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LARGE-VOLUME GRAVID TRAPS ENHANCE COLLECTION OF *CULEX* VECTORS

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ABSTRACT. Gravid mosquito collections were compared among several large-volume (infusion volume ≥35 liters) gravid trap designs and the small-volume (infusion volume = 6 liters) Centers for Disease Control and Prevention (CDC) gravid trap used routinely by vector control districts for vector and pathogen surveillance. The numbers of gravid *Culex quinquefasciatus*, *Cx. tarsalis*, and *Cx. stigmatosoma* collected by large gravid traps were greater than by the CDC gravid trap during nearly all overnight trials. Large-volume gravid traps collected on average 6.6-fold more adult female *Culex* mosquitoes compared to small-volume CDC gravid traps across 3 seasons during the 3 years of the studies. The differences in gravid mosquito collections between large- versus small-volume gravid traps were greatest during spring, when 8- to 56-fold more *Culex* individuals were collected using large-volume gravid traps. The proportion of gravid females in collections did not differ appreciably among the more effective trap designs tested. Important determinants of gravid trap performance were infusion container size and type as well as infusion volume, which determined the distance between the suction trap and the infusion surface. Of lesser importance for gravid trap performance were the number of suction traps, method of suction trap mounting, and infusion concentration. Fermentation of infusions between 1 and 4 wk weakly affected total mosquito collections, with *Cx. stigmatosoma* collections moderately enhanced by comparatively young and organically enriched infusions. A suction trap mounted above 100 liters of organic infusion housed in a 121-liter black plastic container collected the most gravid mosquitoes over the greatest range of experimental conditions, and a 35-liter infusion with side-mounted suction traps was a promising lesser-volume alternative design.

KEY WORDS Arbovirus, mosquitoes, gravid trap, oviposition

INTRODUCTION

Gravid mosquito sampling is a key component of vector surveillance that can enhance pathogen detection rates and improve the precision of predictive models used to guide vector control strategies (Williams and Gingrich 2007, Ginsberg et al. 2010). Gravid adult mosquitoes that have taken a blood meal are more likely to harbor blood-borne pathogens than are nongravid mosquitoes collected by other methods such as light traps and carbon dioxide–baited suction traps (Meyer et al. 2003).

Gravid traps used for *Culex* spp. surveillance have included a variety of trap designs (Allan and Kline 2004, Braks and Cardé 2007, Irish et al. 2013) and oviposition attractants (Reisen and Meyer 1990, Allan and Kline 2004, Irish et al. 2013). The Centers for Disease Control and Prevention (CDC) gravid traps and traps of similar design can effectively collect mosquitoes in the *Culex pipiens* L./*Cx. quinquefasciatus* Say species group that are important vectors of arboviruses in urban environments (Reiter 1983, 1987; Cummings 1992; Braks and Cardé 2007), but standard gravid traps containing ≤6 liters of hay/grass infusion provide variable gravid female interception rates due to interactions among infusion volume, trap surface area, and container color characteristics (Allan and Kline 2004, Irish et al. 2012).

Small-capacity gravid traps often fail to attract the diverse range of mosquito species capable of transmitting arboviruses. Despite different baiting strategies (Reisen and Meyer 1990, Meyer et al. 2003, DiMenna et al. 2006), important vectors such as *Culex tarsalis* Coquillett are rarely collected using standard gravid trap methodologies, even when substantial populations of host-seeking individuals (and presumably gravid individuals) are present (Walton and Workman 1998). Mosquito oviposition is modulated by a variety of physical as well as chemical factors (Clements 1999). *Culex tarsalis* is often associated with comparatively large, structurally complex habitats and responds to different habitat cues than does *Cx. quinquefasciatus*, the larvae of which occur often in comparatively structurally simple and organically enriched environments (Isoe and Millar 1995, Walton et al. 2009). Whereas both mosquito species colonized 1-m^2 microcosms, larvae of each species were associated with different water quality and vegetation regimes (Nguyen et al. 1999, Su and Mulla 1999, Duguma and Walton 2014).

Here we report the results of field trials comparing mosquito collections among 3 designs for large-volume (>35 liters) gravid traps as well as for large-volume gravid traps versus a small-volume gravid trap traditionally used in mosquito surveillance programs. The effects of container

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architecture, suction trap number and mounting, and infusion volume, age, and concentration on gravid mosquito collection were assessed. The performance of each trap design was evaluated using the abundance and reproductive status of adult female mosquitoes in suction trap collections and by the prevalence of egg rafts on the infusion surface.

MATERIALS AND METHODS

Field site

Gravid traps were tested at the University of California at Riverside (UCR) Aquatic and Vector Control Research Facility. The study area was surrounded by eucalyptus trees and citrus groves. Traps were located in an open, sunlit area within the facility. Four 32-m^2 wetland areas containing alkali bulrush (*Schoenoplectus* [=*Scirpus*] *maritimus* [L.] Lye) were adjacent to the study site.

Gravid traps

The CDC gravid trap (Model 1712; John W. Hock Company, Gainesville, FL; Fig. 1) containing 6 liters of oviposition infusion in a light-colored (beige) plastic dishpan (maximum volume = 15 liters, height = 20 cm, length = 39 cm, width = 32 cm) was considered a small-volume design. The CDC gravid trap (Reiter 1983), or a modified version of the trap (Cummings 1992), is widely deployed in mosquito surveillance programs. A single-suction trap was used with each gravid trap, except in a subset of studies during spring 2013, when 2 suction traps were placed over the basin holding the organic infusion.

Large-volume gravid traps were built using 2 types of containers (Fig. 1). Yellow, semitransparent fiberglass cylinders (diameter = 60 cm, height = 100 cm, maximum volume = 225 liters) were used during the 1st and 3rd years of this study. A dark-colored, plastic garbage can (Roughneck®; Rubbermaid, Fairlawn, OH; diameter = 56 cm, height = 72 cm, maximum volume = 121 liters) was the 2nd container type tested. The surface area of infusion reservoir in the large-volume gravid traps was approximately 2.3 (fiberglass cylinder with 150 liters of infusion) and 2.0 (plastic garbage can with 100 liters of infusion) times larger than the CDC gravid trap.

