A NOVEL TRAP CONFIGURATION FOR LIVE CAPTURE OF MOSQUITOES

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ABSTRACT

Diverse mosquito traps are available for mosquito and arbovirus surveillance. The delicate nature of the mosquito body makes them vulnerable to damage as they pass through the trap's fan, which can lead to rapid desiccation or mortality within the capture chamber. This can negatively affect surveillance accuracy, impacting both the precise identification of mosquitoes and the reliable execution of molecular assays for arbovirus detection. In this study, we report a novel modification to three widely used mosquito traps: CDC light trap, BG-Sentinel trap, and CDC gravid trap, incorporating a mesh funnel and updraft design to address these issues. We compared updraft and downdraft configurations of light traps under field conditions and compared the effectiveness of the modified BG and gravid trap to unaltered counterparts in semi-field environments. Subsequently, we conducted field validation of modified mosquito traps to assess their trapping effectiveness in terms of mosquito abundance and species composition in coastal forest and suburban areas. Our findings revealed that there was no significant difference in trapping effectiveness between different fan configurations. The adaptation made to the BG trap exhibited higher recapture rates of *Culex quinquefasciatus* and *Aedes albopictus* in comparison to the unmodified BG-Sentinel trap. The modification of the gravid trap was equivalent to unaltered CDC gravid trap. During our field trial, the modified light traps had a higher collection rate of mosquitoes with a wider spectrum of species diversity than modified BG or gravid traps, regardless of site. The modified BG traps captured more arbovirus vector species (*Culex* and *Aedes* species), with an increase in *Ae. albopictus* (11 times) and *Ae. aegypti* (1.75 times) when compared to the light traps. The modified gravid traps mostly collected *Culex* spp., accounting for over 47% of the collected mosquitoes. The results indicate that the novel trap configuration preserves trap functionality and improves specimen quality by avoiding the death and dismemberment of collected mosquitoes.

Key words: mosquito trap modification, light trap, BG trap, gravid trap, species composition

INTRODUCTION

Mosquito traps are a central part of surveillance and control programs and can be used to measure diversity, and abundance and provide samples for pathogen screening, contributing important data for timely intervention response (Rupp and Jobbins 1969). Many different types of mosquito traps are commercially available, but sampling outcomes may vary from trap design (e.g., suction fan features), types of attractants (e.g., carbon dioxide, light, and chemical lures), and even mosquito physiological state (e.g., gravid mosquito traps). Trap configurations (e.g., updraft vs. downdraft suction), influence mosquito flight and by extension the numbers and species captured (Kline 1999).

Currently, the CDC miniature light trap and the BG-Sentinel trap are two of the most widely used traps for mosquito surveillance (Nguyen et al. 2023). The broad usage of these two traps allows for consistent and comparable data collection across locations and studies (Maciel-de-Freitas et al. 2006, Meeraus et al. 2008). The CDC gravid mosquito trap (Reiter 1983) effectively captures egg-laden females of several *Culex* (*Culex*) spp. and is widely used in the surveillance of West Nile virus (Yee et al. 2022). Previous studies indicated that the infection rate in the mosquitoes collected from gravid traps was more than 30 times higher when compared to those by light trap (Williams and Gingrich 2007). A drawback of most current traps is that female mosquitoes are subjected to physical damage (including loss of wings, legs, and scales) when they pass through the fan blades (light trap, gravid trap) or desiccate in the capture net in the continual airstream (BG-Sentinel). Damage to trapped mosquitoes is a hindrance to morphological identification. For example, a field validation study by Gama et al. (2013) showed that a significantly larger proportion (>80%) of collected *Anopheles* mosquitoes could not be identified because of damage to specimens. The collection bag in the CDC gravid trap poses a challenge when transferring captured mosquitoes, typically causing specimen damage (Russell and Hunter 2010). The collection bag is intentionally designed to collapse, minimizing its space, but this often leads to the damage of captured mosquitoes. Death and desiccation of trapped mosquitoes likely lead to a degradation of DNA and RNA, which negatively impacts downstream molecular assays, such as mosquito DNA barcoding, blood meal analysis and arbovirus detection.

