Journal of Medical Entomology, XX(XX), 2025, 1–7
https://doi.org/10.1093/jme/tjaf019
Research

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Evaluating vector mosquito occurrence in residential rain barrels in central Illinois

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Subject Editor: Donald Yee

Received on 30 August 2024; revised on 8 January 2025; accepted on 30 January 2025

Several species of vector mosquitoes (eg *Culex pipiens* (Linnaeus, 1758), *Aedes albopictus* (Skuse, 1895)) complete juvenile development in artificial containers. Rain barrels are green infrastructure tools used to conserve rainwater for outdoor use, though they may also serve as a source of mosquito habitat in residential neighborhoods. To identify rain barrel features, maintenance habits, and other conditions associated with the presence of juvenile mosquitoes (ie month), we conducted periodic inspections of rain barrels at 53 households in central Illinois, USA between June and September 2016. Additionally, we administered a questionnaire to the household study participants. In the first month of the study, a diversity of mosquito species was detected in household rain barrels, but from July to September juveniles of *Ae. albopictus* were predominant. More than half of inspected households contained at least one mosquito-positive rain barrel within the study period. Using stepwise model selection, the strongest predictors of whether or not mosquito juveniles were detected in rain barrels were the use of a preventative measure (eg *Bti*, chlorine, goldfish), the presence of a mesh covering on the lid of the barrel, and the month of the year. Additionally, the participant questionnaire revealed that the majority of respondents were aware of immediate elimination methods, but few were aware of the need for long-term preventative maintenance of rain barrels against larval mosquito colonization. These findings provide valuable insight into best practices for mosquito prevention in green infrastructure and highlight the importance of proper maintenance and education to minimize juvenile mosquito habitat.

Keywords: vector mosquito, stormwater infrastructure, artificial container, stepwise model selection, mosquito prevention

Introduction

Mosquito-borne diseases remain a public health concern in the United States; West Nile virus (WNV) was first reported in the United States in 1999 in New York City (Nash et al. 2001), and local transmission of the virus has since been reported in every state in the continental United States. In 2021, the Centers for Disease Control and Prevention (CDC) reported 2,911 human cases of WNV, 69% of which were classified as neuroinvasive (CDC 2024). There is currently no vaccine nor treatment available for the prevention of WNV in humans. Considering the significant public health threat posed by mosquitoes and the absence of effective medical interventions for WNV, there is a pressing need for public education and effective control measures for the mitigation of mosquito-borne disease transmission.

The *Culex pipiens* (Linnaeus, 1758) complex of mosquitoes has been determined as primary vectors of WNV in the United States (Farajollahi et al. 2011). Additionally, a multitude of viruses have been isolated from the invasive *Aedes albopictus* (Skuse, 1895) in the United States, including WNV, as well as Eastern Equine Encephalitis, Keystone, La Crosse, and Cache Valley viruses (Garcia-Rejon et al. 2021). Both *Cx. pipiens* and *Ae. albopictus* thrive in urban environments and frequently complete juvenile development in artificial container habitats (Carrieri et al. 2003, Vezzani 2007, Townroe and Callaghan 2014). We define artificial container habitats as engineered structures that are capable of serving as water sources, such as buckets, tires, bird baths, etc. These habitats are frequently found in residential and urban areas with high human activity, bringing vector mosquitoes into close contact with potential human hosts and posing a public health risk when containers are left unmaintained (LaDeau et al. 2015). Understanding the urban ecology of container-breeding mosquitoes is crucial to the development of effective vector control strategies.

Rain barrels (RBs) are a type of artificial container that has gained popularity in the residential landscape due to their capacity to capture and retain rainwater for outdoor use. RBs are green infrastructure tools that can conserve hundreds of gallons of water per year and reduce stormwater runoff (EPA 2013). On average, it is estimated that RBs can supply 44% of the irrigation demand of residential gardens in the United States (Litofsky and Jennings 2014). RBs are more commonly adopted by individuals from high-income neighborhoods with eco-conscious beliefs (Ando and Freitas 2011, Gao et al. 2016) and those who perform additional water conservation practices (eg shorter showers) (Ott et al. 2015).