The number and orientation of suction traps on the large-volume gravid traps differed among the experiments. Either single-suction or multiple (2 or 3)–suction traps (height = 44 cm, inner diameter = 8 cm) with collection nets from CDC gravid traps were used in the trials. When suction traps were oriented vertically above the infusion in the large-volume gravid traps, the wooden rails (length = 40 cm) holding the suction trap were mounted on top of 2 wooden furring strips (1.9 cm × 3.8 cm × 61 cm) customized with shallow grooves to fit into the tops of the large-volume containers and placed 30 cm apart. When multiple suction traps were deployed on top of each large-volume gravid trap, they were placed equidistantly from each other along the length of the customized rails. In some experiments, infusion volume in the large gravid traps was reduced to 35 liters, and suction traps were mounted at approximately 60° through container walls to maintain a distance of 10 cm between suction traps and the infusion surface (Fig. 1). An additional suction trap mounting system composed of traps secured to wooden rails halfway down the insides of the large plastic containers was tested; however, the air flow (i.e., fan exhaust) within the gravid trap presumably caused this design to perform poorly, and it will not be discussed further. Each fan motor was powered by a rechargeable 6-V battery and ran continuously overnight (average duration = 18 h). Photocells on the suction traps were disabled.

Infusions

The infusion concentration and incubation time differed among the experiments. In order to attract species such as *Cx. tarsalis* that prefer oviposition sites containing less organic enrichment than does *Cx. quinquefasciatus* (Walton et al. 2009), the organic infusions used in most of the experiments were less concentrated than recommended for the CDC gravid trap (~4.3 g hay/liter tap water; http://johnwhock.com/products/mosquito-sandfly-traps/cdc-gravid-trap/): 0.1 g of alfalfa rabbit pellets (Nutriphase®; Pacific Coast Distributing, Inc., Phoenix, AZ)/liter of water was used to make low-concentration (LC) infusions. A high-concentration (HC) infusion used 1.0 g of alfalfa rabbit pellets/liter of water. Infusions using 2 g or 4 g of rabbit pellets/liter of water also were tested in some experiments. Infusions were covered and fermented for 1 to 4 wk in the large-volume gravid traps at the study site.

Large-volume fiberglass gravid traps

Two series of experiments tested performance of the large-volume fiberglass gravid traps. In the 1st series of 3 experiments, 8 large-volume fiberglass gravid traps were equally spaced across a 5 m × 2 m concrete slab during September–October 2011. Two treatments were randomly assigned to the gravid traps in each experiment. The 1st experiment compared the number of mosquitoes collected by a single-suction trap above 2 LC volumes: 50 or 150 liters. The 2nd and 3rd experiments compared the efficacy of 1

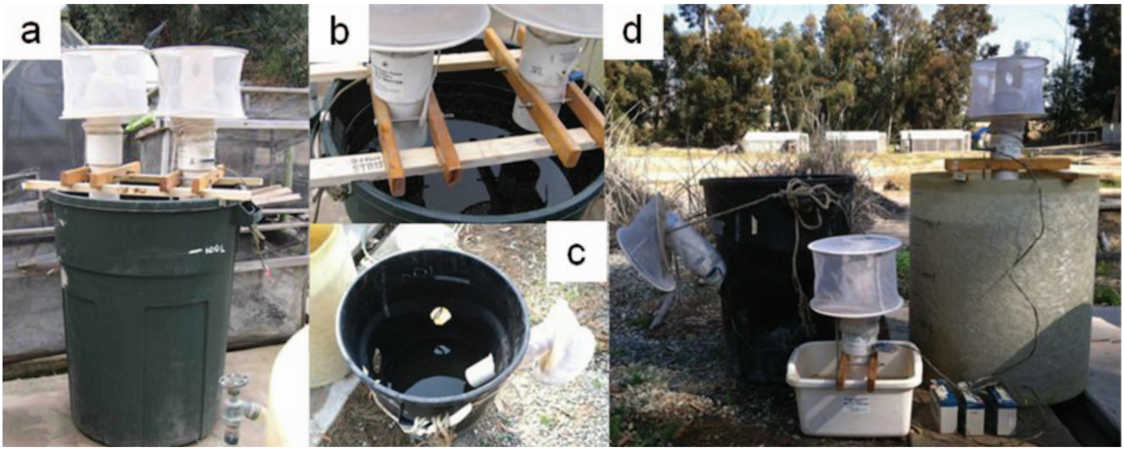

Fig. 1. Examples of gravid trap designs tested at the University of California at Riverside Aquatic and Vector Control Research Facility: (a) large plastic gravid trap with top-mounted suction traps; (b) close-up of top-mounted suction traps; (c) side-mounted suction trap in a large plastic gravid trap; and (d) 3 gravid trap designs: large-volume plastic gravid trap with side-mounted suction traps (left), large-volume fiberglass trap (right), and small-volume Centers for Disease Control and Prevention (CDC) gravid trap (center).

or 2 top-mounted suction traps above the 2 LC infusion volumes (50 vs. 150 liters).

The 2nd series of experiments assessed the effect of infusion age on the number of mosquitoes collected by large-volume fiberglass gravid traps relative to small-volume CDC gravid traps at 4 sites spaced an average of 30 m apart during September–October 2011. Low-concentration infusions were fermented in covered fiberglass drums for 1, 2, 3, or 4 wk. Within each site, each bait–age treatment was assigned randomly to large-volume fiberglass gravid traps that were separated by 2 m. On the 1st evening of the experiment, the large-volume gravid traps were uncovered, and 6 liters of infusion (1.5 liters from each age treatment) were transferred to a paired small-volume gravid trap. A single-suction trap was top-mounted on both large and small gravid traps.

Large-volume plastic gravid traps

Gravid mosquito collections by large-volume black plastic gravid traps containing 100 liters of LC fermented for 10 days were compared to small-volume gravid traps during May–June 2012. Two top-mounted suction traps were used on each large-volume gravid trap. Pairwise comparisons of mosquito collections by large- and small-volume gravid traps at 5 replicate sites were made.

The impact of infusion and trap characteristics on trapping efficacy for the 3 gravid trap designs was examined in experiments carried out between May and October 2013. The 1st series of experiments compared mosquito collections by large-volume gravid traps using different numbers of suction traps (1, 2, or 3), infusion volumes (6, 12, 35, 100, and 150 liters), and infusion concentrations (0.1, 1, 2, and 4 g rabbit pellets/liter). Because a limited number of gravid traps was available for each experiment, a subset of the total treatment combinations was examined in each test. Infusions were aged an average of 9 days. When both large gravid trap designs were deployed simultaneously, an equal volume of infusion (3 liters) was transferred from each large trap design into an adjacent small-volume gravid trap.

