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ORIGINAL PAPER

The effects of simulated rainfall on immature population dynamics of *Aedes albopictus* and female oviposition

Hamady Dieng · G. M. Saifur Rahman · A. Abu Hassan · M. R. Che Salmah · Tomomitsu Satho · Fumio Miake · Michael Boots · AbuBakar Sazaly

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Abstract Larvae of *Aedes albopictus* Skuse typically inhabit natural and artificial containers. Since these larval habitats are replenished by rainfall, *Ae. albopictus* may experience increased loss of immature stages in areas with high levels of rainfall. In this study, we investigated the effects of rainfall and container water level on population density, and oviposition activity of *Ae. albopictus*. In field and laboratory experiments, we found that rainfall resulted in the flushing of breeding habitats. Excess rain negatively impacted larval and pupal retention, especially in small habitats. When filled with water to overflowing, container habitats were significantly repellent to ovipositing females. Taken together, these data suggest that rainfall triggers population loss of *Ae. albopictus* and related species through a direct detrimental effect (flushing out) and an indirect effect (ovipositional repellency).

Keywords *Aedes albopictus* · Container · Rainfall · Population loss · Repellency

H. Dieng (✉) · G. M. S. Rahman · A. Abu Hassan · M. R. Che Salmah
School of Biological Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia
e-mail: hamachan1@yahoo.com

T. Satho · F. Miake
Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan

M. Boots
Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK

A. Sazaly
Department of Medical Microbiology, University of Malaya, Kuala Lumpur, Malaysia

Introduction

A special characteristic of *Aedes* mosquitoes is that their eggs require the retention of enough moisture for successful embryonation (Strickman 1980; Hill et al. 2006). This is typical of *Ae. albopictus*, a species that is increasingly attracting major public health attention. The species has the innate ability to transmit dengue viruses (Shroyer 1986; Mitchell 1991; Gratz 2004; Malavige et al. 2004) which infect up to 50 million people every year, causing more than 20,000 deaths globally (Burke and Monath 2001; WHO http://www.who.int/topics/dengue/en/). Several other pathogens (Konishi 1989; Mitchell et al. 1998) including Chikungunya virus (Roiz et al. 2009; Delatte et al. 2010) are also transmitted by this vector, which has been proven to be a particularly invasive species (Hawley 1988).

Due to the importance of *Ae. albopictus* in public health, a substantial body of works has been directed towards understanding its population dynamics. The larvae of this mosquito typically develop in various aquatic habitats, including phytotelmata and artificial containers (Hawley 1988; Sota et al. 1992; Madon et al. 2003; Simard et al. 2005). The prevalence of the larvae in these habitats depends largely on rainfall, which is therefore the major water source (Fish and Carpenter 1982). Although evidence exists that rainfall is responsible for the abundance of *Ae. albopictus* (Lo and Narimah 1984), heavy rains have negative effects on the egg population (Hornby et al. 1994). It seems likely that there is a trade-off between sufficient rainfall and habitat population. This is because heavy rainfall could create new habitats and the overflowing of existing ones, which may wash out the larvae, thus off-setting their quality in older habitats.

In spite of previous suggestions addressing the negative impacts of rainfall on the eggs (Rozilawati et al. 2007) and immature stages (Foo et al. 1985) of dengue vectors, no research has focused attention on addressing the significance of rainfall as a mortality factor. A few experiments have demonstrated the effects of rainfall on mosquito larvae. Paaijmans et al. (2007) reported that immature populations of malaria mosquitoes suffer high losses during rainfall events, but the authors did not take into consideration the pupal stage, which is known to dwell mostly on the water surface and small habitats; factors that are important in mosquito productivity (Hawley 1988). In another study on container-breeding mosquitoes, Koenraadt and Harrington (2008) showed the effects of rain on *Ae. albopictus* and *Cx. pipiens* populations, but this investigation involved only heavy rains. Therefore, there is little analysis on the significance of the intensity of rainfall on *Ae. albopictus* larval performance and female oviposition.

