A Model to Predict Evaporation Rates in Habitats Used by Container-Dwelling Mosquitoes

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ABSTRACT Container-dwelling mosquitoes use a wide variety of container habitats. The bottle cap is often cited as the smallest container habitat used by container species. When containers are small, the habitat conditions can greatly affect evaporation rates that in turn can affect the species dynamics within the container. An evaporation rate model was adapted to predict evaporation rates in mosquito container habitats. In both the laboratory and field, our model was able to predict actual evaporation rates. Examples of how the model may be applied are provided by examining the likelihood of *Aedes albopictus* (Skuse), *Aedes aegypti* (L.), and *Culex pipiens pipiens* (L.) completing their development within small-volume containers under typical environmental conditions and a range of temperatures. Our model suggests that under minimal direct sunlight exposure, both *Ae. aegypti* and *Ae. albopictus* could develop within a bottle cap before complete evaporation. Our model shows that under the environmental conditions when a plastic field container was sampled, neither *Ae. albopictus* or *Cx. p. pipiens* could complete development in that particular container before the water evaporated. Although rainfall could replenish the habitat, the effects of evaporation would increase larval density, which could in turn further decrease developmental rates.

KEY WORDS evaporation, evaporation rates, containers, bottle cap, container mosquitoes

The size of a container affects which mosquito species will use it for oviposition. Carrieri et al. (2003) found that *Aedes albopictus* (Skuse) were most common in small containers (7.7 ± 28.6 liters), whereas *Culex pipiens* (L.) were most abundant in larger containers (100.2 ± 387.8 liters). However, it is not uncommon to find both species co-occurring in nature. Costanzo et al. (2005) showed that *Ae. albopictus* and *Cx. pipiens* frequently co-occur in tire habitats in Illinois. Carrieri et al. (2003) found *Ae. albopictus* and *Cx. pipiens* co-occurring in tires with a mean volume of 1.2 liters and containers as small as 0.5 liter. The size of a container also can influence the ecology of the container, where large containers provide abundant resources, and small containers are better exploited by species adapted to frequent flooding events.

The frequency of precipitation, as well as evaporation, contributes to egg hatch in container *Aedes* species. Edgerly et al. (1993) found that for most container *Aedes* species, the reduction in dissolved oxygen content, as influenced by an increase in the microbial community, induces egg hatch after a period of flooding. However, because *Ae. albopictus* eggs hatch immediately after flooding, they could feed immediately on the microbes present, increasing the dissolved oxygen content (Edgerly et al. 1993). They also found

that this behavior actually inhibits eggs of other species from hatching, providing a competitive advantage to *Ae. albopictus* in container habitats. However, in the absence of rainfall, *Ae. albopictus* eggs accumulate, which can open up resources to other species (Constanzo et al. 2005).

In education and outreach materials, the bottle cap is often cited as the smallest container habitat used by *Aedes* mosquitoes. A web search (Google, Mountain View, CA) for mosquito and bottle cap produced >207,000 results, mainly pertaining to the bottle cap as a larval habitat for mosquitoes. The bottle cap also is cited in the scientific literature as a source for *Aedes* mosquitoes (Norris 2004). The purpose of this study was to model evaporation rates in common habitats used by container inhabiting mosquitoes and to demonstrate how the model can be used to predict the likelihood of a mosquito [*Ae. albopictus*, *Aedes aegypti* (L.), and *Cx. p. pipiens*) completing development before total evaporation in small volume container habitats, such as a bottle cap and an upside down recycle bin.

Materials and Methods

Evaporation Rate Model. An evaporation rate model was modified from an EPA model [(0.106 * $U^{0.78}$ * $MW^{2/3}$ * A * P)/(82.05 * T)] to predict evaporation rates for hazardous substances (EPA 1987). The EPA model was adjusted to account for metric measurements, and number of hours of exposed sun-

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light. The resulting model was Loss ml/d = $[(U^{0.78} * MW^{2/3} * A * P * H)/(k * T)]$, where U is the wind speed (kilometers per hour [kmph]/1.61), MW is the molecular weight of water, A is the exposed surface area of the container in centimeters, divided by 0.00107, P is the vapor pressure, H is the number of hours of exposed daylight, k is a constant (26000), and T is the temperature in Kelvin. Vapor pressure (P) was calculated as $\log(P) = 8.07131 - ((1730.63)/(233.426 + T))$.

