Habitat Characteristics For Immature Stages of Aedes aegypti In Zanzibar City, Tanzania

Saleh, Fatma; Kitau, Jovin; Konradsen, Flemming; Alifrangis, Michael; Lin, Chia-hsien; Juma, Salim; Mchenga, Salum Seif; Saadaty, Thabit; Schiøler, Karin Linda

Published in:
Journal of the American Mosquito Control Association

DOI:
10.2987/17-6709.1

Publication date:
2018

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Saleh, F., Kitau, J., Konradsen, F., Alifrangis, M., Lin, C., Juma, S., ... Schiøler, K. L. (2018). Habitat Characteristics For Immature Stages of Aedes aegypti In Zanzibar City, Tanzania. *Journal of the American Mosquito Control Association*, *34*(3), 190-200. https://doi.org/10.2987/17-6709.1

Journal of the American Mosquito Control Association, 34(3):190–200, 2018

HABITAT CHARACTERISTICS FOR IMMATURE STAGES OF *AEDES AEGYPTI* IN ZANZIBAR CITY, TANZANIA

FATMA SALEH,[1] JOVIN KITAU,[2] FLEMMING KONRADSEN,[3] MICHAEL ALIFRANGIS,[4] CHIA-HSIEN LIN,[3] SALIM JUMA,[5] SALUM SEIF MCHENGA,[6] THABIT SAADATY[1] AND KARIN LINDA SCHIØLER[3]

ABSTRACT. *Aedes aegypti* is the main vector for dengue, chikungunya, yellow fever, Zika, and other arboviruses of public health importance. The presence of *Ae. aegypti* has never been systematically assessed in Zanzibar, including its preferred larval habitats. In 2016 we conducted a cross-sectional entomological survey to describe the preferred larval habitats of *Ae. aegypti* in Zanzibar City, the main urban area of the Zanzibar archipelago. The surveys for container habitats were conducted for a 17-wk period beginning in January 2016. Immature stages (larvae and pupae) were collected, reared to adulthood, and identified to species. The positive and potential habitats were categorized on the basis of physical, biological, and chemical parameters. A total of 200 samples were collected, of which 124 (62.0%) were positive for immature stages of mosquitoes and 114 (92%) for *Ae. aegypti* larvae and pupae. Presence of vegetation (odds ratio [OR] = 2.11, 95% confidence interval [CI] = 1.19–3.74), organic matter (OR = 2.37, 95% CI = 1.21–4.60), inorganic matter (OR = 1.78, 95% CI = 1.01–3.13), and sun exposure (OR = 2.34, 95% CI = 1.24–4.36) were all significantly associated with the presence of immature stages of *Ae. aegypti*, suggesting that these conditions promote colonization of containers. Plastic containers supported 64% of the immature stages and produced approximately 50% of the pupae. Although immature counts were the highest in discarded artifacts, higher pupal counts were found in domestic water storage containers. Our observations suggest that effective control of *Ae. aegypti* in Zanzibar City must include improved solid waste management (collection and proper disposal of potential container habitats) and reliable supply of domestic water to minimize water-storing practices that provide larval habitats for *Ae. aegypti*.

KEY WORDS *Aedes aegypti*, habitat characteristics, Tanzania, Zanzibar

INTRODUCTION

In sub-Saharan Africa, *Aedes* (*Stegomyia*) *aegypti* (L.) is the main vector for dengue, chikungunya, and urban yellow fever viruses (Harbach 2013). Several dengue and chikungunya outbreaks have been reported in this region in recent decades (Pastorino et al. 2004, Ratsitorahina et al. 2008, Leroy et al. 2009, Amarasinghe et al. 2011). In Tanzania, dengue outbreaks have been reported in 2010, 2012, 2013, and 2014, of which the latter two were major outbreaks with epicenters in the Dar es Salaam region (Mboera et al. 2016). In 2014 alone, there were >2,000 reported cases and 4 deaths (WHO 2014). To date, there has been no reported dengue outbreak in Zanzibar; however, a study conducted at the main hospital, of Mnazi Mmoja, in 2013, found that almost 7% (10/149) of nonmalaria febrile patients attending the outpatient clinic were infected with dengue virus (Ali, unpublished data). Furthermore, dengue immunoglobulin G antibodies were detected in 50.6% of 500 healthy blood donors at Zanzibar National Blood Transfusion Services in 2011 (Vairo et al. 2014). These findings suggest that dengue transmission is endemic in Zanzibar and that symptomatic dengue cases may be misdiagnosed and incorrectly treated as malaria or other febrile illnesses as also suggested in other African settings (Amarasinghe et al. 2011).

A hospital-based study, in the northern part of mainland Tanzania in 2012, identified acute chikungunya virus (CHIKV) infection in 7.9% (55/700) of febrile inpatients, mostly infants and children. In addition, a community-based study completed in 2014, in northeastern Tanzania, reported acute CHIKV infection and presence of CHIKV immunoglobulin M antibodies in 4.2% (11/263) and 12.9% (49/381) of febrile participants, respectively (Kajeguka et al. 2016). These studies suggest that CHIKV is an important but unrecognized cause of febrile illness in the country (Hertz et al. 2012). However, to date there have been no reported studies on chikungunya or official reports of CHIKV infection in Zanzibar.

Yellow fever is not endemic in Tanzania (WHO 2017a), yet the recent outbreaks in neighboring countries, Uganda and Democratic Republic of Congo (WHO 2017b), have raised concerns that future outbreaks could expand into Tanzania. The

[1] Department of Allied Health Sciences, School of Health and Medical Sciences, The State University of Zanzibar, PO Box 1898, Zanzibar, Tanzania.

[2] Department of Parasitology, Kilimanjaro Christian Medical University College, Moshi, Tanzania.

[3] Global Health Section, Department of Public Health, University of Copenhagen, Copenhagen, Denmark.

[4] Center for Medical Parasitology, Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark.

[5] Department of Natural Sciences, The State University of Zanzibar, Zanzibar, Tanzania.

[6] School of Health and Medical Sciences, Department of Medical Laboratory, The State University of Zanzibar, Zanzibar, Tanzania.

risk is considered to be high given substantial regional travels, a largely nonimmunized human population, and the abundance of *Ae. aegypti* in Tanzania (Ellis and Barrett 2008, Mboera et al. 2016).