The potential for inadequately maintained RBs to serve as juvenile mosquito habitats in residential neighborhoods in the U.S. poses an unexplored risk to human health. For comparison, a survey of rainwater harvesting systems conducted in Melbourne, Australia in 2016 detected mosquitoes in 21% of all rainwater tanks inspected (Moglia et al. 2016). Therefore, it is critical to understand and mitigate the risk posed by this new container habitat to ensure the continued safe use of RBs for rainwater harvesting.

RBs possess several key features that may influence mosquito colonization. Properly constructed and maintained RBs should ideally have a sealed lid as well as a fully screened inlet and outlet free of holes or debris (Ott et al. 2018). RB characteristics such as color may influence adult female mosquito oviposition choice, with black being a preferred color of oviposition habitat for *Ae. albopictus* (Hoel et al. 2011, Bartlett-Healy et al. 2012, Gunathilaka et al. 2018). One of the greatest determinants of mosquito abundance remains entirely ungovernable by both RB owner and vector control specialist alike: temporal changes due to species' phenology (Lalubin et al. 2013, Kache et al. 2020). However, RB owners can take extra precautions pertaining to RB maintenance during the months of the highest vector mosquito abundance. It is recommended by many vector control specialists to treat container habitats with approved mosquito prevention methods, such as the bacterial insecticide *Bacillus thuringiensis israelensis* (*Bti*) (Lacey 2007, Ritchie et al. 2010), chlorine (Mackay et al. 2015), or even predators of mosquito larvae such as goldfish (*Carassius auratus*) (Underwood 1901).

We hypothesize that RBs are a frequent but under-recognized artificial container habitat for juvenile mosquitoes in urban and residential environments and that certain features of RBs play a more pertinent role in mosquito prevention than others. For this study, we identified and recruited households with one or more RBs for surveillance of mosquito occurrence. Data were collected on several key RB features, and we surveyed rain barrels for the presence or absence of juvenile mosquitoes approximately every 3 wk during the seasonal period when container-breeding mosquitoes are most active (June to September). To quantify risk factors associated with RB's characteristics, we utilized stepwise model selection to evaluate a set of generalized linear mixed models representing RB features of interest. We assessed the relative importance of RB features in the prevention of mosquito colonization based on the most predictive model output. Additionally, we delivered a questionnaire to our study participants to evaluate behaviors surrounding RB maintenance practices as well as general knowledge about effective mosquito prevention. We synthesized the results of our homeowner survey and field data to determine knowledge gaps in public perception of mosquito ecology and preventative measures pertinent to rain barrel habitats. Our research aims to inform vector mosquito prevention methods in green stormwater infrastructure with the goal of decreasing vector-borne disease risk in residential areas.

Methods

Field Survey

Households in Champaign ($n = 58$) and Piatt ($n = 1$) counties, Illinois, using RBs for rainwater capture were recruited for this study by distributing flyers at municipal rain barrel and horticultural sale events and by posting in the University of Illinois weekly employee email notices and other social media sites related to green infrastructure. At 53 of the households originally recruited for the study, RBs were inspected at approximately 3-wk intervals from 3 June 2016 to 20 September 2016. During the first visit, information was recorded on the color, capacity, and other structural characteristics of the RB and associated components (eg inlet and outlet pipes). On all visits, stored water and any water collected on the RB lid, inlet, or outlet pipes were examined for the presence of mosquito eggs, larvae, pupae, pupae exuviae, and other aquatic macroinvertebrates. Additionally, putative risk factors for mosquito colonization of RBs were assessed, such as the presence and condition of exclusion screens, tightness of the lid seal and pipe connections, or the presence of cracks or holes in the lid or screen. RB owners were informed if mosquitoes were present in their RB(s), after which some households took extra precautionary measures during the course of the study. Some owners adopted additional prevention methods, including the use of the bacterial insecticide *Bti*, chlorine, or goldfish in their RB(s).