The presence–absence (to assess the inherent attractiveness of particular gravid trap design configurations to resting and egg-laying mosquitoes) and orientation of suction traps were examined during September and October 2013. Egg raft abundance in gravid traps with functioning suction traps was compared to gravid traps without suction traps or with suction traps that were present but turned off. Mosquito collections by the large plastic gravid traps that had been modified for side-mounted suction traps were compared to gravid traps with the standard top-mounted suction traps in a 2nd group of experiments. Standard infusion-bait volumes were used for small (6 liters), large plastic (100 liters), and large fiberglass (150 liters) gravid traps. Thirty-five liters of infusion were used in the large plastic gravid traps with side-mounted suction traps to homogenize trap-to-infusion surface distance ($\sim$10 cm). An HC infusion fermented for 9 days was used in these experiments.

Trapping sites for the 2012 and 2013 experiments were separated by an average of 15 m. The different gravid traps were placed in a circle (separated by $\sim$2 m) on the aforementioned concrete slab and at 3 sites northwest of nursery wetlands containing *S. maritimus*.

Sample processing

Adult mosquitoes collected by gravid traps were transported to the laboratory and frozen at −20°C. Specimens were sorted on a chill table by gender and reproductive state (gravid, bloodfed, nongravid, unknown), and to species using the classification of Meyer and Durso (1998). Reproductive state was not assessed in specimens from 2011. Females containing a blood meal and visible eggs were lumped in a single "gravid" category in 2012, but were placed into 2 distinct categories in 2013. Egg rafts, when present, were counted and removed on the morning of trap net collection.

Statistical analysis

Gravid trap data were pooled across suction traps when multiple suction traps were present on, or in, a gravid trap and across dates for a replicate trap when gravid mosquitoes were collected across multiple nights during a particular experiment. Data that met the assumptions of parametric statistical analyses were $\log_{10}(x + 1)$ transformed, and the significance of differences in gravid mosquito collections among trap designs was analyzed by analysis of variance (ANOVA). A post-hoc Tukey's honest significant difference (HSD) test was carried out if the ANOVA was significant ($P < 0.05$). Data that did not meet the assumptions of ANOVA were analyzed using a nonparametric Kruskal–Wallis ANOVA (more than 2 variables) or Mann–Whitney U-test (2 variables). Experiments comparing infusion fermentations and seasonal variation in mosquito collections during 2012 required separation of sample dates in the statistical analysis; a repeated-measures multivariate ANOVA (MANOVA) was carried out. Date effects were tested using Pillai's trace statistic (SYSTAT version 9.0; SPSS Inc., Chicago, IL; SPSS 1998).

To assess the relative importance of the different trap design components on gravid mosquito collection, a standardized effect size was calculated using Cohen's d for pairwise comparisons (Cohen 1977). The number of mosquitoes collected nightly was $\log_{10}(x + 1)$ transformed. The standard deviation of the distribution of difference scores for paired observations at each sampling site was used in calculations. Gravid trap size (large vs. small), number of suction traps per gravid trap, suction trap mounting (top-mounted suction traps above 100 liters of infusion vs. side-mounted suction traps above 35 liters of infusion in large plastic gravid traps), infusion bait volume in large gravid traps (effect of distance between the suction trap and the infusion surface), duration of infusion fermentation (age), and infusion concentration were evaluated. Pairwise comparisons of mosquito collections were made among all infusion ages (older vs. younger) tested on a particular date. Effect size for 6 trap design components was based on the differences in the total number of *Culex* females and for each of the 3 *Culex* species in gravid trap collections. A trap component had a significant impact on gravid mosquito collection if the 95% confidence limits for mean effect size of multiple experiments investigating a particular trap component did not overlap zero.

RESULTS

Large-volume fiberglass gravid traps

The numbers of gravid mosquitoes collected by large-volume fiberglass gravid traps averaged 3 females per trap-night during autumn 2011 and were the lowest of all studies (Fig. 2). Despite low numbers of mosquitoes in collections, large-volume fiberglass gravid traps collected significantly more mosquitoes than did small-volume gravid traps (LC: total *Culex*: $F_{1, 59} = 40.41$, $P < 0.001$; *Cx. quinquefasciatus*: $U = 705.5$, $P < 0.001$) and collected *Cx. tarsalis*, whereas the small-volume gravid traps failed to do so. Very few adult female mosquitoes (<0.1 individuals per trap night) were collected, and egg rafts were not observed in small-volume gravid traps.

The number of mosquitoes collected by large-volume fiberglass gravid traps was affected by infusion volume, but not by the number of suction traps per gravid trap (1 or 2) or by infusion age (LC fermented for 1, 2, 3, or 4 wk). Large-volume gravid traps with 150 liters of infusion collected 3-fold more adult *Cx. quinquefasciatus* females ($U = 20.00$, $P < 0.05$) and 5-fold fewer *Culex* spp. egg rafts than did fiberglass gravid traps with 50 liters of infusion (Fig. 3; $U = 14.0$, $P < 0.05$). The number of suction traps per gravid trap did not significantly alter the number of female mosquitoes collected per gravid trap (Mann–Whitney U-tests, $P > 0.65$); however, the number of egg rafts in gravid traps with 1 suction trap was 5-fold greater compared to fiberglass gravid traps with 2 suction traps ($U = 48.0$, $P < 0.05$). Infusion age did not significantly influence adult mosquito or egg raft collections (infusion age: $F_{3, 59} < 0.03$, $P > 0.8$; trap type × infusion age: $F_{3, 59} < 0.1$, $P > 0.98$).

Large-volume plastic gravid traps

Trap size: Mosquitoes were at intermediate abundance during spring 2012 compared to other years, and large-volume gravid traps collected approximately an order of magnitude more mosquitoes than did small-volume gravid traps using LC as the oviposition attractant (Fig. 2; $F_{1, 8} = 97.34$, $P < 0.001$). The large-volume, dual-suction trap design collected at least an