Laboratory Evaluation. A Darwin Incubator (Darwin Chambers Company, St. Louis, MO) was used for all laboratory studies. A comparison of evaporation rates in oak infused and regular tap water was compared, by using five replicates each over a 6-d period. Oak-infused water consisted of 5 g of oak (*Quercus* sp.) leaves per 8 liters of dechlorinated water, which was placed within a 121-liter (32-gallon) bin over a 1-wk period. All experimental cups (8 cm in diameter), contained a volume of 200 ml. Evaporation rates were calculated daily by taking the weight in grams of the container and subtracting it from the weight 24 h previously. In a second study, evaporation rates were measured in the laboratory by using tap water with 12 different container types, consisting of three plastic, five glass, two paper, and two metal containers. Surface area of the containers ranged in size from 4.9 to 418 cm² and starting volumes ranged from 40 to 634 ml. Nine of the containers had surface areas that were completely exposed. Three containers were partially exposed by cutting sections from the lid. The goal of using different container materials was to make sure container material did not affect the outcome of the overall model. Therefore, the goal was to test different container scenarios to see whether the resulting evaporation rates were similar to that predicted in the model. Each scenario was repeated five times, over a 5-d period at both 24 and 26°C. For each container, the water surface area (centimeters), volume of water (milliliters), opening diameter (centimeters), and container material were recorded. The wind speed (meters per second) within the incubator was calculated using a ball bearing vane anemometer (Davis Instruments, Baltimore, MD) at 10 randomly selected spots within the incubator and averaged to a kmph calculation. Evaporation was allowed to occur in the incubator over a 24-h period. The volume loss (milliliters) for each container was recorded, along with the temperature (°C), photoperiod (L:D), and wind speed (meters per second) in the incubator.

Field Studies. Five different plastic containers, with increasing surface areas (7.6–56.7 cm²), were allowed to evaporate outdoors over a 1-wk period in Monmouth Co., NJ. The amount of direct sunlight was calculated using the U.S. Navy Oceanography's sun and moon data webpage (USNO 2009). Containers were placed in three locations: 1) full sun with 8 h of direct sunlight, 2) partial sun with 4 h of direct sunlight, and 3) partial shade with no direct sunlight. Evaporation rates were calculated during each crepuscular period by taking the weight in grams of the container. At each location, a portable weather station

(ibutton, Maxim innovation delivered, Sunnyvale, CA) calculated the temperature and humidity at 30-min intervals. Average daily temperatures were used. Daily evaporation rates were calculated for 6 d. No rainfall occurred during this period.

Statistical Analysis. The goal of the study was to determine whether the evaporation model could accurately predict evaporation rates in container habitats. Because we accounted for temperature, hours of direct sunlight, and container size and volume in the model, each container was considered a single point in testing the accuracy of the model. Because container material is not a variable included in the model, we compared differences between observed and expected values for each material by using a factorial analysis of variance (ANOVA) with milliliters per day per square centimeter surface area as the dependant variable. The evaporation model was compared with both laboratory and field measurements by using a linear regression analysis (SPSS 18, SPSS Inc., Chicago, IL). For containers that were partially covered, we determined evaporation rates by using surface area of the water and then the surface area of the opening to determine which value should be use in the model.

For field-collected data, we compared differences between shade conditions and container size by using ANOVA. For both field and laboratory data, we performed a curve estimation regression to determine whether the relationship between actual and predicted rates were linear. We ran two separate linear regression analyses for both field and lab experiments, with and without forcing the line through the origin. To determine the likelihood of *Ae. albopictus* or *Cx. p. pipiens* being able to complete development in a field-collected container (5 October), the volume (643.5 ml) and surface area (506.7 cm²) of a container where both species occurred in the field (upside down recycle bin), were inserted into the model at increasing temperatures. This container was chosen because it represents a typical habitat in the field, and represented one of the smallest volume containers where both species occurred. For the model, we used a typical wind speed of 1.61 kmph and a direct sun exposure level of 4 h, both of which were characteristic of the habitat where the container was found. To determine developmental rates for *Ae. albopictus*, temperature-dependent rates were estimated from the literature (Hawley 1988, Alto and Juliano 2001, Kamimura et al. 2002, and Delatte et al. 2009). Developmental rates also were calculated for *Cx. p. pipiens* (Vinogradova 2000) and *Ae. aegypti* (Rueda et al. 1990, Tun-Lin et al. 2000, Bar-Zeev 1958) by using data from the literature. The resulting estimates were included, and curve estimation regressions were performed to determine the shape of the data and to create a continuous set of developmental rates (SPSS 18). We reversed the evaporation model to determine the parameters necessary for *Ae. albopictus* or *Ae. aegypti* to complete development in a bottle cap before total evaporation. The model was used to determine the maximum sun exposure allowed, given a 1.61-kmph wind speed, 7-ml

bottle cap, and temperature-dependent developmental rates of *Ae. albopictus* and *Ae. aegypti*.

