of 1971 m^2 with large front and back yards and many trees and shrubs.

Survey protocol

Catch basins are standard urban structures built to collect wastewater, mainly runoff during rain events (Figure 1B), before it reaches the sewers. To prevent large objects and debris from falling in, metal grids and a siphon are located at the entrance. In the city of Trenton catch basins are mostly built into the curb line of the side walk, are covered with a cast iron grate, and the entrances commonly measure 60.9 by 121.9 cm. Using county highway division maps we documented the location of all the catch basins in our sites, then between 30 June and 8 August 2012, we located and inspected them for immature mosquitoes. In addition, the following environmental parameters were recorded: 1. Depth of the catch basin (distance from the top to the bottom); 2. Width of the grate; 3. Length. If the catch basin was holding water, we also recorded water temperature, water depth, total water volume and pH. All catch basins were sampled 10 times each with a dipper designed to go through the catch basin grates (Calico Enterprises, NJ, USA). Any immature mosquitoes found were transferred into 500 ml plastic containers with water and transported live to the laboratory for identification and enumeration.

Between 30 May and 26 June 2012, all CES in the study sites were documented and inspected for immature mosquitoes (Figure 1C–F). Then during August 2012, a time when the abundance of peridomestic mosquitoes commonly peaks in NJ, all CES were carefully examined and the following environmental parameters were recorded: 1. Facing position of the CES opening (predominantly north, south, east or west), 2. Color of the material (black, white, green or brown); 3. Shape of the CES opening (the outflow end away from the house: circle, rectangle, etc. [Figure 1C and F]); 4. Height (the difference between the bottom of the opening and the ground); and 5. Length (distance from downspout to the open end of the CES). If the CES was holding water, during the 2012 surveys, we also recorded water temperature, total water volume and pH. All CES found to be holding water were completely removed from the housing structure and their contents were emptied into a sampling bucket to determine the presence and abundance of mosquito larvae and pupae, and to obtain a measurement of total water volume. Mosquito larvae and pupae were placed in 500 ml containers and transported to the laboratory for identification and enumeration.

To compare the number *Ae. albopictus* larvae and pupae in CES to nearby mosquito-positive containers such as buckets or plant saucers (hereby defined as 'open' containers), we conducted container surveys in parcels with positive CES. Specifically, in South Clinton in 2012 (10 September to 1 October), we examined the first open containers with water detected by a field crew in parcels with positive CES or, if none were found, in immediately adjacent parcels and collected all immature mosquitoes (regardless of species). The same comparative surveys (open vs CES) were repeated in Ewing in 2013, both during the peak season (26 July-12 August) and the late season (l3–18 September). All larvae and pupae were collected and counted; pupae were allowed to emerge as adults and then identified to species and counted. Early instars were reared to third and forth stage larvae for more accurate identification. Large larvae were preserved in 90% ethanol prior to identification.[7]

Statistical analysis

We performed a logistic regression to examine the effect of environmental parameters on the presence or absence of *Ae. albopictus* in CES. The environmental parameters used were: facing position, color and shape of the opening. We performed a multiple regression analysis to assess if water temperature, water volume, pH, height and length were correlated to the number of larvae and pupae present in CES. Logistic regression analyses were also used to determine the association between five environmental variables (facing position, shape of opening, color, height and length) and the occurrence of water. In addition, we examined the co-occurrence of mosquitoes in CES vs open containers. Because we sampled a corrugated extention spout and an open container in the same or in an adjacent parcel, first we performed correlation analyses to examine if the number of larvae and pupae were associated in the two container types over space. When we did not find a correlation, student's t tests were performed to assess differences in number of larvae and pupae in CES and in

Table 1. Summary of catch basin (CB) surveys in New Jersey, USA during 2012 for *Aedes albopictus*

No. sampled	No. of wet CB (%)	No. of dry CB (%)	No. of larvae/ pupae+CB (%)[a]	No. of *Ae. albopictus* larvae/ pupae+CB (%)[a]	Mean water depth in CB (cm)	Mean volume (L)	Min volume (L)	Max volume (L)	Mean temp (°C)	Min temp (°C)	Max temp (°C)	Mean pH	Min pH	Max pH
252	84 (33.4)	168 (66.6)	6 (7.1)	1 (1.2)	15.5	115	5.6	792.8	27.1	23.4	33.1	6.6	5.2	12.2

CB: catch basin; L: litre; Temp: temperature.
[a] Percentages based on wet catch basins.

open containers. Finally, we examined the relationship between container type (CES vs open) and proportion of immature *Ae. albopictus,* as opposed to other species, using a non-parametric Kruskal-Wallis test for each survey. Analyses were performed using SPSS Statistics 21 (IBM, Armonk, NY, USA) and JMP 8.01 (SAS Institute Inc., Cary, NC, USA).