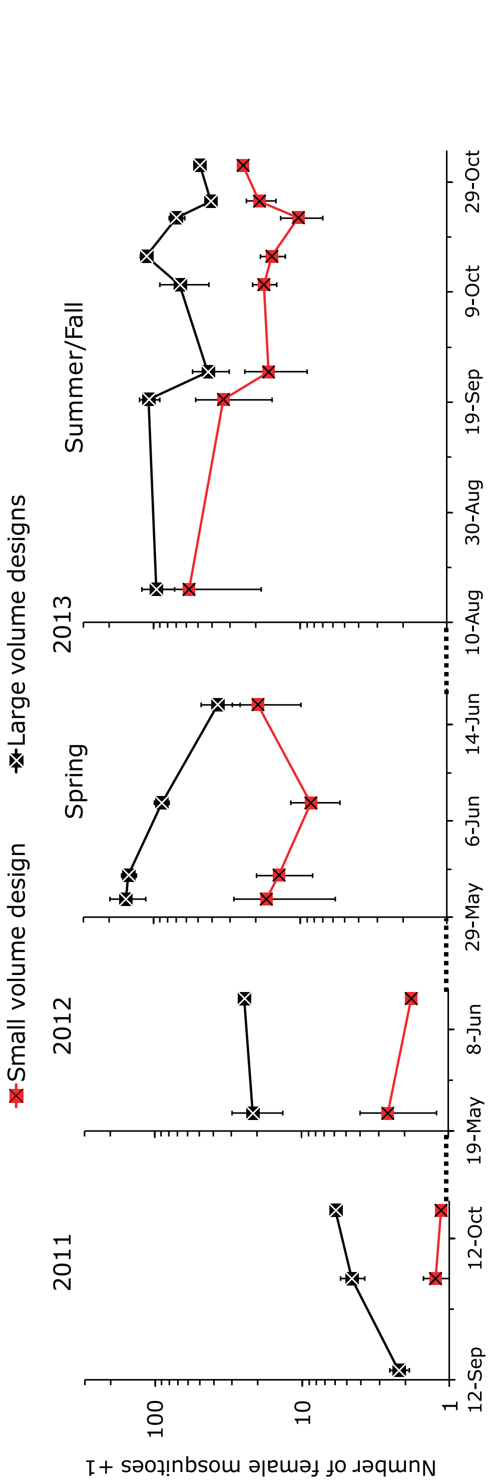

Fig. 2. Abundance (mean ± SE) of adult female *Culex* mosquitoes in small-volume and large-volume gravid traps, 2011–13. Dotted lines indicate where time line is condensed for illustration purposes.

order of magnitude more *Cx. quinquefasciatus* ($F_{1,\ 8} = 111.9$, $P < 0.001$) and *Cx. tarsalis* ($U = 293.5$, $P = 0.002$) females than did the small-volume, single-suction trap design.

Culex quinquefasciatus was most abundant in gravid trap collections (86% of total), followed by *Cx. tarsalis* (7%), *Cx. stigmatosoma* Dyar (4%), and unidentified damaged specimens (3%) in experiments during 2012. About 79% of female mosquitoes were gravid (included bloodfed specimens), 15% were nongravid, and 6% were of unknown reproductive state (i.e., specimen destroyed by the fan; Fig. 4). The percentage of the female mosquitoes that was gravid did not differ by mosquito species, gravid trap design, site, or sample date (F test or Kruskal–Wallis test, $P > 0.10$).

Mosquito abundance during 2013 was the highest of all years in our study, and large-volume gravid traps collected 4-fold greater numbers of gravid mosquitoes compared to small-volume gravid traps (Fig. 2). The relative difference in collection size between trap types varied by sample season, mosquito species, and experimental modifications.

During spring (Fig. 2), the large-volume plastic gravid trap with dual-suction traps and an infusion (LC) volume of 100 liters collected at least 8-fold more adult females of the 3 predominant *Culex* species compared to the small-volume gravid traps ($F_{1,\ 18} = 35.21$, $P < 0.001$). The difference in the number of *Cx. tarsalis* collected between the 2 gravid traps was even more striking (56-fold; $U = 83.00$, $P < 0.005$). Later in the year, the large-volume gravid traps with dual-suction traps collected 2- to 3.6-fold more *Cx. quinquefasciatus* (summer: $F_{1,\ 11} = 6.03$, $P < 0.035$; autumn: $U = 80.0$, $P = 0.013$) and *Cx. stigmatosoma* (autumn: $U = 80.5$, $P = 0.011$) females compared to the small-volume CDC traps. *Culex tarsalis* abundance was comparatively low and did not differ between trap designs during summer and autumn (Mann–Whitney *U*-tests, $P > 0.30$).

Egg rafts were collected from large-volume gravid traps in all trials. Egg rafts were present in the small-volume gravid traps only during spring trials, but they were 40-fold fewer than in the large-volume gravid traps ($U = 89.0$, $P < 0.001$).

Number of top-mounted suction traps

The total number of *Culex* females collected per gravid trap was not influenced by the number of suction traps per large-volume gravid trap (1 vs. 2 suction traps: $U = 24.0$, $P > 0.6$). Large-volume gravid traps with a single-suction trap, however, collected 3-fold more egg rafts than did the dual-suction trap design ($U = 56.0$, $P < 0.001$).

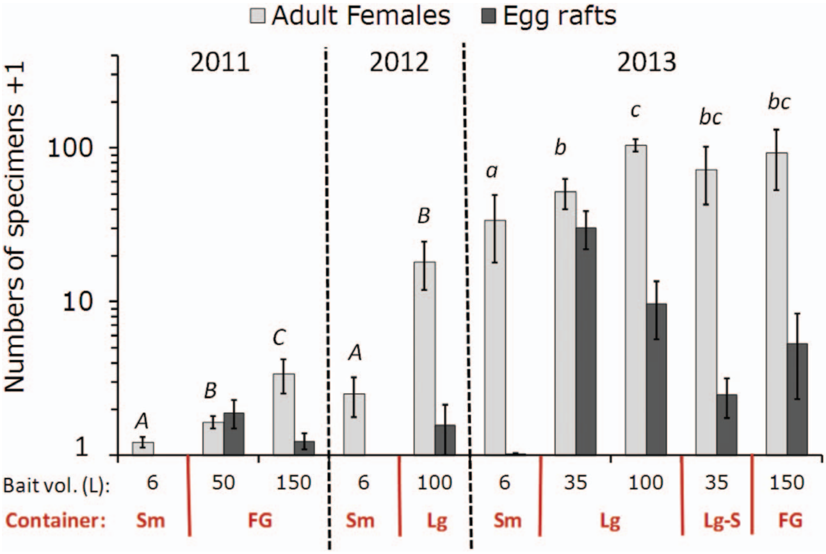

Fig. 3. Abundance (mean ± SE) of female mosquitoes and egg rafts per sample night among years, infusion volumes, and gravid trap designs. Sm = small-volume gravid traps. Lg = large-volume plastic gravid traps with top-mounted suction traps. Lg-S = large-volume plastic gravid traps with side-mounted suction traps. FG = large-volume fiberglass gravid traps. Letters indicate significant differences of gravid mosquito collections: Capital letters indicate Kruskal–Wallis analysis of variance (ANOVA) $P < 0.05$, and lowercase letters indicate Tukey's honest significant difference (HSD) test $P < 0.05$.