Results

The evaporation rate model significantly predicted actual evaporation rates in both laboratory and field situations. We examined the difference between observed (mean $\pm$ SE, 0.40 $\pm$ 0.06) and predicted (0.46 $\pm$ 0.07) milliliters per day per square centimeter of surface area for different container materials and found no significant difference ($F = 0.25$; df = 1, 108; $P = 0.62$). We did not see a significant difference ($F = 1.6$; df = 3, 108; $P = 0.19$) between actual milliliters per day per square centimeter surface area for glass (0.33 $\pm$ 0.1), metal (0.66 $\pm$ 0.2), paper (0.35 $\pm$ 0.2), or plastic (0.33 $\pm$ 0.1) containers. There was no significant difference between daily evaporation rates (milliliters per day) of oak-infused water (mean $\pm$ SE, 10.1 $\pm$ 0.4) versus regular tap water (9.2 $\pm$ 0.4) ($F = 1.8$; df = 1, 39; $P = 0.18$). Based on these data, we continued to use tap water throughout the study for ease of comparison. Given that container material did not significantly affect predicted rates, a single point in the model consisted of observed and predicted evaporation rates, regardless of container material or size. To determine which surface area to use in the model (water surface area, or the area of the container opening that partially exposes water), we compared evaporation rates in containers that were partially covered. We found that exposed surface area ($R^2 = 0.93$) yielded a more accurate prediction than the surface area of the water itself ($R^2 = 0.7$), so this variable was included in all models.

Daily field evaporation rates were significantly different ($F = 7.6$; df = 2, 59; $P = 0.001$) between those in full sun (mean $\pm$ SE, 10.0 $\pm$ 0.4), compared with partial sun (5.2 $\pm$ 0.4) and full shade (4.5 $\pm$ 0.4). As expected, the diameter of the container also affected daily evaporation rates ($F = 27$; df = 4, 59; $P < 0.001$), with increased daily evaporation rates as container diameter increased. Daily evaporation rates were highest in 8.5 cm (mean $\pm$ SE, 14.5 $\pm$ 0.4) compared with 6.2 cm (7.8 $\pm$ 0.4), 4.5 cm (4.5 $\pm$ 0.4), 3.8 cm (3.3 $\pm$ 0.4), and 3.1 cm (2.9 $\pm$ 0.4).

For field observations, the relationship between actual and predicted rates of evaporation was found to be linear ($R^2 = 0.81$; $F = 248$; df = 1, 59; $P < 0.001$). When predicting field observations (Fig. 1), the model was slightly better when forced through the origin ($R^2 = 0.92$; $F = 715.0$; df = 1, 60; $P < 0.001$) than when not forced through the origin ($R^2 = 0.81$; $F = 248.4$; df = 1, 59; $P < 0.001$). The resulting equation was actual evaporative loss = 0.930 * predicted loss, with a 95% confidence interval for the slope between 0.860 and 0.999.

For laboratory observations, the relationship between actual and predicted rates of evaporation was found to be linear ($R^2 = 0.92$; $F = 673$; df = 1, 59; $P < 0.001$). When predicting the laboratory observations (Fig. 2), the model was also better, when forced through the origin ($R^2 = 0.95$; $F = 1,023.8$; df = 1, 60;
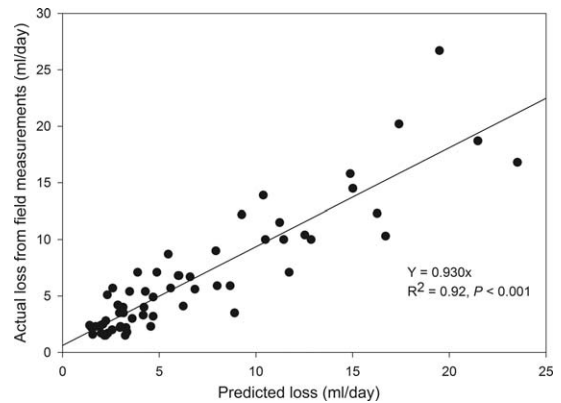

Fig. 1. Regression analysis of the predicted evaporation rates from the model (x-axis) and field measured evaporation rates (y-axis). Resulting equation is actual evaporative loss = 0.930 * predicted loss. The 95% confidence interval for slope was between 0.860 and 0.999.