As in most *Aedes* species, a particular characteristic of *Ae. albopictus* is that the females deposit eggs preferentially on the wet walls of container habitats (Hawley 1988), at sites where there has been standing water previously (Hill et al. 2006) and where flooding will likely to occur at some time in the future (Hill et al. 2006; Clements 1963). The container water level may vary with rainfall. At these sites delimitated by a varying waterline, the newly laid eggs must retain enough moisture for successful embryonation (Strickman 1980; Hill et al. 2006). Clearly, the presence of the waterline is critical to the increase in population of such mosquitoes. Surprisingly, the role of waterline as it affects population dynamics of *Aedes* mosquitoes remains largely unexplored. Here, we investigated the effects of rainfall on *Ae. albopictus* over its larval development, habitat quality and oviposition responses.

Materials and methods

Site of study

The study was carried out in the premises of the Universiti Sains Malaysia, located to the northeast of Penang Island. The premise is near to a wooded peridomestic area, with many trees such *Mischelia champaca*, *Mischelia figo*, *Mangifera indica* and various herbaceous plants. The area is surrounded by administrative buildings including classrooms, many car parking sites, and cafeterias. It is also an open area that receives high amounts of rainfall. Such conditions are typically ideal for the breeding of *Ae. albopictus*. A preliminary survey revealed that containers such as tin cans, flower vases, bottles, trays, plastic bowls, paint cans, broken ceramics, buckets and bucket covers represent the main breeding sites. No tree hole was found in the area. *Ae. albopictus* makes up the great majority of the mosquito fauna (Fig. 1).

Fig. 1 Study site

Mosquito rearing

The *Ae. albopictus* colony used in this study was collected as pupae from outdoor containers in 2009 in Penang Island (between latitude 5°8′N - 5°35′N and longitude 100°8′E - 100°32′E) (Ahmad et al. 2006) and maintained at 29±3°C, 75±10% humidity and photoperiod 13:10 h, 1 h of dusk in the insectary of the School of Biological Sciences, Universiti Sains Malaysia. Emerging adults were kept in standard mosquito rearing cages (30 cm×30 cm×30 cm, length × breadth × height) and provided with a 10% glucose solution. Approximately 2–3 days after eclosion, females were blood-fed on restrained mice. Gravid females were given the opportunity to lay eggs in oviposition devices. An oviposition device corresponds to a 300 mL capacity plastic container (height 7 cm, upper diameter 9 cm, lower diameter 5.5 cm) half-filled with tap water and lined with an oviposition substrate (3.5 cm×25 cm piece of filter paper, Whatman® #1; Whatman International, Maidstone, UK). Subsequently, eggs were dried under insectary conditions and kept as stock colonies.

The production of experimental gravid females and immature stages

To routinely obtain experimental gravid females, eggs from the egg bank were hatched in cool boiled tap water in accordance with the procedure previously described elsewhere (Dieng et al. 2006). Larval feeding was standardized to avoid variability in larval populations; 100 larvae were fed 2.5 g dried yeast at eclosion and on day 4 post-eclosion. The resulting adults were kept in cages and fed 10% glucose solution from a cotton wick. Two days after emergence, females were offered blood meals from restrained mice. To routinely obtain experimental larvae and pupae, samples from the stored eggs were hatched in deionized water and the eclosed larvae were reared following Dieng et al. (2002).

Mimics of natural habitats of *Ae. albopictus* using plastic containers

In northern peninsular Malaysia, *Ae. albopictus* uses a variety of artificial containers that collect rainwater as habitats (Zakaria et al. 2009). This mosquito is an opportunistic container user, which can utilize both natural and artificial container habitats. It has the ability to acclimate to a wide range of confined water bodies and to develop in very small collections of water (Hawley 1988; Dieng et al. 2007). In Penang, the habitats frequently infested by *Ae. albopictus* include plastic containers of diverse sizes. Here, we used two types of disposable plastic cups: a large one (5.5 cm×7 cm×9 cm, capacity 300 mL) and a small one (5 cm×2.5 cm×6 cm, capacity 60 mL). These containers are pertinent mimics of those encountered in nature.