Results

We inspected 252 catch basins, of which 168 were dry (66.6%,168/252) and 84 were holding water (33.4%, 84/252) (Figure 1B). The minimum amount of water in wet basins was approximately 5 litres. Of those catch basins containing water, 6 (7%, 6/84) were positive for immature mosquitoes (Table 1). The most common species collected was *Culex pipiens*. We found one *Ae. albopictus* larvae in one catch basin.

We did not find immature mosquitoes during documentation and inspection of all CES in the study sites in the early season (May-June), therefore those data were not included in Table 2. During the peak season in 2012 (14–21 August) we inspected 220 CES, of which 151 were dry (68.6%, 151/220) and 69 were holding water (31.3%, 69/220) (Figure 1C–F). Of those CES containing water, 36 (52.1%, 36/69) were positive for immature mosquitoes and we collected a total of 1888 specimens (Table 2). The most common species collected was *Ae. albopictus* (99%, 68/69 of the total) followed by only one other species, *Aedes japonicus japonicus* (Theobald) (2%, 1/69). The average number of immature *Ae. albopictus* collected per CES was 52.4±8.2 (SE). Most (72%, 26/360) of mosquito-positive CES had square openings while the remainder had round openings (Table 2). The average water temperature in positive CES was 24.6°C±4.1 with the average pH was 5.3±0.1. None of the five environmental parameters were significantly associated with the number of *Ae. albopictus* in CES. A logistic regression did not indicate a significant association between any of the five environmental parameters measured and the presence of *Ae. albopictus*. However, there was a significant association between presence of water in CES and the length and height of the CES (χ^2=5.2, df=4, p=0.03; χ^2=7.5, df=4, p=0.006, respectively). The average length of dry CES was 103.1±7.5 cm (mean±SE), and average length of CES with water was 149.9±24.8 cm (Table 2).

In the fall of 2012, we surveyed CES and compared the species and number of immature mosquitoes in CES to those in nearby open containers. We sampled a total of 41 positive open containers near 41 positive CES in South Clinton (Table 3). Although *Ae. albopictus* was the only species collected in CES during 2012, we also collected *Ae. j. japonicus*, *Aedes triseriatus* (Say), *Culex pipiens* L. and *Culex restuans* Theobald, in addition to *Ae. albopictus*, from open containers. We did not detect significant differences in total number of larvae and pupae between CES and open containers. In 2013, we inspected an additional 40 positive open containers near 40 positive CES during the peak season (July-August). We found one CES with *Ae. j. japonicus,* while all other samples contained only *Ae. albopictus* (Table 3.). In contrast, we collected *Ae. albopictus*, *Aedes atropalpus* (Coquillett), *Ae. j. japonicus*, *Cx. pipiens*, *Psorophora ferox* (von Humboldt) and *Toxorhynchites rutilus septentrionalis* (Dyar and Knab) from open containers (Table 3). During this survey, the number of *Ae. albopictus* in CES was significantly higher than those in open containers (Table 3).

Table 2. Summary of corrugated extension spout (CES) surveys for *Aedes albopictus* in New Jersey, USA during 2012

	No. sampled	No. of wet CES (%)	No. of dry CES (%)	No. of larvae/ pupae+CES (%)[a]	No. with circle opening (%)	No. with square opening (%)	Mean length (cm)	Mean height (cm)	Mean volume (ml)	Min volume (ml)	Max volume (ml)	Mean temp (°C)	Min temp (°C)	Max temp (°C)	Mean pH	Min pH	Max pH
Dry and wet CES	220	69 (31.3)	153 (69.5)	36 (16.4)	51 (23.2)	169 (76.8)	117±9	21±2.6	NA[b]	NA	NA	NA	NA	NA	NA	NA	NA
Mosquito positive CES	36	36	0	36	10 (27.7)	26 (72.2)	173±46	13±2.4	1344±549	100	20 000	24.5±0.4	21.1	30.8	5.3±0.1	4.3	6.5
CES holding water[c]	69	69	0	36 (52.1)	15 (21.7)	54 (78.3)	149±25	11±1.6	890±636	50	20 000	25.1±0.3	20.8	31.2	5.6±0.1	3.4	8
CES facing North	84	27 (32.1)	56 (67.9)	14 (51.8)	21 (77.8)	63 (54.6)	105.5±4	6±5.1	574±128	50	2 500	24.7±0.5	20.8	29	5.6±0.12	3.4	6.8
CES facing South	88	21 (23.8)	67 (76.2)	11 (52.3)	16 (9.5)	72 (90.5)	86±8	13±3.5	660±192	50	3000	25.5±0.7	21.9	30.8	5.8±0.2	4.3	7.9
CES facing West	21	10 (47.6)	11 (52.4)	8 (80)	7 (20)	21 (80)	308±157	2±2.7	2600±194	100	20 000	24.7±0.8	22.3	29.2	5.3±0.3	4.2	6.2
CES facing East	28	11 (39.2)	17 (60.8)	3 (27.3)	7 (45.4)	13 (54.6)	386±264	20±5.1	454±639.8	100	1500	25.5±0.9	21.8	31.2	5.6±0.3	3.5	7.2