Infusion concentration

Adult female numbers in traps above HC bait (2 g/liter) were 2- to 4-fold more abundant than in traps above LC bait (0.1 g/liter), and this differential was significant for *Cx. quinquefasciatus* and *Cx. stigmatosoma* (Mann–Whitney *U*-tests, $P < 0.01$). Egg raft numbers in gravid traps were not related to infusion concentration (Mann–Whitney *U*, $P = 0.665$)

Infusion volume

Large-volume and small-volume traps with single-suction traps placed over 6 liters of infusion trapped similar adult numbers ($F_{1,\ 17} = 1.29$, $P > 0.27$), even though egg rafts were abundant in the large-volume gravid traps and were absent in the small-volume gravid traps ($U = 90.0$, $P < 0.001$). During autumn 2013, the large-volume plastic gravid trap with a top-mounted suction trap (100 liters of HC infusion) was the most effective mosquito collector of 5 gravid trap designs tested (all top-mounted suction traps: large-volume plastic trap with 12 or 35 liters of infusion, large-volume fiberglass traps with 150 liters of infusion, CDC gravid traps) and averaged 2- to 6-fold more adult female mosquitoes than the small-volume gravid traps ($F_{4,\ 55} = 21.94$, $P < 0.001$). Significantly greater numbers of each of the 3 *Culex* species were collected by the large-volume traps as compared to the small-volume gravid trap (*Cx. quinquefasciatus*: $F_{4,\ 55} = 23.20$, $P < 0.001$; *Cx. tarsalis*: $H_4 = 12.8$, $P = 0.012$; *Cx. stigmatosoma*: $H_4 = 25.7$, $P < 0.001$).

Egg raft abundance in large-volume gravid traps was overall higher than in small-volume gravid traps during 2013 (Fig. 3). Large-volume plastic gravid traps with 35 liters of infusion and top-mounted suction traps contained 4-fold more egg rafts compared to the large-volume gravid trap design with the next highest number of egg rafts (100 liters of infusion; $H_4 = 32.67$, $P < 0.01$).

Inherent structural attraction

When suction traps were absent, large-volume plastic gravid traps containing 35 liters of infusion also collected the most egg rafts (ANOVA, $P < 0.05$; Fig. 5); however, gravid traps with nonoperational suction traps (top-mounted) over 35 liters of infusion collected similar numbers of egg rafts compared to gravid traps holding 100 liters of infusion (Tukey's HSD, $P > 0.05$). Nonoperational side-mounted suction traps did not significantly alter oviposition (data not shown).

Suction trap mounting

Side-mounted suction traps above 35 liters of infusion and top-mounted traps above 100 liters of infusion captured similar quantities of adult mosquitoes in experiments during October–November

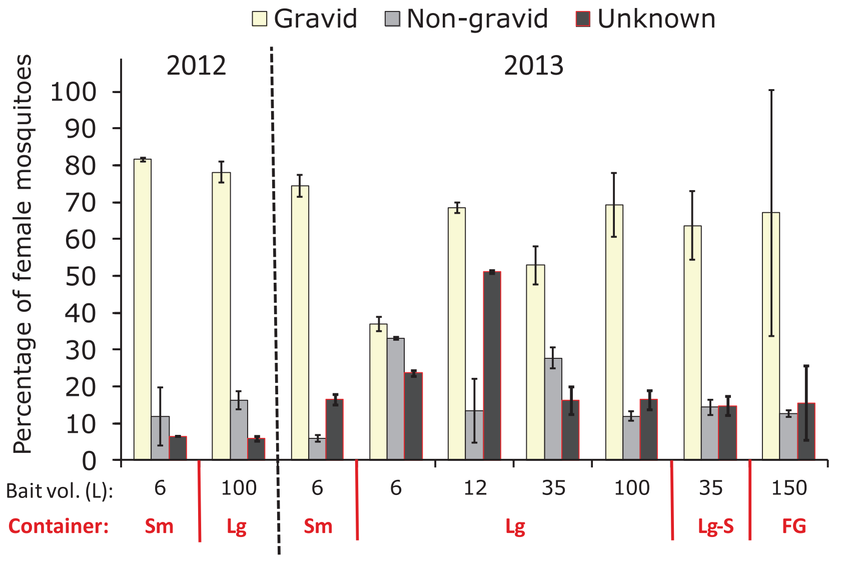

Fig. 4. Percentages (mean ± SE) of gravid, nongravid, and unknown fecundity for female mosquitoes in gravid trap collections from 2012 and 2013. Gravid numbers included females with visible eggs or blood meals. Sm = small-volume gravid traps. Lg = large-volume plastic gravid traps with top-mounted suction traps. Lg-S = large-volume plastic gravid traps with side-mounted suction traps. FG = large-volume fiberglass gravid traps.

2013 (Fig. 3). The numbers of total *Culex* females, *Cx. quinquefasciatus*, and *Cx. stigmatosoma* females in both large-volume trap designs exceeded the small-volume traps by more than 7-fold (total *Culex*: $F_{2,\ 15} = 28.94$, $P < 0.001$; *Cx. quinquefasciatus*: $F_{2,\ 15} = 30.91$, $P < 0.001$; *Cx. stigmatosoma*: $H_2 = 12.94$, $P < 0.01$); however, the abundance of *Cx. tarsalis* did not differ statistically among the 3 trap designs ($H_2 = 2.38$, $P > 0.30$). The number of side-mounted suction traps (1, 2, or 4) per container did not affect the numbers of adult mosquitoes or egg rafts collected per gravid trap (*U*-tests, $P > 0.13$). *Culex quinquefasciatus* was the predominant species (78% of total), with lesser abundance of *Cx. stigmatosoma* (9%), *Cx. tarsalis* (7%), *Culiseta* spp. (2%: *Cs. inornata* [Williston] and *Cs. incidens* [Thomson]), and unidentified damaged specimens (4%).

Side-mounted suction traps reduced the number of egg rafts in the gravid traps by more than half ($H_2 = 10.88$, $P = 0.004$) compared to the top-mounted suction traps on the large-volume gravid trap. Gravid traps with >2 suction traps were nearly devoid of egg rafts, while gravid traps with 1 suction trap contained an average of 2 egg rafts per night. Egg rafts were absent in the small-volume gravid traps during this series of experiments.