When juvenile mosquitoes were detected in the reservoir of RBs, the relative abundance of both larvae and pupae was visually estimated and a representative sample was collected to determine the species composition. A single sweep across the water surface with an aquarium net (12.7 × 10.2 cm) was performed in RBs with a removable lid. For other RB designs where access to the reservoir was restricted to vent plugs or inlet holes, a smaller net, pipette, or hand pump was used to collect a sample. All larvae and pupae present in auxiliary structures (eg lid surface, outlet pipes) were also collected. All mosquito larvae collected were identified as species, and mosquito pupae were identified as genus (Fig. 1).

Statistical analyses were conducted using R version 4.2.1 (R Core Team 2022) to determine the relative effects of 19 RB features of interest (Table 1) on the presence or absence of mosquito juveniles at the time of inspection. Binomial generalized linear mixed models were chosen as each model incorporated a spatial exponential covariance structure using the decimal degree coordinates associated with each address. We justify the spatial random effect to account for the non-independence of residual spatial autocorrelation as evidenced by a statistically significant Moran's I test on the residuals of the final model (see Results).

We performed a stepwise model selection using the function buildglmmTMB in the R package buildmer (Voeten 2023). Backward stepwise regression was chosen for model selection due to its effectiveness in ecological studies with complex datasets, as demonstrated by Adjemian et al. (2006) and Lopez et al. (2024). This method starts with the most complex model that can be fitted and then iteratively removes non-significant explanatory variables based on changes in log-likelihood until the optimal model is achieved.

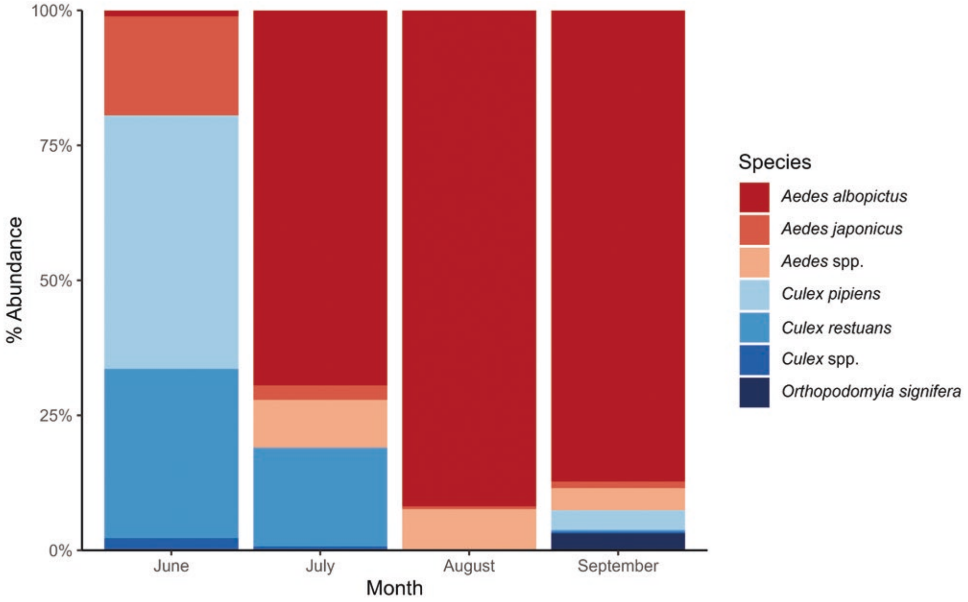

Fig. 1. Observed juvenile mosquito composition in RBs by month. Labels "*Aedes* spp." and "*Culex* spp." represent both pupal specimens and larvae only identified to genus. *Cx.* spp. represented a majority of juvenile mosquitoes detected in June, whereas a larger percentage of *Ae.* spp. were observed July to September. Samples were non-exhaustive; therefore, these values do not necessarily reflect the true distribution of species observed in RBs.

Table 1. List of 19 fixed effects parameters used in the stepwise model selection.