Until recently, information on *Aedes* mosquito ecology in Tanzania was limited to habitat identification surveys carried out in several areas of Dar es Salaam city in the early 1970s (Trpis 1972, 1973; Rao et al. 1973). At the time *Ae. aegypti* was found in artificial containers, in particular in discarded artifacts (tin cans, broken bottles, mud pots), automobile parts (car tires, motor parts), and indoor water containers (metal tanks, drums, barrels, and water pots), but also in natural habitats such as coconut shells, coral rock holes, snail shells, and tree holes (Trpis 1972, 1973; Rao et al. 1973). More recently, used tires were reported as the most important larval habitats for *Ae. aegypti* in Dar es Salaam, followed by domestic water storage containers and natural habitats (Philbert and Ijumba 2013, Mathias et al. 2017). Likewise, Mboera and colleagues discovered that used tires, discarded plastic containers, and flowerpots were the most important larval habitats for *Ae. aegypti* in Dar es Salaam (Mboera et al. 2016).

While there is recent, though limited, literature on *Ae. aegypti* larval habitats in Tanzania mainland, only 1 entomological survey has been conducted in Zanzibar. This unpublished study by Zanzibar Malaria Elimination Program (ZAMEP) was conducted in rural areas of Unguja Island in 2014 and confirmed the existence of *Aedes* mosquitoes on the island (ZAMEP, unpublished data). However, the survey did not identify the species or the habitat characteristics, nor did it investigate for *Aedes* sp. in the urban areas of Zanzibar.

Current vector control activities in Zanzibar focus on malaria vectors only, and are largely limited to indoor residual spraying and distribution of long-lasting insecticide-treated nets (LLINs). Both methods are hampered by the development of insecticide resistance (Haji et al. 2013), while evidence of LLINs' effect against *Ae. aegypti* is limited. Thus, in order to prevent dengue and other *Ae. aegypti*–transmitted diseases, it is important to identify the most productive *Ae. aegypti* larval habitats and target these to reduce adult vector abundance. The aim of this study was to identify the preferred larval habitats of *Ae. aegypti* in Zanzibar City.

MATERIALS AND METHODS

Study sites

This study was conducted on the tropical island of Unguja, in the Tanzanian archipelago of Zanzibar off the coast of East Africa. Zanzibar archipelago comprises Unguja and Pemba islands. During the study period (January–June 2016), Unguja experienced minimum and maximum average temperatures of 25.1°C and 32.5°C, respectively. The mean relative humidity was between 61% and 92% with total rainfall of 995.8 mm (Tanzania Meteorological Agency, unpublished data).

The study was carried out in the *Shehia*s of Malindi and Kikwajuni located in Zanzibar City along the western coast of Unguja Island, Zanzibar. The city has a total of 45 *Shehia*s. *Shehia* is the lowest government administrative unit that demarcates the district, and the leader of this unit is called *Sheha*. The sites were purposely selected to represent the different urban characteristics of Zanzibar City. Malindi is situated at the heart of Stone Town (6°09′32.6″S, 39°11′36.8″E) (Fig. 1) and has a population of 3,204 (The United Republic of Tanzania 2013). Stone Town constitutes the historical core of Zanzibar City and is the main urban and commercial center of the archipelago. Stone Town was declared a World Heritage Site by the United Nations Educational, Scientific and Cultural Organization in 2000 due to its unique 19th-century townscape. Houses in Stone Town are mainly large blocks made of concrete, stones, or timber, and there are a high number of tourist hotels, public buildings (mosques, hospitals, schools, markets), and commercially operated compounds (restaurants, shops, garages) within the city limits. Kikwajuni consists of 2 subareas, namely upper and lower Kikwajuni. It is located at the southern border of Stone Town (6°10′03.8″S, 39°11′42.1″E) and has a population of 4,665 inhabitants (The United Republic of Tanzania 2013) (Fig. 1). It is a planned residential area with townhouses and a few blocks made of concrete and stones, and there are a number of gardens and open parks. There are also a large number of trees in and around its perimeters. Both areas (Malindi and Kikwajuni) are poorly serviced in terms of water supply coverage and solid waste collection.

Sampling procedures

A cross-sectional, entomological survey to identify and characterize habitats of immature *Ae. aegypti* was conducted from January to the 1st week of June 2016. The immature samples were collected weekly over a period of 17 wk during dry (January–February 2016) and wet (April to 1st week of June 2016) seasons. The research team carefully screened the study area for potential habitats, including indoor and outdoor spaces of residential, public (e.g., mosques and parks), and commercial entities (e.g., guest houses, hotels, garages, and markets). Potential habitats were defined as any uncovered or incompletely covered water container of all sizes likely to hold stagnant water for >3 days (Nyamah et al. 2010). Once identified, water containers were examined for the presence of immature stages and the water then examined for gross physical and biological features, as well as chemical parameters as described below. A water container containing at

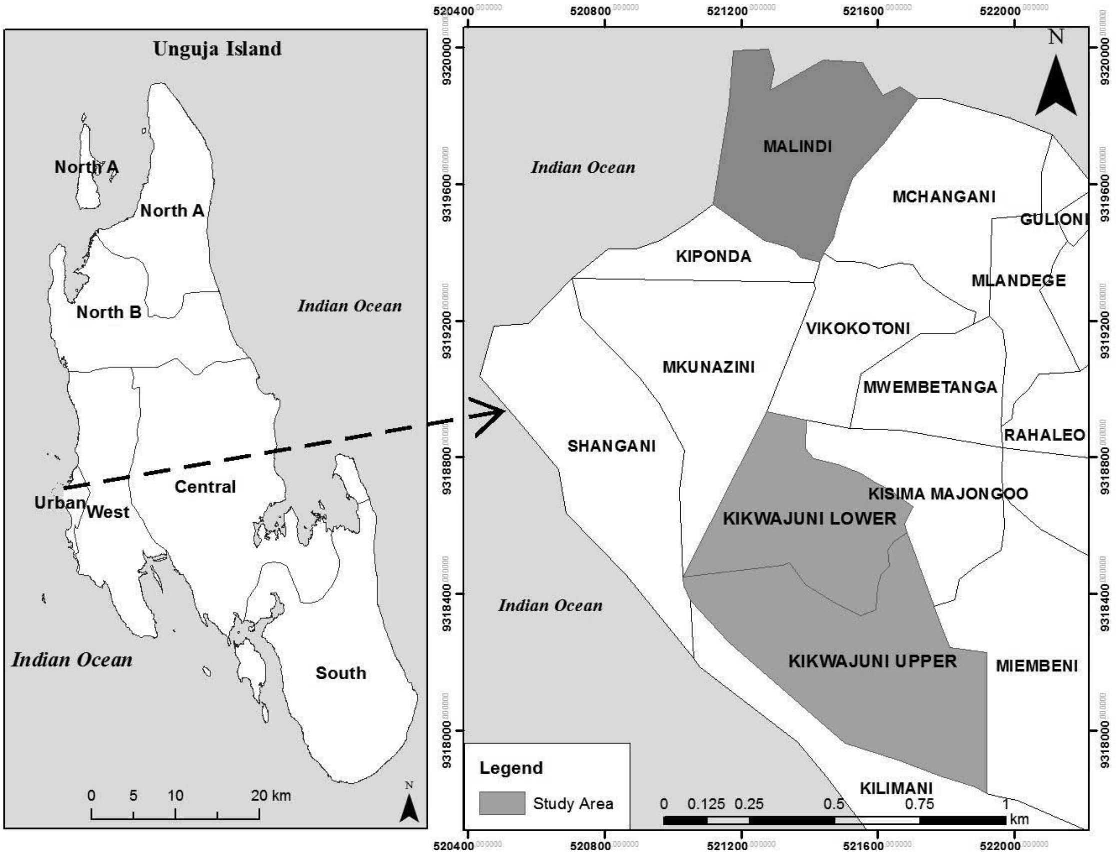

Fig. 1. A map showing study areas within Zanzibar City.

least 1 larva or pupa was considered positive. Productivity denoted the abundance of pupae in a given type of habitat (WHO 2011).