Side-mounted suction traps collected twice as many adult males compared to the trap design with top-mounted suction traps (ANOVA: $F_{2,\ 78} = 93.76$, $P < 0.001$; Tukey HSD: $P = 0.026$). Males were rare in small-volume gravid traps.

Reproductive state of females

During 2013, the reproductive profile of trapped female mosquitoes was 56% gravid, 10% with recent blood meal, 15% nongravid, and 19% of unknown reproductive status. In general, small- and large-volume (>6 liters of infusion) gravid traps collected similar proportions of gravid and bloodfed *Culex* mosquitoes (Fig. 4) and the dominant species, *Cx. quinquefasciatus* (Mann–Whitney *U*-tests, $P > 0.40$); however, the proportions of gravid and blood-fed *Cx. stigmatosoma* and *Cx. tarsalis* in collections from the large-volume gravid traps were between 2- and 4-fold higher compared to small-volume gravid traps (Mann–Whitney *U*-tests, $P < 0.02$). Interestingly, the proportions of gravid plus bloodfed *Cx. quinquefasciatus* females collected by large-volume gravid traps declined markedly when the infusion volume was 6 liters (Fig. 4; 40% vs. 73%; Mann–Whitney *U*-tests, $P < 0.01$). Regardless of infusion volume, the numbers of nongravid *Culex* mosquitoes, as well as each of the 3 *Culex* species, were 2 to 3 times higher in large-volume gravid traps compared to the small-volume traps (Mann–Whitney *U*-tests, $P < 0.02$).

The number and position of suction traps affected the distribution of *Culex* females among the reproductive status categories collected by large-volume gravid traps. The large-volume gravid trap with a 100-liter (LC) infusion and a single top-mounted suction trap collected proportionally fewer gravid *Cx. quinquefasciatus* and a greater abundance of nongravid mosquitoes compared to the same trap with 2 suction

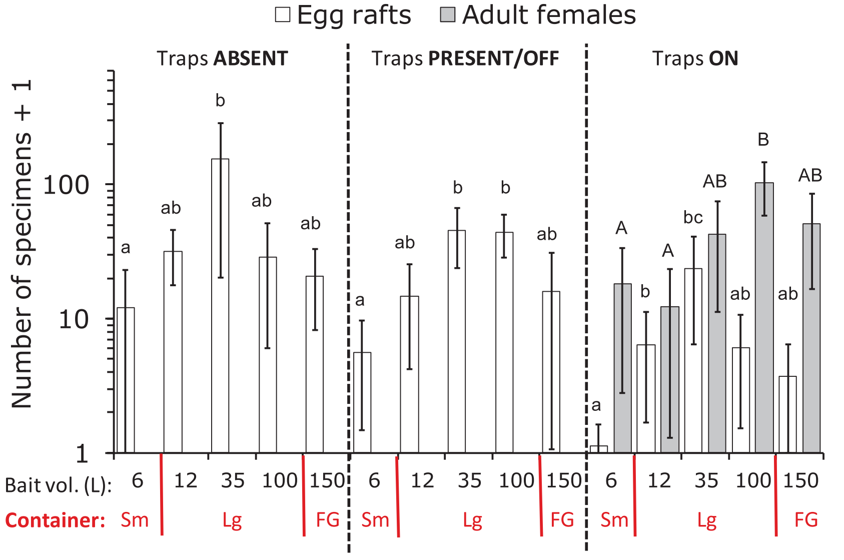

Fig. 5. Abundance (mean ± SD) of egg rafts and/or female mosquitoes during autumn 2013 when suction traps were absent (left panel), were present and turned off (middle panel), and were turned on (right panel) within different gravid traps and infusion volumes. Sm = small-volume gravid trap. Lg = large-volume plastic gravid trap. FG = fiberglass gravid trap. Letters indicate significant differences of gravid mosquito collections: lowercase (egg rafts) and capital (adults) letters indicate Tukey's honest significant difference (HSD) test $P < 0.05$.

traps (Mann–Whitney *U*-tests, $P < 0.03$). The opposite trend was found for large-volume gravid traps with 35-liter infusions and side-mounted suction traps. Single side-mounted suction traps collected larger numbers of gravid *Cx. quinquefasciatus* and smaller numbers of nongravid mosquitoes than did traps with 2 side-mounted suction traps (Mann-Whitney *U*, $P < 0.05$). The proportions of mosquitoes in the categories of reproductive status did not differ significantly when either 1 or 2 suction traps were used on small-volume gravid traps (Mann–Whitney *U*, $P > 0.40$). Infusion concentration did not affect the proportions of gravid and nongravid mosquitoes in large-volume gravid traps with top-mounted suction traps (Mann–Whitney *U*, $P > 0.10$).

Relative importance of gravid trap components

Large-volume gravid traps collected more *Culex* mosquitoes, and more of each of the 3 *Culex* species, than did small-volume gravid traps across experiments during the 3 years of investigation, with effect size ranging between 1.00 and 2.45 (Table 1: Size). Infusion volume in the large-volume gravid traps had the largest effect on the total number of *Culex* mosquitoes in collections and relates to the distance between the suction trap and the infusion surface. Suction traps positioned approximately 10 cm above the infusion surface collected more gravid mosquitoes than when the distance between the infusion surface and the opening to the suction trap was >10 cm. Collections of total *Culex* females for the 2 best gravid trap designs (top-mounted suction traps above 100 liters of infusion vs. side-mounted suction traps above 35 liters of infusion) did not differ; although top-mounted suction traps collected markedly more *Cx. stigmatosoma* mosquitoes (Table 1: Suction trap mounting). Averaging effect size across multiple experiments, large gravid traps with 1 suction trap collected more *Culex* mosquitoes than did gravid traps with multiple suction traps, but these effects were based on comparatively few experiments (Table 1).

Comparatively concentrated infusions (2 g/liter vs. 0.1 g/liter) strongly attracted *Cx. stigmatosoma*. A large effect of infusion concentration was also observed for *Cx. quinquefasciatus*; however, despite a response of gravid females that differed from random (black plastic gravid trap infusion concentration: Mann–Whitney *U*), the 95% confidence limits of effect size barely overlapped 0.

Gravid trap size was the most important trap design component influencing the size of *Cx. tarsalis* and *Cx. quinquefasciatus* collections. For *Cx. stigmatosoma*, organic matter in the infusion and top-mounted suction traps on large-volume gravid traps had larger effects on mosquito collections than did gravid trap size, which nevertheless was highly significant. The numbers of *Culex* (species combined), *Cx. tarsalis*, and *Cx. stigmatosoma* females collected above younger infusions were larger than above comparatively older infusions, but these effects were weak compared to gravid trap size.