Category	Predictor variable	Response
Component	Month	"June" "July" "August" "September"
Barrel	RB color	"beige/brown" "black" "blue" "green" "gray" "red" "white"
	Water source	"end of inlet pipe within RB" "end of inlet pipe exterior to RB, discharges onto surface of screened lid"
	RB capacity (L)	"151.4" "170.3" "204.4" "208.2" "214.0"
	% filled	"0%" "25%" "50%" "75%" "100%" (NA if view obstructed)
	% bottom surface covered by sediment	"0" "<10%" "10-50%" "50-90%" ">90%" (NA if view obstructed)
	Water algae	"Yes" "No" (NA if view obstructed)
	Other invertebrate	"Yes" "No" (NA if view obstructed)
	Prevention method	"Yes" "No"
Lid	Lid sealed	"Yes" "No"
	Lid water	"Yes" "No"
	Lid mesh presence	"Yes" "No"
	Lid mesh type	"fiber glass" "aluminum" "plastic" "homemade" (NA if no mesh)
	Lid mesh intact	"Yes" "No" (NA if no mesh)
	Lid removable	"Yes" "No" "No lid"
Inlet	Inlet mesh	"Yes" "No" (NA if no inlet)
Outlet	Outlet presence	"Yes" "No"
	Outlet screen	"Yes" "No" (NA if no screen)
	Outlet discharge location	"Back to downspout" "Next barrel" "Adjacent Ground" "Bucket" (NA if no outlet)

After assessing the final model parameters for multicollinearity using variance inflation factors (VIF), we then used the final model to predict the observed data and 2 theoretical scenarios to predict the highest and the lowest expected frequency of mosquito presence in rain barrels assuming none or all of the homeowners used one or more prevention methods (ie screened rain barrel lid, and/or use of *Bti*, chlorine or goldfish), respectively, using the function lsmeans in the R package emmeans.

Homeowner Survey

Residents who participated in the homeowner survey were asked to disclose if they had observed juvenile mosquitoes in their RB(s) in the past. Homeowners who indicated in the affirmative were asked what, if any, action they took after making this observation. All homeowners were asked the following multiple-choice question: "If you found mosquito larvae inside your rain barrels, what action would you take? Please check all that apply," with options including (i) "None (no action would be taken)"; (ii) "Empty water from rain barrel"; (iii) "Thoroughly clean rain barrel by manually scrubbing inner walls"; (iv) "Treat water in rain barrel with an insecticide (example: "mosquito dunks")"; and (v) "Other", with the option to write in a response. The responses were then categorized by homeowners' effective knowledge of short-term prevention, long-term prevention, both, or neither (Table 2). We define short-term

Table 2. Responses from homeowner survey classified by knowledge of effective mosquito prevention methods.

Homeowner knowledge of effective mosquito prevention methods	N	Example Response
Homeowner understands long-term prevention only	2	"Cover the lids or overflow spout with mesh material to prevent mosquito from going in."
Homeowner understands short-term prevention only	12	"Empty water from rain barrel"
Homeowners shows knowledge of both long-term and short-term prevention	20	"Treat water in rain barrel with an insecticide (example: "mosquito dunks")"
Homeowner lacks knowledge of short-term and long-term prevention	19	"None (no action would be taken)"; "I really don't know what to do."

prevention methods here as any action that immediately removes juvenile mosquitoes currently on the property (eg dumping standing water), while long-term prevention methods seek to prevent mosquito colonization or development (eg installing mesh screens over water holding structures, *Bti* application). Responses from residents who participated in the homeowner survey but who did not participate in the field survey were excluded from the analysis.

The homeowner data were integrated with the field survey to provide a comparison between households who indicated having previously observed larvae in their RB(s) and those confirmed positive by the research team during the study. A Fischer's exact test was performed to determine the probability of prior juvenile mosquito detection by homeowners given a positive RB at that household.

Results

Over the course of the field survey, a total of 115 RBs were inspected at 53 households. The average number of RBs per household was 2.2 with a range of 1 to 8. Over the entire sampling period, 52.2% ($n = 60/115$) of RBs were observed with juvenile mosquitoes present, and 58.5% ($n = 31/53$) of households contained at least one RB with juvenile mosquitoes present.