Collection of immature stages

The immature stages were collected by standard dipping (300-ml dipper) method for water containers with wide openings holding >1 liter of water, and pipetting (5-ml plastic pipette) was used for smaller containers such as coconut shells, tree holes, discarded tins, and tires (Mboera et al. 2016). Movable or manageably sized containers positive for immature stages were emptied and left overturned; in the case of functional containers, permission was obtained from the owner/occupier of the given premises. For each positive habitat (artificial and natural), samples of immature stages were collected, placed in labeled glass vials with loose screw caps, then transported in cool boxes to the laboratory at the State University of Zanzibar.

Rearing and species identification

At the laboratory, the collected immature mosquitoes were counted and placed in plastic petri dishes filled with daily-refreshed water and kept in netted cages according to their respective sample identification for rearing and species identification. On emergence, adult mosquitoes were fed with 10% glucose solution soaked in cotton balls that were plugged on a 30-ml vial placed in each cage and changed daily as previously described (Imam et al. 2014). About 75% of the reared immature mosquitoes sampled in the field emerged as adults. The adults were aspirated from cages using standard mouth aspirator and transferred to paper cups covered with mosquito nets, killed by freezing, and then sorted into genera. *Aedes aegypti* adults were identified using a dissecting microscope and the morphological identification key by Rueda (2004), and the mosquito identification guide by University of Florida Medical Entomology Laboratory (date unknown). All identified mosquito genera and *Ae. aegypti* from each positive habitat were counted and recorded.

Characterization of larval habitats

The larval habitats were categorized by location, function, material, water volume, and environment. The location was divided into indoor and outdoor spaces. Indoor space was defined as any part of the house or building covered by a roof (intradomestic space) (WHO 2011), including the main house and other buildings within the surrounding compound. Outdoor habitats were defined as containers located in open areas such as housing yard or peri-domestic area, pedestrian streets, roads, unmanaged spaces, and public spaces including parks and playgrounds.

The function was divided into 3 categories:

a) Discarded artifacts (e.g., tires, car parts, tins, cans, bottles, broken pots, cups, buckets, and similar containers).
b) Water containers for domestic purposes (e.g., drums, buckets, basins, tanks made of plastic, metal, or cement) such as basic household functions, pets drinking/bathing, chicken/ducks drinking, and drains.
c) Other water containers including natural habitats (e.g., coconut shells, tree holes, and puddles), ornamental containers such as flowerpots and vases, and water containers for gardening and construction purposes.

The materials were classified into 5 categories, namely plastic, ceramic and cement, metal, rubber (tire), and natural habitats. The water volume was divided into low (<5 liter), medium (5–20 liter), and high (>20 liter) volumes. The environment was categorized into housing compound (the area within buildings and surrounding compound), unmanaged public spaces (e.g., pedestrian streets/roads and other open spaces with no specific ownership), and managed public spaces (e.g., parks and playgrounds).

Measurement of water quality

The physical, biological, and chemical characteristics of the larval habitats were examined on-site at the time of entomological survey. The physical parameters included visually observed light conditions (duration of sun exposure per day as either less or more than 50% exposed), organic or inorganic mater (presence or absence), water temperature, and turbidity. The biological parameters included macrofauna (presence or absence), and vegetation in the form of algae, floating or submerged plants, short or tall plants in and around habitats (presence or absence). The chemical parameters comprised pH, salinity, conductivity, dissolved oxygen, and total hardness. The chemical parameters (except for total hardness) and temperature were measured using the HACH HQ40d portable multiparameter checker with respective probes according to the general instructions of the manufacturer (HACH Company, Loveland, CO). Turbidity and total hardness were measured using HACH 2100Q portable turbidity meter (HACH Company) and HACH total hardness test kit Model HA-71A (HACH Company), respectively.

Data analysis

Statistical analyses were conducted for presence and abundance of immature stages. Relationships between the presence of immature *Ae. aegypti* and season, location, environment, vegetation, macrofauna, sun exposure, and water content were estimated using odds ratios (ORs) with 95% confidence intervals (CIs). To test for equality of mean number of immature stages among materials, functions, and water volume levels, 1-way analysis of variance (ANOVA) was applied. Where significant associations were observed, the Games-Howell post hoc test was applied to determine the means that were significantly different from each other. Student's *t*-test (for normal distribution) and Mann–Whitney *U*-test (for nonnormal distribution) were used to compare the physico-chemical variables (temperature, pH, turbidity, total hardness, salinity, conductivity, and dissolved oxygen) between positive and negative water receptacles. Data were analyzed using SPSS 15.0 for Windows Evaluation Version (SPSS, Cary, NC) using a significance level of 0.05.

Ethical considerations

The study protocol was approved by the Research Committee of the State University of Zanzibar by the letter dated January 11, 2016, with reference number SUZA/RPC/RP/3. Permission to carry out the study was sought from community leaders, and informed consent was verbally sought from the heads of households before entrance for inspection.

RESULTS

A total of 200 samples of water containers were identified in the 2 study sites. Given the relatively low number of samples and as there were no major differences in sample distribution (Malindi = 94 and Kikwajuni = 106), location, and type of habitats in the 2 sites, the results are based on pooled analysis of samples.

Number of water containers by function and type of materials

Of the 200 containers, 80 (40%) constituted containers for domestic use, 74 (37%) were discarded artifacts, and 46 (23%) were other kinds of habitats (natural habitats, ornamental containers, gardening, and construction site containers). In terms of materials, 109 (54.5%) were made of plastic, 32 (16%) ceramic/cement, 25 (12.5%) metal, 23 (11.5%) rubber (tires), and 11 (5.5%) were natural habitats.