Obtaining simulated rains

Penang is an equatorial state and receives heavy rainfall during the rainy season, with annual rainfall varying from 2,000 to 2,500 mm (Ahmad et al. 2006). To mimic this natural pattern simulated, rainfall was produced as shown in Fig. 2. Water flow from the tap water was channeled to a shower head (Techplas, Malaysia), using a PVC pipe (1.5 cm). The shower head used in this experiment had the following characteristics: round shaped, diameter of 9 cm and containing 60 holes. The distal part of the pipe holding the shower head was hung on a wooden support at a height of 10 m. The shower head was directed upward, such that the water flow from the shower was released as rain drops. The shower head was adjusted so that the vast majority of raindrops fell within the approximate surface area of 1 m^2. Preliminary trials showed that the intensity at which the jets of water were ejected from the holes of the shower head depended on the degree to which the valves of the tap were opened. We also observed that the quantity of water runoff resulting from the rain shower was a function of time. Based on this information, we used two rainfall regimes: (1) tap valves were one-quarter open (90° to the vertical or "off" position for 10 min and (2): tap valves were fully open (360°) to the vertical or "off" position for 25 min. Two sets each of 20 replicates of both small and large containers were exposed to each of the rainfall regimes.

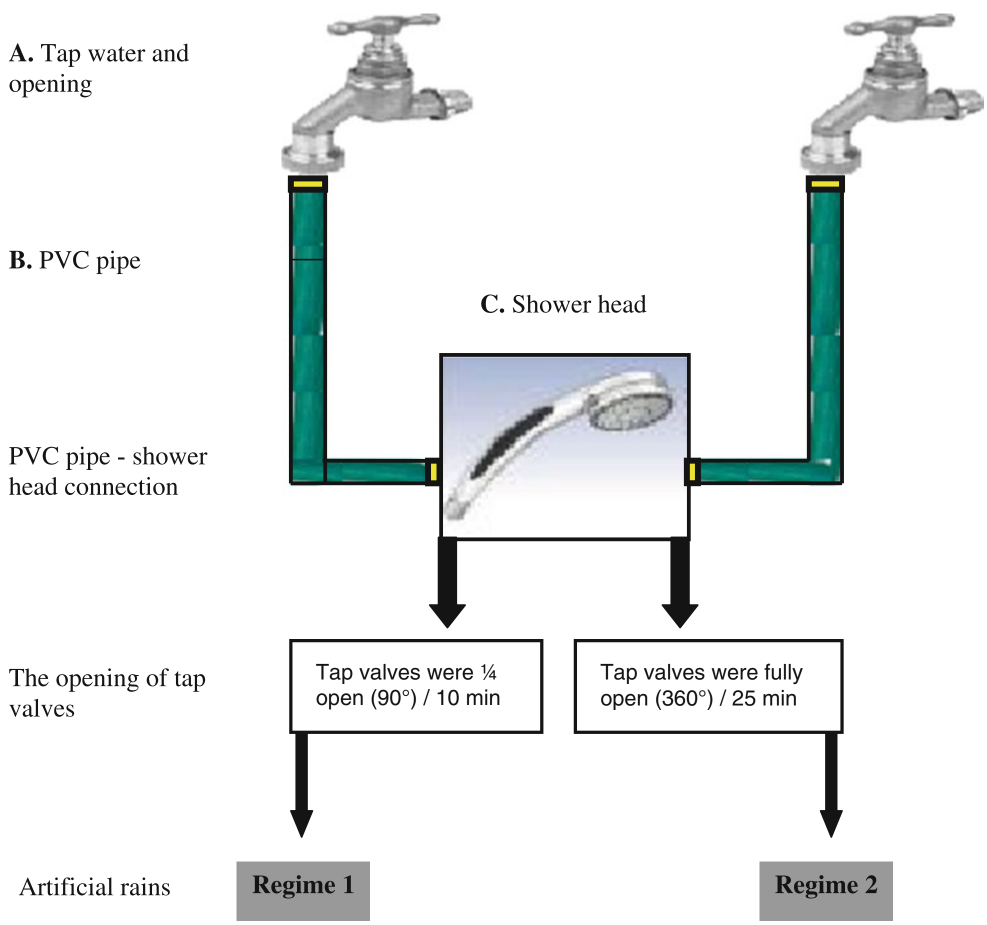

Fig. 2 Experimental design. Tap water (*A*) is connected to a PVC pipe (*B*), which is connected to the shower tap water (*C*). The *boxes* indicate the opening directions and opening intensities of the taps

Experiments

Simulated rainfall and developing stages of Ae. albopictus