Juvenile mosquito species composition in RBs varied greatly over the course of the field survey. In the month of June, a majority of mosquito juveniles collected were *Culex* spp. (80.6%); by July, *Aedes albopictus* juveniles became the predominant species detected (70.5%) and continued to dominate RB species composition through August (91.7%) and September (75.3%).

Based on the results from stepwise model selection, the model including lid mesh, prevention method, and month was the best fit to the observed data. A Moran's I test performed on the residuals of the model was significant ($z = 3.37$, $P < 0.001$), thus a spatial exponential covariance structure was included in the final model. The VIF scores for each explanatory variable of the final model did not exceed 1.2, indicating no evidence of multicollinearity, thus all predictors were retained. The final model parameter estimates, standard errors, z-values, and *P*-values for each predictor can be found in Supplementary Table S1. The predictive capacity of this model performed well compared to the observed proportion of RBs containing juvenile mosquitoes over the duration of the study (Fig. 2A). Based on model coefficients, peak seasonal positivity of mosquito juveniles in rain barrels is predicted to exceed 75% in the complete absence of prevention practices (Fig. 2B) but remain below 4.5% if both lid mesh and at least one prevention method (*Bti*, chlorine, or fish) are used by homeowners (Fig. 2C).

Pairwise tests using the best-performing model from the model selection confirm the directionality and provide log-odds ratios for each of the predictors. Based on the model, RBs without mesh screens were 8.34 (SE = 4.42) times more likely to be positive for mosquitoes than RBs with intact mesh screens ($P < 0.001$). RBs using any method of prevention, either *Bti*, chlorine, or goldfish, were 7.57 (SE = 6.63) times less likely to have mosquito larvae ($P = 0.021$). Positive mosquito observations in RBs increased by month over the course of the study. Mosquitoes were 12.22 (SE = 7.96) times more likely to be found in July than in June ($P < 0.001$), and 3.09 (SE = 1.21) times more likely to be found in August than July ($P = 0.020$). Mosquito observations did not significantly differ between August and September ($P = 0.98$).

The homeowner survey revealed a majority of homeowners possessed some understanding of the role that RBs could play in serving as habitats for juvenile mosquitoes; 64.2% ($n = 34/53$) of homeowners demonstrated knowledge of short-term prevention methods, long-term prevention methods, or both (Table 2). The results from the homeowner survey combined with the field data allowed us to find that RB owners, however, were not able to assess the presence of mosquito larvae in RBs efficiently. At homes where we detected juvenile mosquitoes (31/53), homeowners were no more likely to report previously observing mosquito larvae (4/31) in their RBs compared with homes where we did not detect juvenile mosquitoes (3/22) (Fisher's exact test, $P = 1$).

Discussion

Despite their beneficial use as green stormwater infrastructure tools, residential RBs were found to be frequently colonized by mosquitoes in this study. We explored risk factors associated with RB characteristics and quantified their relative contributions to mosquito positivity. The parameters chosen for model selection were based on the prior knowledge of the capacity to physically prevent juvenile mosquito colonization (eg lid mesh, prevention method), or the mosquito life cycle (eg month). Using stepwise model selection, we found the presence of lid mesh, the use by the homeowner of a mosquito prevention method, and the month were the best predictors of juvenile mosquitoes in residential RBs. The finding that an intact lid mesh being present is an important prevention factor is consistent with the average body size of an adult *Culex* spp. or *Ae. albopictus* mosquito (McCann et al. 2009, Davis et al. 2016), which would be too large to bypass the 1.4 mm or smaller diameter mesh covering present in many RBs. Water treatment-based prevention methods used by homeowners in this study (ie *Bti*, chlorine, or goldfish) were combined during model selection due to low sample size, but more evidence is needed to compare the effectiveness between treatment methods.