Table 1. Mean effect size (lower 95% confidence interval [CI], upper 95% CI; number of experiments) of trap design components on the number of mosquitoes collected by gravid traps. Significant effects are in bold font.

Trap design component	*Culex* (species combined)	*Cx. tarsalis*	*Cx. quinquefasciatus*	*Cx. stigmatosoma*
Infusion age[1]	**−0.26 (−0.50, −0.02; 13)**	**−0.16 (−0.29, −0.02; 13)**	−0.15 (−0.44, 0.14; 13)	**−0.69 (−1.03, −0.36; 3)**
Infusion concentration[2]	**2.02 (0.38, 3.65; 3)**	1.09 (−0.82, 3.00; 3)	1.94 (−0.03, 3.90; 3)	**2.15 (0.87, 3.43; 3)**
Infusion volume (large gravid traps)[3]	**2.86 (0.05, 5.68; 13)**	**0.85 (0.19, 1.51; 11)**	3.08 (−0.74, 6.89; 12)	**1.43 (0.068, 2.18; 10)**
Suction trap mounting[4]	0.44 (−0.15, 1.04; 7)	**0.54 (0.07, 1.02; 7)**	0.29 (−0.30, 0.89; 7)	**2.01 (0.80, 3.24; 7)**
Number of suction traps[5]	**−0.99 (−1.85, −0.12; 5)**	−2.26 (−3.27, 0.75; 4)	**−0.95 (−1.50, −0.40; 5)**	**−0.74 (−1.44, −0.04; 5)**
Size[6]	**2.41 (1.89, 2.94; 31)**	**1.00 (0.47, 1.54; 28)**	**2.45 (1.83, 3.07; 31)**	**1.63 (1.17, 2.09; 26)**

[1] Effect size comparison: older vs. younger for infusions aged 1, 2, 3, or 4 wk.
[2] Effect size comparison: more concentrated vs. less concentrated for 4, 2, and 0.1 g rabbit pellets/liter.
[3] Effect size comparison: larger volume vs. smaller volume for 100 versus 35, or 12, and 150 versus 50 liters.
[4] Effect size comparison: top-mounted suction traps, 100 liters vs. side-mounted suction traps, 35 liters in black plastic gravid traps.
[5] Effect size comparison: 2 (or 3) suction traps vs. 1 suction trap per gravid trap.
[6] Effect size comparison: large gravid trap (infusion = 100 or 38 liters) vs. small gravid trap (infusion = 6 liters).

DISCUSSION

Large-volume gravid traps collected on average 6.6-fold (based on back-transformed means) more adult female *Culex* mosquitoes compared to small-volume CDC gravid traps during the 3 years of these studies. Infusion container size and infusion volume within large-volume gravid traps were significantly associated with changes in the size and species composition of mosquito collections. Twice as many *Cx. quinquefasciatus* and *Cx. stigmatosoma* females were collected above comparatively concentrated infusions (2 g vs. 0.1 g rabbit pellets/liter). Suction trap number and mounting style, and infusion age, contributed less to the observed differences in mosquito collections between gravid trap designs.

Among the large-volume trap design component combinations tested, 2 black-plastic container designs, each with a different suction trap mounting style and infusion volume, were most effective at gravid mosquito monitoring. Top-mounted suction traps and 100 liters of infusion provided the greatest yields for the combined abundance of all mosquito species as well as gravid *Cx. tarsalis* and *Cx. stigmatosoma* females. This gravid trap configuration was the most effective at collecting mosquitoes across an order-of-magnitude increase in mosquito abundance between 2012 and 2013. The differences in the numbers and species of mosquitoes collected by large-volume gravid traps and CDC gravid traps were more pronounced in the spring compared to summer and autumn. This evidence suggests large-volume gravid traps filled with infusion volumes >6 liters have potential to enhance gravid mosquito collection and the detection of infected mosquitoes early in the annual activity period of vector mosquitoes. Early-season detections of arbovirus-infected mosquitoes can aid in the prediction of late-season severity of diseases such as those caused by West Nile virus (Ginsberg et al. 2010).

Black-plastic gravid traps with side-mounted suction traps over 35 liters of infusion and with top-mounted suction traps over 100 liters of infusion collected similar numbers of gravid *Culex* females. One likely reason for this result was that the side-mounted suction traps were positioned at the same distance (about 10 cm) from the surface of the infusion as were the top-mounted traps placed above the surface of the 100-liter infusion. Suction trap to bait surface distance was a critical factor influencing adult mosquito catches. When infusion volume was reduced (<100 liters), gravid traps with top-mounted suction traps collected fewer gravid mosquitoes, a larger proportion of nongravid females, and more egg rafts. Increasing the distance from the suction trap to the bait surface reduced the suction force at the egg-laying surface and presumably facilitated escape and egg laying.

Large-volume black-plastic gravid traps with intermediate volumes of organic infusion may have also created a humid microenvironment within the trap that was favorable for mosquito resting and egg laying. High humidity is commonly preferred by resting adult mosquitoes and is associated with increased survival and fecundity (Clements 1963, Yamana and Eltahir 2013). The interior of the large-volume gravid trap was a region of comparatively high humidity. A poststudy comparison indicated that the 35-liter infusion formed a 20-cm-tall zone of constant relative humidity in the infusion container that was 10% higher than that measured concurrently near the surface of the 100-liter infusion (D. Popko, personal observation). Egg raft numbers in containers (suction traps absent) with 35 liters of infusion were about 10-fold larger compared to gravid traps with 100 liters of infusion. A calm microenvironment may have further affected the plume signal of trap baits and created an enriched zone of volatile chemical cues emitted during bait fermentation important in both long- and short-range mosquito oviposition behaviors (Isoe and Millar 1995, Cooperband and Cardé 2006).

The number of suction traps per large-volume gravid trap was not directly related to the total mosquito numbers in collections, such that total mosquito catches by 2 suction traps regardless of container type and mounting design did not exceed that by a single-suction trap. However, suction trap number was inversely related to the numbers of egg rafts on the infusion surface and the relative abundance of nongravid mosquitoes in collections from the large-volume gravid traps. For gravid traps with top-mounted suction traps and 100 liters of infusion, 2 suction traps decreased egg rafts and the proportion of nongravid female mosquitoes, especially *Cx. quinquefasciatus*, in collections compared to 1 suction trap. Dual-suction traps were probably more efficient than a single-suction trap at intercepting gravid mosquitoes before oviposition. Within particular experiments, suction trap number, however, was not linked to differences among collections of gravid *Cx. tarsalis* and *Cx. stigmatosoma* females. Yet, considering the effect size for suction trap number based on a small number of comparisons across the entire study, a single-suction trap appeared to perform better than did multiple suction traps per large-volume gravid trap for collecting gravid and nongravid *Cx. quinquefasciatus* and *Cx. stigmatosoma* mosquitoes. Further studies are needed.