To assess the effects of rainfall on immature stages of *Ae. albopictus*, two rainfall intensity regimes (regime 1 and regime 2) were evaluated against second, third and fourth instar larvae and pupae in large plastic containers in the field. Each container contained 50 larvae of same stage The number of replicates of containers for each developing stage was either 8 or 10. All containers were placed in a tray. Eight test tubes (2.3 cm×15 cm) placed at the same height above the ground level and at the vicinity of the experimental containers were used as gauges to measure rainfall. The quantity of rainfall was measured by the conventional method of rain gauge. It was calculated (in mm) using the formula: $h = V / \pi * R^2$, where V is the volume of water (mL) collected on the surface area of the opening of the glass tube, $\pi=3.14$, and R is the radius of the glass tube's opening. In order to assess the effect of rainfall on the larvae in different sized containers, the experiment was repeated using small containers. In this experiment, the numbers of immature stages removed from both large and small containers were counted after rainfall. In the text, we considered second instar larvae (L2) as young, while the third and fourth developmental stages (L3–L4) were considered as old larvae.

Water level and oviposition responses of Ae. albopictus

This experiment was carried out to examine the effects of water level on the oviposition responses of *Ae. albopictus*. A total of 20 gravid females were placed in a cage holding 10% glucose solution source and four oviposition cups. Two cups were filled with water to overflowing and the remaining two were half-filled. A circular piece of cardboard box, with a diameter of 24 cm was placed at the middle of the oviposition cage. On the circle were delimited four circles (diameter 8 cm) so that each circle was of equal distance from the nearest circle. The four oviposition cups were placed on these circles as shown in Fig. 3. This experiment was replicated four times with one positioning of the four cups corresponding to one replicate. After a 24-h oviposition period, the numbers of eggs laid in each cup were counted and recorded.