Aedes aegypti–positive containers by function of container and type of material

In total, 114 of 200 (57.0%) samples were positive for immature stages of *Ae. aegypti*. Of the 114

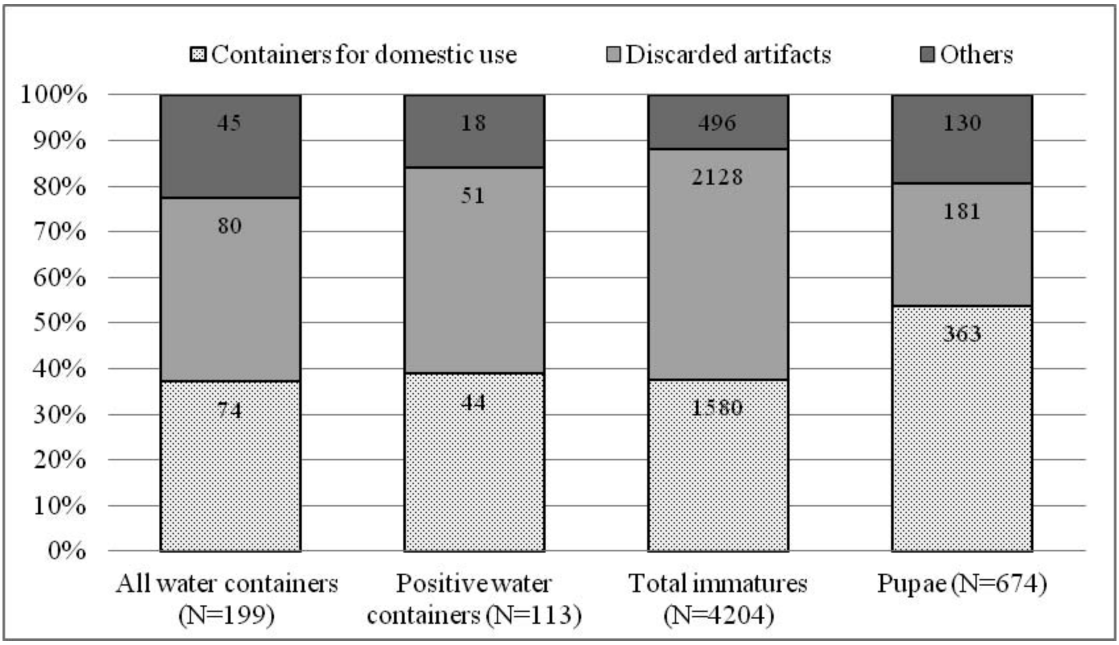

Fig. 2. Distribution and abundance of *Aedes aegypti* immature stages and pupae by function of water container.

positive samples, 51 (44.7%), 44 (38.6%), and 19 (16.7%) were discarded artifacts, domestic water containers, and "others," respectively. Almost all houses visited had 1 or more types of water storage container. The most common ones included plastic containers, metal tanks/buckets, and concrete troughs. As for materials, 62 out of 114 positive samples (54.8%) were plastic, 17 (14.9%) were ceramic/cement, 15 (13.0%) were metal, 15 (13.0%) were rubber (tires), and 5 (4.3%) were natural habitats.

Types of mosquitoes collected

Of the 200 samples, 124 (62.0%) were positive for immature mosquitoes. Of these, 90 (72.6%) samples had *Aedes* sp. only, 10 (8.1%) samples had *Culex* only, while *Aedes* sp. and *Culex* sp. coexisted in 24 (19.4%) samples. In total, 114 (92%) of the positive habitats contained immature stages of *Ae. aegypti*. Notably, large numbers of *Culex* sp. and *Ae. aegypti* immature stages were found cohabiting in 1 large underground water trough (>1,000 liters) at a construction site. This sample was identified as an extreme outlier and was excluded from the immature count. The included positive samples ($N = 113$) consisted of 4,733 immature mosquitoes, including 4,204 (88.8%) *Aedes* sp. and 529 (11.2%) *Culex* sp. Of the 4,204 immature *Aedes* sp. collected, 674 were pupae. In addition, uncountable numbers of immature *Culex* sp. were found in manholes and septic tanks. However, immature *Culex* sp. were not further investigated in this study. Furthermore, this study did not find any immature stages of *Ae. albopictus* (Skuse) or *Anopheles* sp. This paper focused only on *Ae. aegypti*.

Aedes aegypti immature counts by container function and type of material

Of the total 4,204 *Aedes* immature stages, 50.6% were identified in discarded artifacts, 37.6% in domestic water receptacles, and 11.8% in "others." However, for the 674 pupae specifically, a different distribution was observed, as 53.9%, 26.9%, and 19.3% were identified in domestic water receptacles, discarded artifacts, and "others," respectively (Fig. 2). The total immature stages for the materials were as follows: 64.0% were found in plastic, 11.5% in ceramic/cement, 11.2% in metal, 11.2% in rubber (tires), and 2.1% in natural habitats. The pupae counts were 48.4% in plastic containers, 24.5% in metal containers, 18.8% in ceramic/cement containers, 5.8% in rubber (tires), and 2.5% in natural habitats (Fig. 3).

Associations between immature stages and container function, material, and water volume

The ANOVA test showed a statistically significant difference ($P < 0.05$) in mean numbers of immature *Ae. aegypti* among the 3 functions ($F = 4.34$, $P = 0.015$) and among the 3 water volume categories ($F = 5.20$, $P = 0.008$). Games-Howell post hoc test further indicated that the mean number of immature *Ae. aegypti* obtained from discarded artifacts (mean = 26.60, SD = 36.35) was significantly higher than the category "others" (mean = 11.02, SD = 20.46) while