CES: corrugated extension spout; NA: not applicable; Temp: temperature.

[a] These CES includes both mosquito immature positive and negative samplings.

[b] Combination of dry and wet container for volume, temperature and pH will be misleading, therefore they were not presented in the table.

[c] Percentages based on wet CES.

Table 3. Summary of open and corrugated extension spout (CES) container larval and pupal surveys for *Aedes albopictus*, New Jersey, USA

				No. of *Ae. albopictus*			
Year	Container type	Species composition (%)[a]	No. of containers	Min	Max	Mean±SE	p-value[b]
2012 LS	Open container	*Ae. albopictus*, *Ae. j. japonicus*, *Ae. triseriatus*, *Cx. p. pipiens*, *Cx. restuans*	41	0	204	47.4±7.9	NS
	CES	*Ae. albopictus*	41	2	162	37.1±6.1	
2013 PS	Open container	*Ae. albopictus*, *Ae. atropalpus*, *Ae. j. japonicus*, *Ps. ferox*, *Tx. r. septentrionalis*	40	0	54	9.4±1.9	0.047
	CES	*Ae. albopictus*, *Ae. j. japonicus* (5)	40	1	107	16.8±3.8	
2013 LS	Open container	*Ae. albopictus*, *Ae. j. japonicus*, *Ae. triseriatus*, *Tx. r.septentrionalis*	46	0	57	10.3±1.8	NS
	CES	*Ae. albopictus*, *Ae. j. japonicus* (10.8), *Cx. p. pipiens* (2.17), *Tx. r. septentrionalis* (4.3)	46	1	82	14.5±2.4	

LS: late season; NS: not significant; PS: peak season; SE: standard error.

[a] Percentages are indicated in the table for the species other than *Ae. albopictus* represented in CESs.

[b] p-value calculated using Student's t test.

Finally, a total of 46 open containers near positive CES were inspected during late-season in 2013. Surprisingly, during these surveys, we collected *Ae. albopictus*, *Ae. j. japonicus*, *Cx. pipiens* and *Tx. r. septentrionalis* from CES, but like in the previous two surveys, *Ae. albopictus* was the overwhelmingly predominant species (Table 3). Open containers yielded a more even mix of *Ae. albopictus*, *Ae. j. japonicus*, *Ae. triseriatus* and *Tx. r. septentrionalis* (Table 3). Again, during this fall survey we did not detect significant differences between open containers and CES for the total number of *Ae. albopictus* larvae and pupae. However, for all three surveys Kruskal-Wallis analysis showed a significant relationship between container type and proportion of *Ae. albopictus* (χ^2=17.9, df=1, p=0.001; χ^2=25.2, df=1, p=0.001; χ^2=10.6, df=1, p=0.001, respectively) indicating that the proportion of mosquitoes that were *Ae. albopictus* was always significantly higher in CES than in open containers.

Discussion

Our study shows that immature *Ae. albopictus* do not utilize catch basins in our region but instead utilize CES. Because *C. pipiens,* the primary vector of West Nile virus, is known to thrive in catch basins, in Trenton, they are routinely treated with insecticides.[16] On its own this fact may explain the relative low occurrence of immature mosquitoes in catch basins in our sites,[16] but as we have found before,[7] immature *Ae. albopictus* were still significantly less common in catch basins than immature *Culex*.