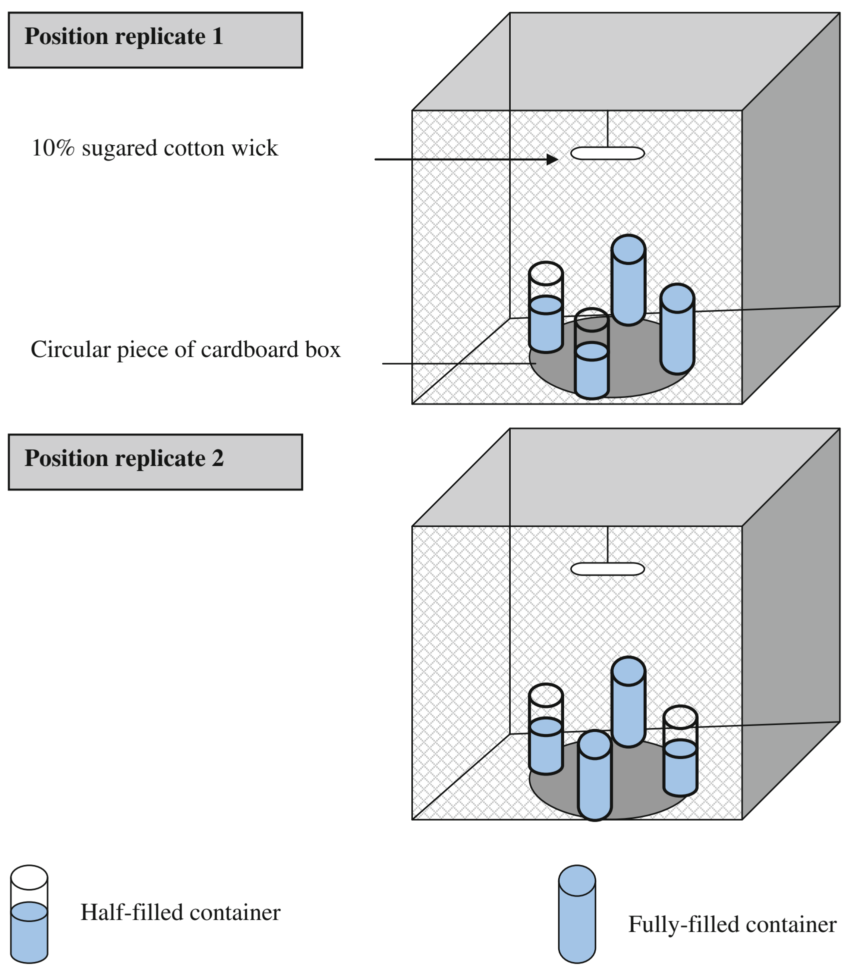

Fig. 3 Oviposition bioassay design. The two positions of the two types of containers scored one oviposition bioassay replicate

Data collection and analysis

In the first experiment, the numbers of immature stages removed from both large and small containers were counted after the simulated rainfalls. In the second experiment, eggs laid were counted by inspecting oviposition substrates under a dissecting microscope (Meiji EMZ; Meiji Techno, Tokyo, Japan). We considered the rate of removal of larvae and pupae to be the number individuals flushed out divided by the initial number of individuals. We evaluated the effects of rainfall intensity regime on immature stages by comparing the flushing out patterns of immature stages due to the light and heavy rainfalls for each container size. The effects of container size on immature stage due to rainfall were determined by comparing the flushing out profiles from large and small containers. The numbers of eggs laid on oviposition substrates and on water surface were scored as oviposition responses. Comparisons were performed by analysis of variance from the SYSTAT®13 statistical software package (Systat 2009).

Results

Experimental rains

The rainfall quantity was significantly (F=54.52, df=1, P< 0.001) higher during the second regime than during the first regime. Based on this evidence, we considered the regime 2 rain as “heavy rainfall (HRF)” and regime 1 as “light rainfall (LRF)” (Table 1).

Effects of rainfall on developing stages of *Ae. albopictus*

Under LRF, all developing stages were flushed out from the large containers, but in small numbers. In the small containers (SC), however, only young larvae were ejected during LRF. There was no significant difference between the rates of ejection of *Ae. albopictus* from SC and large containers (LC) (young larvae: F=2.14, df=1, P=0.160; old larvae: F=2.25, df=1, P=0.151; pupae: F= 2.25, df=1, P=0.151). Under HRF, all developing stages were flushed out from all the containers, but at greater rates by far from SC. Significant differences were noted between the rates of flushing out of *Ae. albopictus* from SC and LC (young larvae: F=53.28, df=1, P<0.001; old larvae: F=49.63, df=1, P<0.001; pupae: F=11.02, df=1, P=0.004). In both container types, ejection rate of all developmental stages increased with rainfall intensity. In LC, the rate of flushing out of young larvae did not differ statistically between the two rain intensities (F=4.04, df= 1, P=0.059), but was numerically higher during HRF. The rates of flushing out were significantly higher during HRF for both old larvae (F=8.03, df=1, P=0.011) and the pupal stage (F=14.44, df=1, P=0.001). The patterns of flushing out from SC were similar to those observed in LC. Significantly, the rates of flushing out of young larvae (F=88.29, df=1, P<0.001), old larvae (F=75.24, df=1, P<0.001) and pupae (F=27.15, df=1, P<0.001) were higher by far during HRF than LRF (Fig. 4). Clearly, rainfalls result in the loss of the immature stages of *Ae. albopictus* and this effect was stronger during HRF.