$P < 0.001$) than when not forced through the origin ($R^2 = 0.92$; $F = 673.4$; df = 1, 59; $P < 0.001$). The resulting equation was actual evaporative loss = 0.926 * predicted loss, with a 95% confidence interval between 0.868 and 0.983.

In addition, we wanted to use the model to predict whether *Ae. albopictus* and *Cx. p. pipiens* could complete development in a typical container (Fig. 3) found in the field at different temperatures that are experienced throughout the mosquito season. For temperature-dependent rates of *Ae. albopictus*, a power curve was significant ($R^2 = 0.992$; $F = 361$, $P < 0.0001$) and allowed us to determine rates [(development rate = (16201) * (temperature)$^{-2.287}$) based on temperature. A similar curve was significant ($R^2 = 0.84$; $F = 73.5$, $P < 0.0001$) for *Cx. p. pipiens* [(development rate = (6099.5) * (temperature)$^{-1.924}$)], and *Ae. aegypti* ($R^2 = 0.88$; $F = 123.5$, $P < 0.0001$), [(development rate = (12209) * (temperature)$^{-2.169}$)].
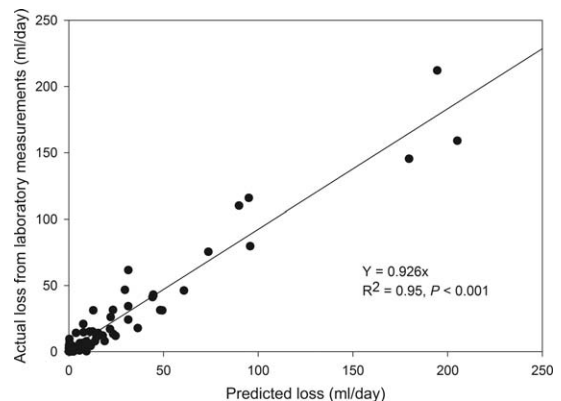

Fig. 2. Regression analysis of the predicted evaporation rates from the model (x-axis) and laboratory measured evaporation rates (y-axis). Resulting equation is actual evaporative loss = 0.926 * predicted loss. The 95% confidence interval for slope was between 0.868 and 0.983.
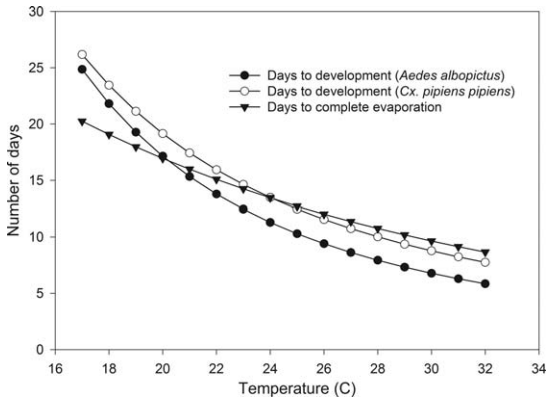
Fig. 3. Predicted evaporation rates in a field-collected container compared with length of development (days) for *Ae. albopictus* and *Cx. p. pipiens*. Lines with circles indicate temperature-dependent developmental rates for *Ae. albopictus* (black) and *Cx. p. pipiens* (white). The line with the triangle symbol indicates temperature dependent evaporation rates, by using parameters at collection site (1.61-kmph wind speed and 4 h of direct sunlight exposure).
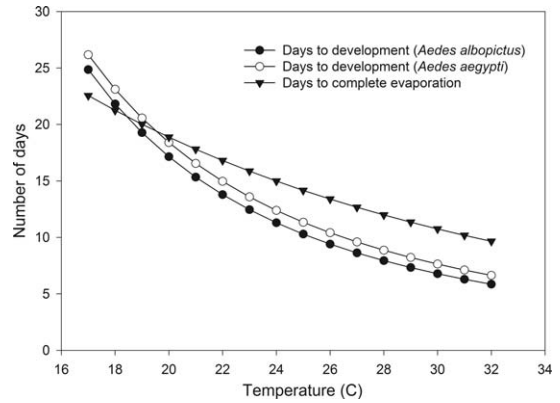
Fig. 4. Predicted evaporation rates in a bottle cap compared with length of development (days) for *Ae. albopictus* and *Ae. aegypti*. Lines with circles indicate temperature-dependent developmental rates for *Ae. albopictus* (black) and *Ae. aegypti* (white). The line with the triangle symbol indicates temperature dependent evaporation rates in the bottle cap by using 2.8 as the maximum number of hours of exposed sunlight for mosquitoes to complete their development at temperatures >19°C (average fall temperatures in New Jersey), before complete evaporation.