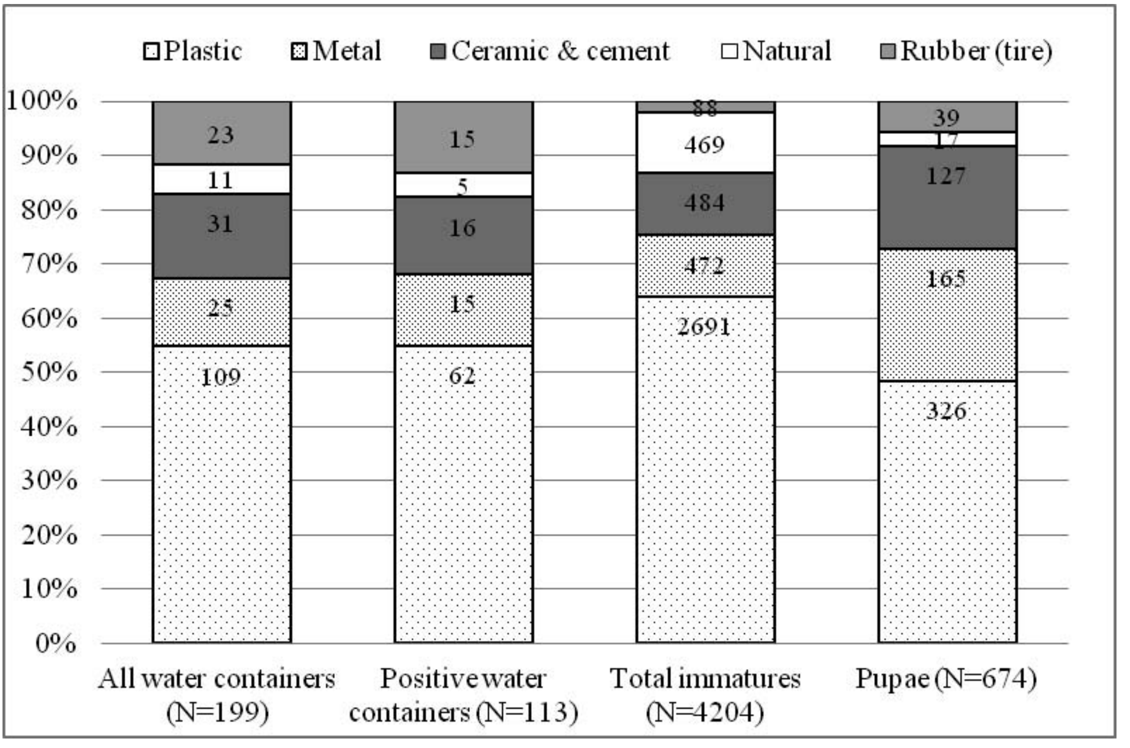

Fig. 3. Distribution and abundance of *Aedes aegypti* immature stages and pupae by type of water container materials.

the mean number of immature *Ae. aegypti* in low-volume (<5 liter) was significantly lower than the medium-volume (5–20 liter), with mean = 14.94 (SD = 22.87) and mean = 44.12 (SD = 57.81), respectively. There was statistically no significant difference between the observed means for the 5 container material types.

Associations between *Ae. aegypti*–positive samples and season, location, biological, and physico-chemical parameters

Almost twice as many samples were observed in the wet season ($N = 133$; 80 positive, 53 negative) as compared to the dry season ($N = 67$; 34 positive, 33 negative) (Table 1). However, the odds of positive samples were not significantly different between seasons (OR = 1.47, 95% CI = 0.81–2.64). The same pattern was observed for location, where the number of samples collected outdoors ($N = 131$; 73 positive, 58 negative) were higher than those collected indoors ($N = 69$; 41 positive, 28 negative), yet the odds of finding positive samples did not differ significantly between the 2 locations (OR = 1.16, 95% CI = 0.64–2.10).

Of the 8 physical and biological parameters examined (Table 1) for the presence or absence of immature *Ae. aegypti*, 4 were statistically significant, including vegetation (OR = 2.11, 95% CI = 1.19–3.75), <50% sunlight exposure per day (OR = 2.34, 95% CI = 1.24–4.36), organic content (OR = 2.37, 95% CI = 1.20–4.60), and inorganic content (OR = 1.78, 95% CI = 1.01–3.13) (Table 1). Of the physico-chemical parameters (temperature, turbidity, pH, salinity, conductivity, dissolved oxygen, and total hardness) tested for 92.0% of the samples, there were no significant differences, in terms of mean or median measures for the 7 parameters, between positive and negative water receptacles. However, temperature (°C) ($t = 1.76$, $P = 0.080$) and salinity (parts per thousand [ppt]) (U-value = 3,454.00, $P = 0.060$) were considered to be borderline statistically significant (Fig. 4).

DISCUSSION

We report here on the abundance, and preferred habitats of *Ae. aegypti* in the city of Zanzibar. To the best of our knowledge, this is the 1st systematic study of the presence of *Ae. aegypti* in the archipelago. Similar studies in Dar es Salaam region have recently demonstrated the presence of *Ae. aegypti* in urban areas of Tanzania (Philbert and Ijumba 2013, Mboera et al. 2016). The occurrence of *Ae. aegypti* in these areas was attributed to the existence of a large variety of water-holding appliances favorable to container-inhabiting mosquitoes. In this study, we identified most habitats of immature *Ae. aegypti* as manmade, with only a few natural habitats observed. Among the most commonly observed habitats were plastic