Effects of water level on oviposition responses of *Ae. albopictus*

The mean number of eggs deposited by *Ae. albopictus* varied significantly with container water level (F=16.88, df=4, P< 0.05). The oviposition response was significantly greater in half-filled containers than in those filled to overflowing (Fig. 5).

Discussion

Mosquito larvae and pupae live in water and reach the air–water interface from time to time to obtain oxygen (Paaijmans et al. 2007). During rains, rain drops hit the water surface of containers, thus splashing some water away from the containers. With splashing water, the larvae and pupae present in the container could be swept out. During container flushing, immature stages may be carried over the edge of the container while attached (via the larval siphon or pupal trumpets) to the water surface. This is more prominent

Table 1 Characteristics of the artificial rainfalls used in this study. Mean values with the same lower case letter do not show a significant difference (P<0.05) based on ANOVA

	Regime 1	Regime 2
Tap valves	One-quarter opened (90°)	Fully opened (360°)
Duration (min)	10	25
Replicates	20	20
Rainfall quantity (mm)	6.28±0.61 a	62.45±7.58 b
Range	4.3–9.1	15.6–84.3
Characterization	Light	Heavy

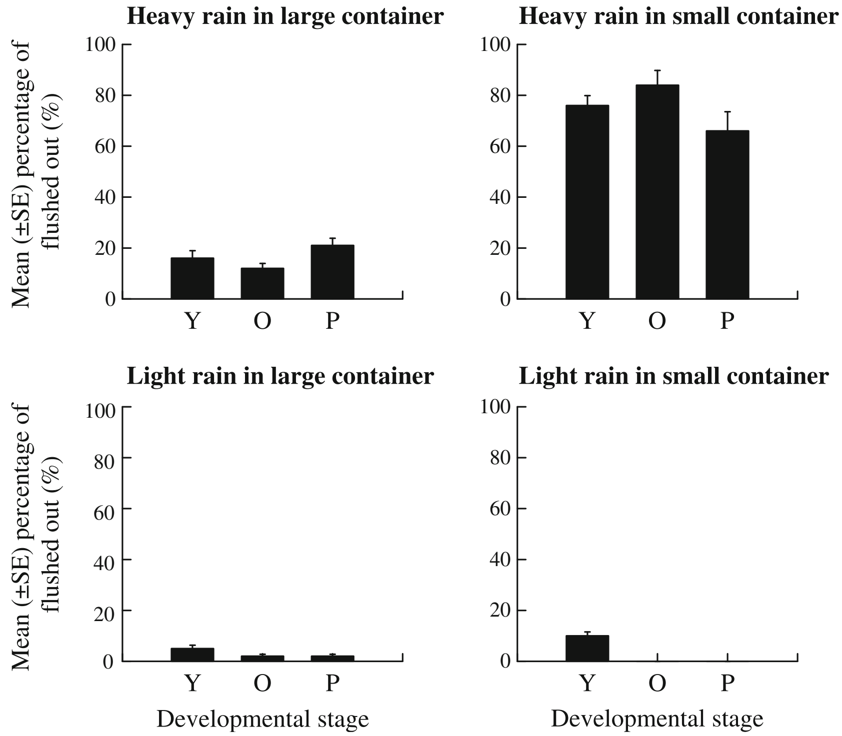

Fig. 4 Mean (± SE) percentage of *Aedes albopictus* flushed out from containers under heavy and light rainfalls. *Y* Young larvae (second stages), *O* old larvae (third and fourth stages), *P* pupae. Bar charts with the same letter or number do not show a significant difference ($P<0.05$) based on Tukey's test

during strong rains, which have larger and more frequent raindrops than light rain. Therefore, rainfall could have direct and negative effects on the number of larvae and pupae in the containers.