Two predictor variables we explored that were dropped from the model in the last 2 iterations of the stepwise elimination were "lid sealed," indicating if the lid of the RB was fully sealed or not

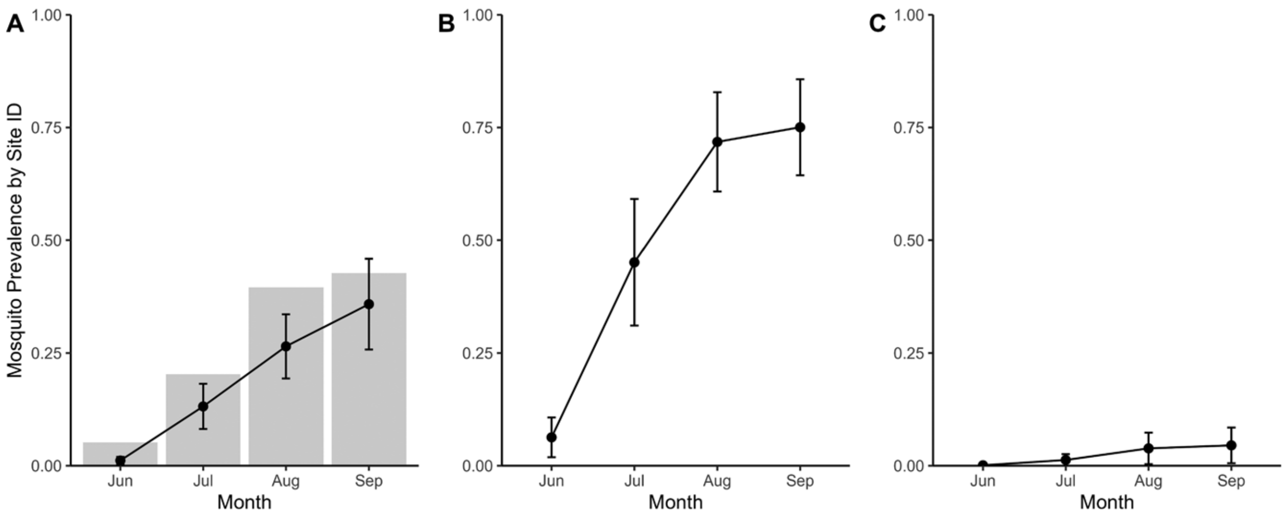

Fig. 2. Model predictions for proportion of positive mosquito observations. Panel (A) shows the predictive model (points/lines) performs well against the observed data (bars) for mosquito positivity by Site ID each month. Model predictions to maximize mosquito productivity (B) were generated by assuming all RBs did not have a mesh screen over the lid nor used a preventative measure. In (C), model predictions minimize mosquito productivity by assuming all RBs had a mesh screen over the lid and used a preventative measure. Error bars represent standard errors of standard errors of the estimated marginal means.

upon inspection, and "lid water," which indicated that the lid of the RB was both sealed and had water pooled on the lid. It is likely that these 2 predictors fell out of the model due to several convoluting factors. While having a sealed lid over the RB creates a physical barrier preventing female mosquitoes from laying eggs in the barrel itself, the lid may become a secondary container if water is allowed to pool within the rim. The converse is also true; if an RB was observed with water pooled on the lid, it meant that the rain barrel was sealed. The relationship between the lid of the RB and mosquito presence is meaningful regardless of its predictive ability in the final model but should be understood in the context of confounders.

Our findings are consistent with other studies that indicate a public health risk of mosquito reproduction with residential rainwater harvesting. In a survey of multiple types of artificial container habitats found in residential yards in upstate New York, Tuiten et al. (2009) detected juveniles of multiple WNV vector mosquito species in nearly half of inspected rain barrels. Likewise, in a survey conducted in Lelystad, Netherlands, rain barrels were the most frequently detected juvenile habitat for *Ae. japonicus* (Ibañez-Justicia et al. 2018). In a survey of rainwater harvesting structures in Tamil Nadu, India, Mariappan et al. (2008) found across 3 seasons that 14.2% to 34.6% of these structures had defects, and 5.7% to 11.9% were positive for *Aedes* spp. mosquitoes. A survey of water storage systems in Melbourne, Australia found mosquitoes in 21% of all rainwater tanks inspected and determined the absence of mesh on the tank inlet or overflow pipe to be the most common access routes into the tank systems (Moglia et al. 2016). While inlet and outlet screens were not significant predictors of juvenile mosquito presence in this study, the size of inlet and outlet pipes may contribute to oviposition success, with smaller diameters and longer pipe lengths previously shown to reduce oviposition success (Harbison et al. 2008). Thus, globally, there is considerable potential for rainwater harvesting structures to serve as mosquito habitats, creating urgency for homeowner education in mosquito prevention.