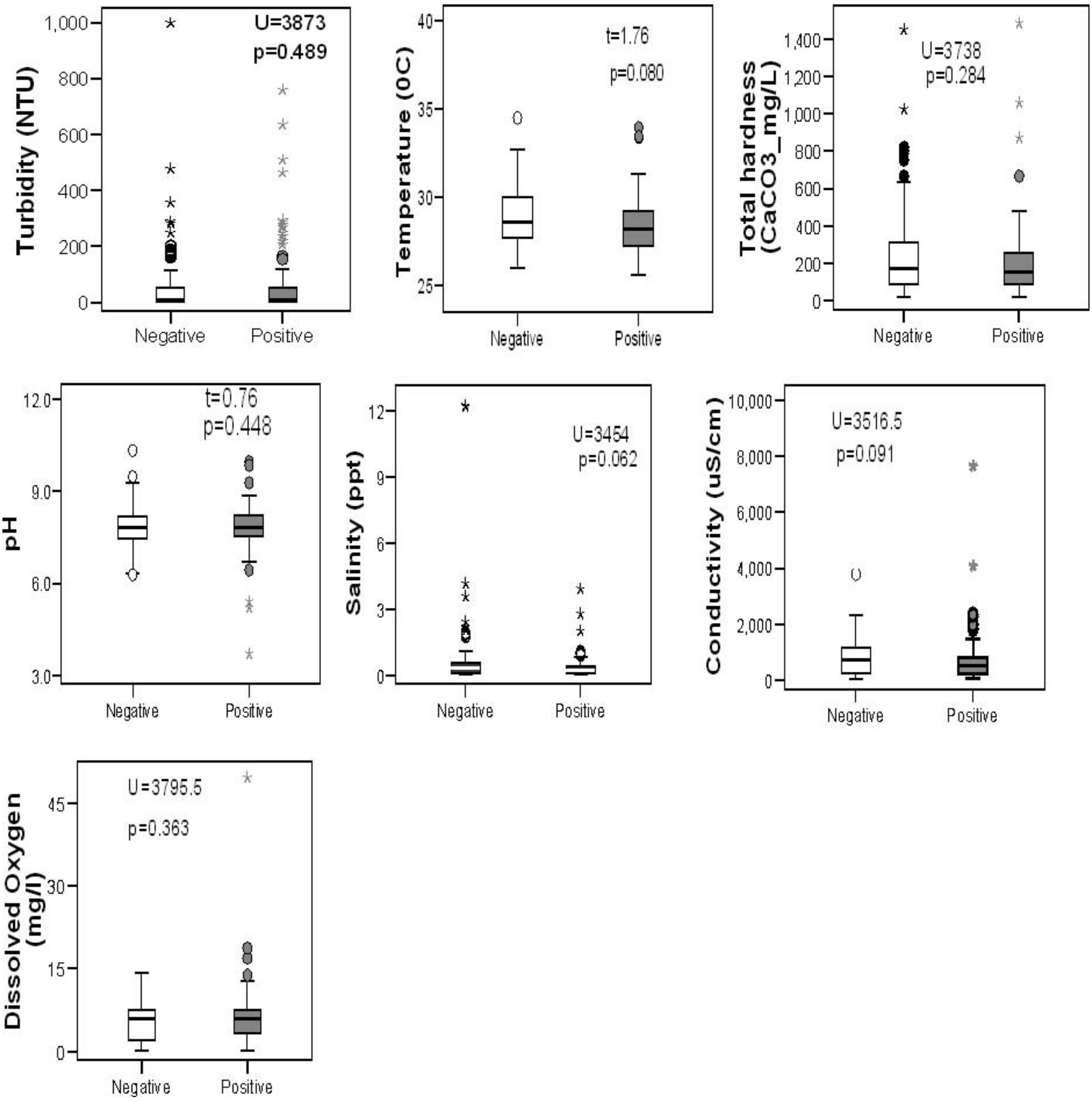

Fig. 4. Differences between water containers positive or negative for immature *Aedes aegypti* in terms of physicochemical parameters (*t*-test for mean comparisons and Mann–Whitney *U*-test for median comparisons).

containers of different makes and sizes, metal tanks/buckets, used tires, tin cans, water bowls for pets, ablution tanks, and flowerpots.

Poor solid waste management coupled with littering practices accounted for the majority of outdoor habitats for immature *Ae. aegypti*. Almost all houses visited had 1 or more types of water storage devices with or without covers. The most common storage appliances included plastic jerry cans and buckets (5–20 liter), plastic drums and metal tanks (50–200 liter), elevated tanks (1,000–5,000 liter), and concrete tanks/troughs (>1,000 liter). According to the Zanzibar Water Authority (ZAWA), water supply coverage in Malindi and Kikwajuni areas is low and unreliable, with only about 40% of residents in Kikwajuni and 50% in Malindi having access to water supply (ZAWA officer, personal communication). Thus, the constant water supply shortages compel residents to store water in their households for a considerable duration of time, thereby creating *Ae. aegypti* habitats.

In this study, we found domestic water receptacles to be of similar importance to that of discarded artifacts in terms of breeding preference by *Ae. aegypti*. However, in terms of productivity, domestic water appliances were more productive than discarded artifacts and materials considered as "others." Although total immature counts (larvae and pupae) were the highest in discarded artifacts, higher pupal counts were found in domestic water storage

Table 1. Number of water containers positive or negative for immature *Aedes aegypti* by physical and biological parameters.[1]

Parameter	Category	*n* (%) Positive	*n* (%) Negative	Total	OR	95% CI
Season	Wet	80 (60.2)	53 (39.8)	133	1.47	(0.81, 2.64)
	Dry	34 (50.7)	33 (49.3)	67		
Location	Indoor	41 (59.4)	28 (40.6)	69	1.16	(0.64, 2.10)
	Outdoor	73 (55.7)	58 (44.3)	131		
Environment	Housing compound	60 (57.7)	44 (42.3)	104	1.14	(0.55, 2.31)
	Unmanaged public spaces	30 (57.7)	22 (42.3)	52	1.14	(0.50, 2.55)
	Managed public spaces	24 (54.5)	20 (45.5)	44	1.00[2]	
Vegetation	Yes	75 (64.7)	41 (35.3)	116	2.11*	(1.19, 3.74)
	No	39 (46.4)	45 (53.6)	84		
Fauna	Yes	13 (59.1)	09 (40.9)	22	1.1	(0.44, 2.70)
	No	101 (56.7)	77 (43.3)	178		
Sun exposure[3]	$<50\%$	90 (62.9)	53 (37.1)	143	2.34*	(1.24, 4.36)
	$\geq 50\%$	24 (42.1)	33 (57.9)	57		
Water content						
Inorganic matter	Yes	68 (63.6)	39 (36.4)	107	1.78*	(1.01, 3.13)
	No	46 (49.5)	47 (50.5)	93		
Organic matter	Yes	40 (71.4)	16 (28.6)	56	2.37*	(1.21, 4.60)
	No	74 (51.4)	70 (48.6)	144		

[1] OR, odds ratio; CI, confidence interval.
[2] Managed public spaces used as reference (OR = 1).
[3] Duration (%) of direct sun exposure per day.
* Statistically significant at the 0.05 level.

containers. This may be due to the fact that domestic water storage is more stable and longer in duration compared to discarded artifacts. This finding is somewhat different to what was reported in Dar es Salaam, where household water storage containers were considered of secondary importance to discarded containers and tires (Philbert and Ijumba 2013, Mboera et al. 2016). Similarly, a study in rural Vietnam found a positive correlation between the numbers of household water storage containers and the prevalence and abundance of *Ae. aegypti* immature stages where domestic water containers accounted for >90% of all late instars (III/IV) and pupae (Nguyen et al. 2011).

With respect to materials, plastic containers were the predominant choice of both larvae and pupae, followed by metal containers, containers made of ceramic or cement, rubber (tires), and natural habitats. Similar findings were reported in Dar es Salaam especially in terms of plastic containers (Mboera et al. 2016). The observed importance of plastic materials–inhabiting *Aedes* mosquitoes is also supported by a study in Kolkata, India, where plastic containers were reported as the most productive habitats for *Aedes* sp. (Banerjee et al. 2013). The reason for this finding is unclear. A laboratory study, by Alvarado-Moreno et al. (2013), evaluated the efficiency of 5 different types of plastic films (vinyl, high- and low-density polyethylene, cellophane, and polyvinyl chloride) for adhesion and hatching of *Ae. aegypti* eggs. The study found that vinyl and low-density polyethylene resulted in 90% or more ovicidal activity, thus concluding that ovitraps using these types of plastics could reduce abundance of *Ae. aegypti* (Alvarado-Moreno et al. 2013). Our study did not consider the type of plastics. There may be certain physical characteristics that make plastic containers either favorable or inhibitory to *Ae. aegypti* breeding. More studies are needed to investigate the importance and mechanism of different types of plastics in reduction or propagation of *Ae. aegypti*.

Low-volume (<5 liter) containers were found to be the most common breeding habitats for *Ae. aegypti*. However, the medium-sized containers (5–20 liter) had higher number of immature mosquitoes in comparison to other water volume levels—as supported by previous findings (Mboera et al. 2016). Contrary to other studies in Tanzania (Trpis 1972, Philbert and Ijumba 2013, Mboera et al. 2016, Mathias et al. 2017), tires did not appear to be the most important source of *Ae. aegypti* in the present study, with only 5.8% and 11.2% of total pupae and total immature counts, respectively. This might be attributed to the low number of tires (only 11.5% of all water receptacles) encountered during the study, which aligns with the findings from India (Banerjee et al. 2013). Likewise, natural habitats appeared to be the least important source of *Ae. aegypti* in this study, which might be due to the fact that natural habitats constituted a smallest part of the sampling because of the urban nature of the study sites.