To purposely target *Cx. tarsalis*, the infusion concentration used for our tests was typically lower than the 4 g/liter of organic matter/liter recommended for CDC-style traps; however, less concentrated baits were not more attractive, nor did strong baits (≤2 g/liter of organic matter) inhibit *Cx. tarsalis* collection. A possible consequence of using LC was to reduce the numbers of dirty-water species such as *Cx. quinquefasciatus* and *Cx. stigmatosoma*, which responded to infusion composition more readily than did *Cx. tarsalis*, as evident in this and other studies (Reisen and Meyer 1990, Rodcharoen et al. 1997). Interestingly, the large-volume gravid trap with the least concentrated bait (0.1 g/liter) collected 10-fold more *Cx. quinquefasciatus* mosquitoes than did the CDC gravid traps with the most concentrated (4 g/liter) infusion that was tested. This trend was consistent with the difference of gravid mosquito collections between large- and small-volume gravid traps and suggested infusion concentration played a relatively minor role among different gravid trap designs under field conditions.

Bait fermentation age was not a significant factor in the performance of fiberglass large-volume or small-volume gravid traps in 2011. This finding was unexpected considering addition of organic matter changed the abundance and species composition of *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis* collections in developmental sites over the course of several weeks (Beehler and Mulla 1995, Su and Mulla 1999, Jiannino and Walton 2004, Walton et al. 2009). Transient peaks in oviposition of the aforementioned studies were in open-water systems with extensive top-down (e.g., consumption by larval mosquitoes) and/or bottom-up (e.g., reduction of nutrients across time) regulation of microbial growth that could have altered the chemical signature of oviposition sites. The lack of a transition in mosquito abundance and/or species in our experiments may have been related to the predominately sealed, semitransparent nature of the fiberglass containers. Dense algal mats thrived (D. Popko, field observations) in the sunlight-exposed environment devoid of filter-feeding invertebrates such as mosquitoes. The proximity of different treatments in each replicate block of the experiment also may have interacted to reduce differences in the volatile signals related to bait age. Last, the overall low levels of mosquito populations during autumn 2011 could have precluded the detection of bait age effects. Across the 3 years of our study, infusion age had a small to moderate effect on the number of mosquitoes collected by gravid traps: *Cx. tarsalis* and *Cx. stigmatosoma* collections were larger over comparatively younger infusions. Age-based differences in the performance profile of baits in large-volume gravid traps should be examined further.

Color and water surface area were other variables that may have altered the effectiveness of the gravid traps to collect different mosquito species. Gravid *Cx. quinquefasciatus* females are known to prefer dark-colored bait to light-colored bait in the laboratory (Beehler et al. 1993), and dark-colored habitat has been associated with increased mosquito numbers in field mesocosms (Belton 1967). O'Meara et al. (1989) found that *Cx. nigripalpus* Theobald laid more egg rafts in 0.25-m^2 vats than in smaller jars (1-quart glass Mason jar; ~0.003 m^2), whereas *Cx. quinquefasciatus* females preferred to lay eggs in the jars. Allan and Kline (2004) reported a 3-fold increase in *Cx. quinquefasciatus* adult abundance in dark-colored gravid traps with twice the surface area and volume than light-colored traps, but they found no differences in the numbers of mosquitoes collected by gravid traps with dark-colored (black or green) versus beige infusion containers. Irish et al. (2012) demonstrated no change in *Cx. quinquefasciatus* adult numbers in traps of the same color and surface area that differed 2-fold in volume. However, Lewis et al. (1974) found that gravid traps with a larger infusion volume collected more *Cx. quinquefasciatus* mosquitoes than did traps with less infusion. In our study, the differences in color and surface area between large-volume plastic and small-volume gravid traps were expected to be related to differences in gravid mosquito collections between designs; however, adult mosquito abundance was similar when bait volume was the same (6 liters) in both types of gravid traps. The proportion of nongravid females and egg raft numbers differed between the 2 gravid trap designs. Also surprising was that light-colored fiberglass cylinders performed poorly compared to the maximum-volume large plastic design despite a 1/3 greater volume and nearly 20% greater surface area.

The gravid trap with side-mounted suction traps and 35 liters of infusion could be particularly useful for vector control monitoring programs because it would be a more practical option than the gravid trap using top-mounted suction traps and 100 liters of infusion, especially at sites where water supply is limited. The smaller infusion volume was easier to store, transport, and manipulate than the larger infusion volume. Side-facing suction traps were more secure than the top-mounted suction traps.

In conclusion, large-volume gravid traps offer a promising alternative to gravid traps used traditionally for mosquito-borne disease surveillance. The small-volume CDC gravid traps are more portable and practical than are the large-volume gravid traps for collecting species such as *Cx. pipiens* and *Cx. quinquefasciatus*. The large-volume gravid traps collect more gravid mosquitoes and, at times during the annual activity period of *Culex* adults, collect significant numbers of principal arbovirus vectors such as *Cx. tarsalis*, especially during the spring when mosquito populations and arbovirus populations are increasing. The effect of gravid trap size on *Cx. tarsalis* collections is even greater than indicated by our study because there were sampling dates when *Cx. tarsalis* was collected by the large-volume gravid traps but not by the small gravid traps; consequently, effect size could not be calculated for these sampling dates. The plastic garbage cans are more portable than in-ground oviposition pools (Surgeoner and Helson 1978) and are relatively inexpensive and easier to manage (i.e., stackable) than are the fiberglass containers. Box traps (Cummings 1992, Braks and Cardé 2007) could be fitted across the top of the infusion reservoir to reduce the damage to mosquitoes by the fan blade. The disadvantages of using large infusion volumes might be offset by using infusions for multiple-week deployments and covering the infusion basin between gravid mosquito collections. Whether the benefits of the enhanced collections of gravid mosquitoes for arbovirus surveillance provide a significant improvement in early warning for disease outbreaks and outweigh the disadvantages of large infusion volumes and costs (capital and labor) associated with large-volume traps remains to be evaluated.

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