Homeowners' understanding of both short- and long-term methods of mosquito prevention is essential to residential mosquito control. While most homeowners in this study possessed some knowledge of short-term prevention, long-term prevention, or both, integrating the homeowner survey with the field data revealed the lack of proficiency in RB owners' ability to identify the presence of mosquito larvae. This finding highlights the importance of outreach and education surrounding mosquito biology, including mosquito morphology and the mosquito life cycle, in addition to the education of mosquito prevention methods. Community outreach programs held by educational institutions, public health districts, and mosquito abatement districts should be more accessible to the public and include training on how to reduce the availability of juvenile habitats in residential environments.

The addition of green infrastructure tools in urban environments has many benefits for water management and conservation, including energy cost savings and reduction in water pollution (Ghimire and Johnston 2017). A recent simulation conducted using 4 major U.S. cities predicted that urban rainwater harvesting would be able to reduce potable water demand by over 65% and roof runoff by over 75% (Rostad et al. 2016). However, it is important for RB owners to take the proper precautions to prevent colonization by mosquitoes and minimize the risk of mosquito-borne illnesses. Our study indicates that maintaining the integrity of the lid screen, and application of an approved, long-lasting larvicide (eg *Bti* dunks, methoprene), chlorine bleach, or larvivorous fish, can effectively reduce the potential for RBs to support mosquito production. Other preventative measures to consider include regular cleaning of the RBs, removing any standing water that may accumulate on RB lids, and use of a mesh screen to cover inlet and outlet pipes. Additionally, it is essential that RB owners regularly inspect their RBs for juvenile mosquitoes and apply appropriate countermeasures when necessary (eg dumping of water, lid screen replacement, etc.), particularly during the months of heightened mosquito activity (July to September). Our model predictions (Fig. 2C) suggest single practices may not be sufficient to entirely prevent colonization by mosquitoes; a combination of prevention methods may be required to completely eliminate risk. Taking these preventative measures will reduce potential health risks and ensure that RBs remain a safe, effective, and environmentally sustainable tool for managing stormwater runoff.

Acknowledgments

Special thanks to Eliana Brown, Leslie Mitchell, Alex Nagy, Nancy Taylor, Scott Tess, Emily Dejka, Hei Chan, Madison Moore, Erica

Cimo Dean, and all of the Champaign County residents who participated in the study.

Supplementary material

Supplementary material is available at *Journal of Medical Entomology* online.

Funding

Support for this study was provided by the Institute for Sustainability, Energy, and the Environment at the University of Illinois Urbana-Champaign, the NSF Institute for Geospatial Understanding through an Integrative Discovery Environment, and NSF DEB 1754115.

Author contributions

Rebecca Cloud (Formal analysis [lead], Visualization [lead], Writing—original draft [lead], Writing—review & editing [equal]), Andrew Mackay (Conceptualization [equal], Data curation [lead], Investigation [equal], Methodology [lead], Supervision [equal], Validation [lead], Writing—review & editing [equal]), Maeli Sanchez (Formal analysis [supporting], Writing—review & editing [equal]), Catherine Wangen (Data curation [equal], Investigation [equal], Validation [equal], Writing—review & editing [equal]), and Brian Allan (Conceptualization [equal], Funding acquisition [equal], Methodology [equal], Supervision [equal], Writing—review & editing [equal])

Conflicts of interest. None declared.

Data Availability

Data from this study are available from Dryad Digital Repository: doi:10.5061/dryad.n2z34tn77. Cloud, 2025.

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