Among the 8 explanatory variables examined in this study, we found vegetation, sun exposure, organic matter, and inorganic matter to be significantly associated with the presence of immature *Ae. aegypti*. Notably, the presence of vegetation (algae, short grasses, tall plants) in and around habitats increased the risk of immature *Aedes* mosquitoes. This observation supports earlier reports of association between presence of immature *Ae. aegypti* and the amount of vegetation in the surrounding area as vegetation cover attracts oviposition and provides nutrients and shade needed for development and survival of immature mosquitoes (Philbert and Ijumba 2013).

In addition, we found that receptacles exposed to sunlight <50% of a day and those containing organic matter were more likely to be positive for immature mosquitoes. This finding is in line with the study in Sri Lanka in which vegetation and light exposure were shown to be important determinants of breeding success even for other types of mosquitoes (Piyaratne et al. 2005). Although the association was weak, we did observe that habitats with inorganic materials (e.g., sand and suspended plastic bags) were more likely to harbor *Ae. aegypti* immature stages than those holding clean water. This could be due to increased availability of nutrition in the form of microscopic organic substances that adhere to the surface of these inorganic materials. It should be noted that, due to the low numbers of pupae, statistical analyses were performed for total immature stages rather than for pupae and larvae separately. In a sufficiently large study, it would be important to stratify the analysis for different immature stages as they portray productivity and risk differently.

It is worth noting that, in this study, we found unusually large numbers (>1,000) of immature *Ae. aegypti* (and *Culex* sp.) in a single water container in the form of an underground water trough at a construction site. The trough was made of concrete, had a high vegetation level, and was fully shaded. The construction sector has long been recognized for creating potential breeding habitats for *Ae. aegypti* (Teng and Singh 2001, Chang et al. 2011). In Zanzibar, urban construction activities are widespread and usually uncontrolled and unregulated. Based on our finding and in the absence of regular entomological surveillance, it is suggested that the construction sector may contribute substantially to *Ae. aegypti* production in Zanzibar, enhancing the risk of arboviral disease transmission. Given that ground/underground concrete water tanks are common at household level in Zanzibar City, there is also a need for larger studies to ascertain their relative importance in terms of *Ae. aegypti* production, in order to deduce appropriate vector surveillance and source reduction strategies.

Notably, this study did not find any significant association between the presence of immature *Ae. aegypti* and any of the following parameters: season (wet/dry), location (indoor/outdoor), environment (housing compound/public spaces), or fauna (presence/absence of other aquatic fauna). This was the same for the 7 physico-chemical parameters, namely pH, turbidity, total hardness, conductivity, dissolved oxygen, temperature, and salinity, although the latter 2 displayed a borderline significant association with immature stages. To the best of our knowledge, there are no other published reports on physico-chemical parameters of *Ae. aegypti* breeding habitats in Tanzania. However, studies on other mosquito species, conducted elsewhere, have found significant associations between breeding and certain physico-chemical parameters. For instance, a study in Egypt found that temperature, pH, dissolved oxygen, and nitrite were directly related to the densities of *Cx. pipiens* (L.) and *Cx. perexiguus* Theobald, whereas salinity and turbidity were indirectly related (Kenawy and Ammar 2013). Likewise, a study in Sri Lanka revealed that the abundance of some *Anopheles* spp. was positively associated with temperature and pH (Piyaratne et al. 2005). Further, sufficiently large studies are needed to clarify the true influence of physico-chemical parameters on mosquito breeding and development in Zanzibar and mainland Tanzania.

A clear limitation of this study was the fact that the screening for potential habitats did not include all floors in multistory houses or the elevated spaces, omitting, for example, gutters and roofs. Furthermore, we did not obtain permission to enter a number of houses ($N = 9$), as the owners were unavailable during the time of the survey. Lastly, interobserver bias cannot be excluded in spite of thorough training of all research assistants. Nevertheless, the fact that field technicians were randomly allocated to new search areas each sampling day suggests an evening out of any observer-related biases. Notably, we did not investigate the peri-urban settings and rural villages of Zanzibar, which may present with different vector profiles in terms of species composition, habitat availability, and preferences. Further studies are needed in order to assess the mosquito vector profile and opportunities for surveillance and control in these settings.

The present study provides a baseline for further studies on *Ae. aegypti*, but may also inform the development of a surveillance system for immature *Ae. aegypti* as well as targeted control interventions in the urban settings of Zanzibar and other similar settings. Findings from this study, largely, support previous observations on *Ae. aegypti* habitat and larvae/pupae productivity from Dar es Salaam (Philbert and Ijumba 2013, Mboera et al. 2016). This suggests that the breeding behavior of *Ae. aegypti* follows a consistent pattern across the urbanized coastal areas of Tanzania, and that similar approaches to *Aedes* mosquito control may apply throughout. As such, and as domestic water receptacles exhibited higher pupal productivity, strategies for mosquito control should focus not only on environmental management and solid waste management, but also on improved island-wide domestic water supply to minimize the water-storing practices that provide larval habitats for *Ae. aegypti*.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to DANIDA/Building Stronger Universities project (BSU II) for funding this work. We also thank Abdalla Ibrahim for his participation in entomological data collection and Juma Mcha (entomologists at Zanzibar Malaria Elimination Program) for sharing technical expertise and experience on entomological surveys and involvement in data collection.

REFERENCES CITED

Alvarado-Moreno MS, Laguna-Aguilar M, Rodriguez OSS, Sanchez-Casas RM, Ramirez-Jimenez R, Zarate-Nahón EA, Achee N, Grieco JP, Fernandez-Salas I. 2013. Potential community-based control by use of plastic film to block *Aedes aegypti* (L.) egg adhesion. *Southwest Entomol* 38:605–614.

Amarasinghe A, Kuritsky J, Letson G, Margolis H. 2011. Dengue virus infection in Africa. *Emerg Infect Dis* 17:1349–1354.

Banerjee S, Aditya G, Saha GK. 2013. Pupal productivity of dengue vectors in Kolkata, India: implications for vector management. *Indian J Med Res* 137:549–559.

Chang MS, Christophel EM, Gopinath D, Abdur RM, Moh CT, Chang S. 2011. Challenges and future perspective for dengue vector control in the Western Pacific Region. *Western Pac Surveill Response J* 2:9–16.

Ellis BR, Barrett ADT. 2008. The enigma of yellow fever in East Africa. *Rev Med Virol* 18:331–346.