Furthermore, rains may also affect the fitness of the container for breeding mosquito larvae and pupae. During heavy rains, the nutrients in the container habitat may be lost with the splashing water, making the container habitat impoverished of food resources for the larvae (Dieng et al. 2003). Similarly, the disturbance of the water surface due to falling raindrops may increase energy loss by the larvae, as they attempt to escape from the effects of rain drops when they come to the water surface for oxygen (Paaijmans et al. 2007). These factors could reduce the fitness of the larvae, pupae, and the emerging adults. On the other hand, the removal of some larvae by raindrops during heavy rains results into reduced competition for food, thus increasing body size at emergence and fitness of the larvae remaining in the container. However, in areas with high rainfall, since the field populations of developing larvae are exposed to frequent rains, such positive effects are more unlikely.

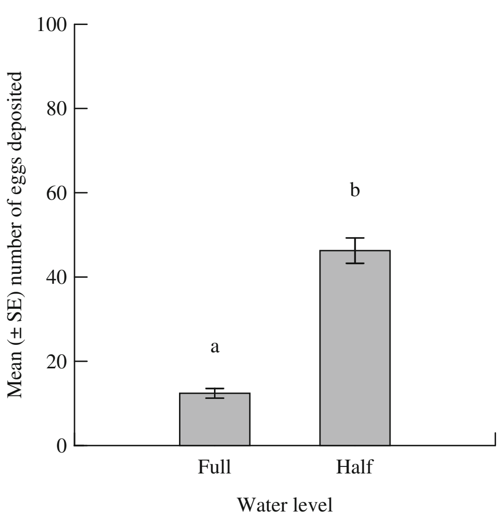

Fig. 5 Mean (± SE) number of eggs laid by *Ae. albopictus* in containers fully- and half-filled with water. Bar charts with the same letter or number do not show a significant difference ($P<0.05$)

Once a female mosquito becomes gravid, it goes through a period of oviposition site seeking. *Ae. albopictus* females were shown to elicit increased egg retention under low-moisture conditions (Rahman et al. 2010) and decreased egg deposition in the presence of oviposition deterrents (Xue et al. 2001) and food scarcity (Dieng et al. 2002). In Japan, females of *Ae. aegypti* were reported to deposit eggs preferentially in open-type tree holes (Tsuda et al. 1994) to protect larvae during drought. This behavior could be an adaptation to prevent unsuccessful egg development and better survival of the species. In our study, it was observed that *Ae. albopictus* females preferred to lay eggs in half-filled containers. During rainfall, the overflow events tend to occur more rapidly in fully filled containers than in half-filled ones. Since overflow has direct detrimental effects on mosquito larvae through splashing out, *Ae. albopictus* may avoid fully filled containers for oviposition. Paaijmans et al.

(2007) observed increased mortality and flushing out of immature stages of *An. gambiae*. With reference to these observations, it seems likely that *Ae. albopictus* females respond to their habitat overflow potential, with respect to its suitability for larval development. The observed increased egg deposition in half-filled containers could likely be an adaptation adopted to prevent the loss of their immature stages, since habitat overflow has a negative effect on survival (Paaijmans et al. 2007).

The flushing out of immature stages from containers by tap water from shower heads was used to mimic flushing out by rainfall larvae. Strong water input by rainfall, which provokes increased frequency of overflow events, would tend to decrease the probability of larval development completion. With frequent overflowing events, larvae have a higher probability of decreased lifespan, as feeding activities would be interrupted, thus reducing feeding rates. Since such a phenomenon is dependent on the size of droplets of water and the pressure and velocity of the droplets, the lack of consideration of these physical properties could be considered a drawback of our approach.

Conclusion

Heavy rains resulted in high *Ae. albopictus* larval and pupal loss, especially from small containers. *Ae. albopictus*, which prefer to lay eggs in partly filled containers rather than fully filled containers. These effects may limit the population size of the adults—the stage that vectors diseases. This study used simulated rainfall using tap water. However, we did not take into consideration the difference in quality between tap water and rainwater because our experiment was focused on the mechanical effects of rainfall on larval population dynamics. Additional studies are required to demonstrate whether or not the physical properties of our artificial rainfall were equivalent to those of real rain.

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