Without factoring in resources and competition, based on this model and the field conditions where this habitat occurred (15.5°C), there would not be sufficient time for either *Ae. albopictus* or *Cx. p. pipiens* to complete development before complete evaporation within the container occurred (Fig. 3).

To determine whether either *Ae. albopictus* or *Ae. aegypti* could use a bottle cap as a larval habitat, we reversed the model to determine the necessary parameters for both species to complete development. Using a wind speed of 1.61 kmph, we determined the maximum number of hours of exposed sunlight the bottle cap could be subjected to for both species to complete development before complete evaporation at temperatures >19°C (when we typically find *Ae. albopictus* larvae). This ranged from 2.7 (19°C) to 4 h (32°C) for *Ae. aegypti* and from 2.9 (19°C) to 4.6 h (32°C) for *Ae. albopictus*. At 2.8 h of sun exposure, both *Ae. aegypti* and *Ae. albopictus* could complete development in a bottle cap before complete evaporation at temperatures >19°C (Fig. 4).

Discussion

Our model significantly predicted actual field and laboratory evaporation rates. Although the model was tested in both the laboratory and the field, and with different container materials and diameters, it provided a useful estimate of daily evaporation rates. In the model, we did not account for precipitation that would naturally replenish containers throughout the season. As anticipated, there were slight variations between actual and predicted evaporation rates. One contributing factor is that the model assumes constant temperature and wind speed, and means were used in the calculations. However, variations occur in both time and space. In the field there could be slight variations in vegetation and shading that can create

microclimates. When measuring wind speed and temperature in the laboratory incubator, we also discovered slight microclimates. To compensate for these slight variations, we continually adjusted the position of containers in both the laboratory and the field.

We used a field-collected container (upside down recycle bin) as an example of a habitat where *Ae. albopictus* and *Cx. p. pipiens* can co-occur in nature. Our model predicts that it is unlikely that either species could develop into adults within this container habitat. However, there are situations (e.g., high amounts of shade), where mosquito larvae would have more time to complete development, due to lower evaporation rates. An example would be a situation where the container is sheltered from direct sunlight and wind, such as being hidden in the bushes, or having a cover to reduce evaporation. The container was sampled in fall in New Jersey, when mean temperatures are well <18°C, whereas summer mean temperatures range from 25 to 29.4°C. Therefore, during summer, this container would be a suitable habitat to complete development before total evaporation. It is also important to realize that developmental rates for *Ae. albopictus* and *Cx. p. pipiens* were determined from studies in the literature, which can vary based on habitat conditions, resources, and the strain of the mosquito. Limited resources and larval density can both affect developmental rates (Vinogradova 2000). Given that larval densities increase as water levels decrease, it is even more unlikely that either species could have developed within this habitat at the time it was sampled.

The bottle cap is often used as an example in outreach and education to reinforce the message that *Aedes* container species are capable of finding and exploiting small cryptic containers. Although we

found >207,000 Google hits citing the bottle cap as a potential larval habitat, we did not find any scientific studies demonstrating that mosquitoes could complete their development within this habitat. We determined that throughout most of the summer and fall in the United States, where mean temperatures are >19°C, both *Ae. albopictus* and *Ae. aegypti* could complete development within a bottle cap before total evaporation, as long as the bottle cap had minimal direct sunlight exposure (<2.9 h/d).

This evaporation model has many applied uses in mosquito biology and control. It can be used to determine the likelihood of a habitat producing mosquitoes, determining suitable container sizes for laboratory and field studies, determining how often to refill gravid traps and ovitraps in the field, determining whether larviciding a habitat is necessary given the current environmental conditions and can be incorporated into ecological modeling of different mosquito species.

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