Haji KA, Khatib BO, Smith S, Ali AS, Devine GJ, Coetzee M, Majambere S. 2013. Challenges for malaria elimination in Zanzibar: pyrethroid resistance in malaria vectors and poor performance of long-lasting insecticide nets. *Parasit Vectors* 6:1–9.

Harbach R. 2013. Genus *Aedes* Meigen, 1818. *Mosquito taxonomic inventory* [Internet]. London, United Kingdom: Natural History Museum [accessed July 15, 2016]. Available from: http://mosquito-taxonomic-inventory.info/node/12738.

Hertz J, Munishi O, Ooi E, Howe S, Lim W, Chow A. 2012. Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. *Am J Trop Med Hyg* 86:171–177.

Imam H, Zarnigar, Sofi G, Seikh A. 2014. The basic rules and methods of mosquito rearing (*Aedes aegypti*). *Trop Parasitol* 4:53–55.

Kajeguka DC, Kaaya RD, Mwakalinga S, Ndossi R, Ndaro A, Chilongola JO, Mosha FW, Schiøler KL, Kavishe RA, Alifrangis M. 2016. Prevalence of dengue and chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. *BMC Infect Dis* 16:183.

Kenawy MA, Ammar SE. 2013. Physico-chemical characteristics of the mosquito breeding water in two urban areas of Cairo Governorate, Egypt. *J Entomol Acarol Res* 45:96–100.

Leroy E, Nkoghe D, Ollomo B, Nxe-Nkogue C, Becquart P, Grard G. 2009. Concurrent Chikungunya and dengue virus infections during simultaneous outbreaks, Gabon. *Emerg Infect Dis* 15:591–593.

Mathias L, Baraka V, Philbert A, Innocent E, Francis F, Nkwengulila G, Kweka EJ. 2017. Habitat productivity and pyrethroid susceptibility status of *Aedes aegypti* mosquitoes in Dar es Salaam, Tanzania. *Infect Dis Poverty* 6:1–10.

Mboera LEG, Mweya CN, Rumisha SF, Tungu PK, Stanley G, Makange MR, Misinzo G, De Nardo P, Vairo F, Oriyo NM. 2016. The risk of dengue virus transmission in Dar es Salaam, Tanzania during an epidemic period of 2014. *PLoS Negl Trop Dis* 10:e0004313.

Nguyen LAP, Clements ACA, Jeffery JAL, Yen NT, Nam VS, Vaughan G, Shinkfield R, Kutcher SC, Gatton ML, Kay BH, Ryan PA. 2011. Abundance and prevalence of *Aedes aegypti* immatures and relationships with household water storage in rural areas in southern Viet Nam. *Int Health* 3:115–125.

Nyamah MA, Sulaiman S, Omar B. 2010. Categorization of potential breeding sites of dengue vectors in Johor, Malaysia. *Trop Biomed* 27:33–40.

Pastorino B, Muyembe-Tamfum J, Bessaud M, Tock F, Tolou H, Durand J, Peyrefitte C. 2004. Epidemic resurgence of Chikungunya virus in Democratic Republic of the Congo: identification of a new central African strain. *J Med Virol* 74:277–282.

Philbert A, Ijumba JN. 2013. Preferred breeding habitats of *Aedes Aegypti* (Diptera- Culicidae) Mosquito and its public health implications in Dar es Salaam, Tanzania. *E3 J Environ Res Manage* 4:344–351.

Piyaratne MK, Amerasinghe FP, Amerasinghe PH, Konradsen F. 2005. Physico-chemical characteristics of *Anopheles culicifacies* and *Anopheles varuna* breeding water in a dry zone stream in Sri Lanka. *J Vector Borne Dis* 42:61–67.

Rao TR, Trpis M, Gillett JD, Teesdale C, Tonn RJ. 1973. Breeding places and seasonal incidence of *Aedes aegypti*, as assessed by the single-larva survey method. *Bull WHO* 48:615–622.

Ratsitorahina M, Harisoa J, Ratovonjato J, Biacabe S, Reynes J. 2008. Outbreak of dengue and chikungunya fevers, Toamasina, Madagascar. *Emerg Infect Dis* 14:1135–1137.

Rueda LM. 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission. *Zootaxa* 589:1–60.

Teng AK, Singh S. 2001. Epidemiology and new initiatives in the prevention and control of dengue in Malaysia. *Dengue Bull* 25:7–14.

The United Republic of Tanzania. 2013. *The 2012 population and housing census. Population distribution by administrative areas*. Dar es Salaam, Tanzania: National Bureau of Statistics, The United Republic of Tanzania.

Trpis M. 1972. Seasonal changes in the larval populations of *Aedes aegypti* in two biotopes in Dar es Salaam, Tanzania. *Bull WHO* 47:245–255.

Trpis M. 1973. Ecological studies on the breeding of *Aedes aegypti* and other mosquitos in shells of the giant African snail *Achatina fulica*. *Bull WHO* 48:447–453.

University of Florida Medical Entomology Laboratory. [Date unknown]. *Mosquito identification guide* [Internet]. Vero Beach, FL: Univ. of Florida Medical Entomology Laboratory [accessed July 2, 2016]. Available from: http://fmel.ifas.ufl.edu/key/.

Vairo F, Nicastri E, Yussuf SM, Cannas A, Meschi S, Mahmoud MAA, Mohamed AH, Maiko PM, Nardo PDe, Bevilacqua N, Castilletti C, Caro ADi, Racalbuto V, Ippolito G. 2014. IgG against dengue virus in healthy blood donors, Zanzibar, Tanzania. *Emerg Infect Dis* 20:465–468.

WHO [World Health Organization]. 2011. *Operational guide for assessing the productivity of* Aedes aegypti *breeding sites*. WHO Library Cataloguing-in-Publication Data. Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 2014. *Dengue outbreak in the United Republic of Tanzania (Situation as of 30 May 2014)* [Internet]. Brazzaville, Republic of Congo: WHO Regional Office for Africa [accessed June 17, 2017]. Available from: http://www.afro.who.int/en/disease-outbreaks/outbreak-news/4155-dengue-outbreak-in-the-united-republic-oftanzania-30-may-2014.html.

WHO [World Health Organization]. 2017a. *Countries with risk of yellow fever transmission and countries requiring yellow fever vaccination. International travel and health, Annex 1 update as of 16 February 2017* [Internet]. Geneva, Switzerland: World Health Organization [accessed May 10, 2018]. Available from: http://www.who.int/ith/2017-ith-annex1.pdf?ua=1.

WHO [World Health Organization]. 2017b. Yellow fever in Africa and the Americas, 2016. *Wkly Epidemiol Rec* [Internet] 92:437–452 [accessed May 10, 2018]. Available from: http://apps.who.int/iris/bitstream/handle/10665/258707/WER9232.pdf?sequence=1.