Both the high relative abundance of *Ae. albopictus* in CES and the fact that they were overwhelmingly the primary mosquito species there were surprising. Other studies have reported the common co-occurrence of *Ae. albopictus* with other container mosquitoes.[7,8,17,18] Indeed, as in previous studies examining open containers, we too have documented the co-existence of *Ae. albopictus* with *Ae. j. japonicus*, *Cx. pipiens* or *Cx. restuans*.[7] However, in this study we found *Ae. albopictus* almost exclusively in CES and hardly any other species, suggesting that *Ae. albopictus* may be uniquely able to exploit the CES. A limitation of our sudy was that, as mentioned, we measured total water volumes by emptying the entire CES into a bucket so the volume of each isolated compartment was not measured. Although a clogged CES can sustain a large volume of water, visual inspections revealed water inside a CES was distributed into small volumes in each of the accordion folds (Figure 1F). Supporting our findings, previous studies clearly showed that *Ae. albopictus* is more likely to be found in smaller volumes of water, whereas *Culex* species were more likely to be found in larger volumes such as in catch basins and swimming pools.[7,8,19]

During surveys in Réunion Island researchers did not observe any association between exposure, water quality, presence of organic matter, season, or presence of other species and the number of *Ae. albopictus*.[20] They attributed their findings to the fact that *Ae. albopictus* populations in Réunion might not have a history of adaptation to a particular type of larval habitat because they have been relatively recently introduced.[20] However, Bartlett-Healy et al. in studies also developed in NJ, found that *Ae. albopictus* and *Ae. j. japonicus* pupae (as opposed to *Culex*) were mostly found in containers with cooler temperatures, which in our area are predominantly located in the southwestern quadrants of parcels (southeastern quadrants had containers with the highest temperatures). In our studies focusing on CES we found a random distribution of *Ae. albopictus* among CES facing different directions and with different color, opening shape, water temperature, water volume, height and length. However, instead of lack of preference our results may indicate instead that, regardless of facing location, opening shape etc., CES maintain lower and more stable water temperatures because they create shade and a semi-enclosed environment with lower evaporation rates.[21] These lowered evaporation rates may reduce the likelihood that the small volumes of water in each of the CES folds will dry during the hot summer and allow the development of *Ae. albopictus*.[21] A related finding is that longer CES and those close to the ground are more likely to contain pockets of water. Longer CES are often flat over the ground, allowing water to accumulate in the grooves. Pooled water in the CES is a requirement for the presence of immature mosquitos and clearly the length and pitch of the CES are characteristics that can be easily controlled.

The overwhelming representation of *Ae. albopictus* in CES to the detriment of other species, which were common in the nearby open containers, may be the result of the small volumes of water involved, as well as a combination of differential oviposition and differential mortality. Unlike *Culex,* female *Ae. albopictus* skip oviposit,[22] and they may be more likely to choose to lay some of their eggs in the small water volumes characteristic of CES. Once present, the known competitiveness of larval *Ae. albopictus* may reduce the likelihood that immature of other species will survive.[23] In addition, although no differences were found in the number of immature *Ae. albopictus* in open containers and nearby CES during the fall surveys in 2012 or 2013, the number of immature *Ae. albopictus* was higher in CES during the summer, supporting our assertion that CES may provide a protected environment leading to high productivity.

In conclusion, from an applied standpoint, the use of CES by immature *Ae. albopictus* raises new concerns for management of this species, especially regarding any other hidden pockets of water that they may be exploiting. Of note, we found that CES are relatively common in residential backyards in Trenton, NJ, but are mostly overlooked by mosquito control professionals. Although area-wide truck-mounted applications of larvicides may be useful as an alternative to reduce the time and effort needed to apply larvicides in backyards,[24] penetration into cryptic habitats such as CES would most likely be very limited. Furthermore, even if mosquito control programs have the ability to apply larvicides to each CES, rainwater flushing through the downspout will impact and possibly nullify the residual effects of pesticides, requiring the need for reapplication after each rain event. Importantly, correct CES installation can prevent them from holding water and therefore from being a source of mosquitoes. In addition, physical measures such as drilling holes for increased drainage or emptying the CES assiduously, should lead to the reduced usage of these habitats by *Ae. albopictus*. Our results suggest that unless they are specifically targeted, even if extensive source reduction of open containers is implemented, CES will continue to be an important source of adult *Ae. albopictus* production in residential properties. A limitation of this study is that all our study sites were located in or near the city of Trenton in New Jersey, USA, and therefore further research should examine the prevalence of mosquitoes in CES in other geographical areas and levels of urban development in order to ascertain the relevance and widespread significance of our findings.

Authors'contributions: IU, AF, NI and DMF conceived the study and designed the protocol; IU and NI conducted data collection and entry; IU and DMF analyzed the data; IU, AF, NI and DMF drafted the manuscript. All authors read and approved the final manuscript. IU is the guarantor of the paper.

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Competing interests: None declared.

Ethical approval: All surveys and collections were made by county mosquito control professionals. Private yards and other private lands were entered only after oral and/or written consent was obtained from the individual owners/residents. No specific permits were required for the collection of mosquitoes and these studies did not involve endangered or protected species. Ethical Committee approval was not required for this study.

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