Collecting mosquitoes alive and without physical damage is highly desirable for examining insecticide resistance, capture-mark-release-recapture experiments, and arbovirus detection (Verhulst et al. 2015). For example, the laboratory-based vector competence assay, utilized for assessing a mosquito's physiological capacity to become infected with and transmit arboviruses, sometimes necessitates live adult mosquitoes sourced from wild-type populations. Collecting and rearing larvae from natural breeding sites is not practical for many vector groups (e.g., *Anopheles*), as immature stages (eggs, larvae, pupae) of these insects are notoriously difficult to collect, rear, and maintain in laboratory conditions (Coluzzi 1964, Verhoef et al. 2014).

Here, we report the results of field and semi-field studies of evaluating a novel trap configuration to improve the survival and condition of adult mosquitoes. The new configuration consists primarily of a mesh cone (made of standard window screen), placed upstream of the fan, such that mosquitoes are funneled into a protective capture chamber without passing through the fan. We applied this modification to commonly used mosquito traps including the CDC light trap, BG-Sentinel trap, and CDC gravid trap. We compared the efficacy of updraft and downdraft configurations of light traps under field settings. Modified traps were tested compared to the commercially available (unaltered) BG-Sentinel or gravid traps in semi-field enclosures. Finally, we performed field validation of modified mosquito traps to quantify the trapping efficacy (abundance and species composition).

MATERIALS AND METHODS

The “trap body” of all traps consisted of a square prism-shaped tube made of sections of standard polyvinyl chloride (PVC) hollow fence post joined by clear panels (Figure 1), with suction created by a square 12-V brushless fan powered by 12-V rechargeable batteries. Each end section of square PVC (Fiber Composites, New London, NC, USA) measured 10.0 cm x 10.0 cm (wide) x 9.0 cm (high). The middle section of the trap body consisting of transparent polypropylene (ClearBags, El Dorado Hills, CA, USA) measured 10.0 cm x 10.0 cm (wide) x 20.0 cm (high) (Figure 2). A 12-volt motor fan (Model number: 06020SA, 0.090A; NMB Technologies Corporation, San Jose, CA, USA) powered by a rechargeable battery (12V-12Ah; Duracell Inc., Bethel, CT, USA) produced suction through the mesh funnel. The funnel, made from a standard window screen (18x14 apertures per square inch; Phifer, Tuscaloosa, AL, USA), possesses an opening radius of 6.5 cm and a height of 20.0 cm. The apical 1.5 cm portion of the cone was cut away and discarded. The resulting mesh funnel was secured in place with silicone glue. The mesh funnel aperture (outflow) was affixed using silicone glue into a transparent polypropylene exit chute (ClearBags, El Dorado Hills, CA, USA) measuring 8.0 cm × 1.5 cm × 1.5 cm. This chute acts as an exit pathway for mosquitoes into the capture chamber. A small hole (1.5 cm) was cut into the chute opposite to the funnel aperture and covered with no-see-um netting. This permitted air to flow through the exit chute but prevented the passage of mosquitoes. At the termination of the flight tube, a UV-LED light (Model Number: C503B-BAS-CY0C0461, 3.2V-470nm; CreeLED, Inc., Durham, NC, USA) was attached to lure mosquitoes towards a capture chamber constructed from clear PVC measuring 20.0 x 7.0 cm, and the capture chamber was fortified with screen mesh to prevent mosquito escape while facilitating air exhaust.

The novel configuration light trap consisted of a trap body, as described above fitted with a commercially-available UV-LED array (Model Number: 2770, 6.0V-390 nm; BioQuip Products Inc., Rancho Dominguez, CA, USA). The UV-LED array was inserted into a bayonet base socket (Super Bright LEDs, Earth City, MO, USA) affixed near the trap intake with small screws. A rain shield with a diameter of 33.0 cm was situated atop the trap (Figure 2A). Updraft and downdraft configurations of the light trap were produced by reversing the orientation of the trap body relative to the rain shield. The updraft and downdraft configurations of the light trap were compared under field settings in Vero Beach, FL, USA. Trapping was performed in a coastal forest environment and replicated over 12 trap nights. Each trap was operated for 14 hours (1700 to 0700) and then position rotated to minimize location bias. Light traps were baited with ~1kg of dry ice in an insulated thermos. Collected mosquitoes were identified to species using published keys (Darsie and Ward 2005).

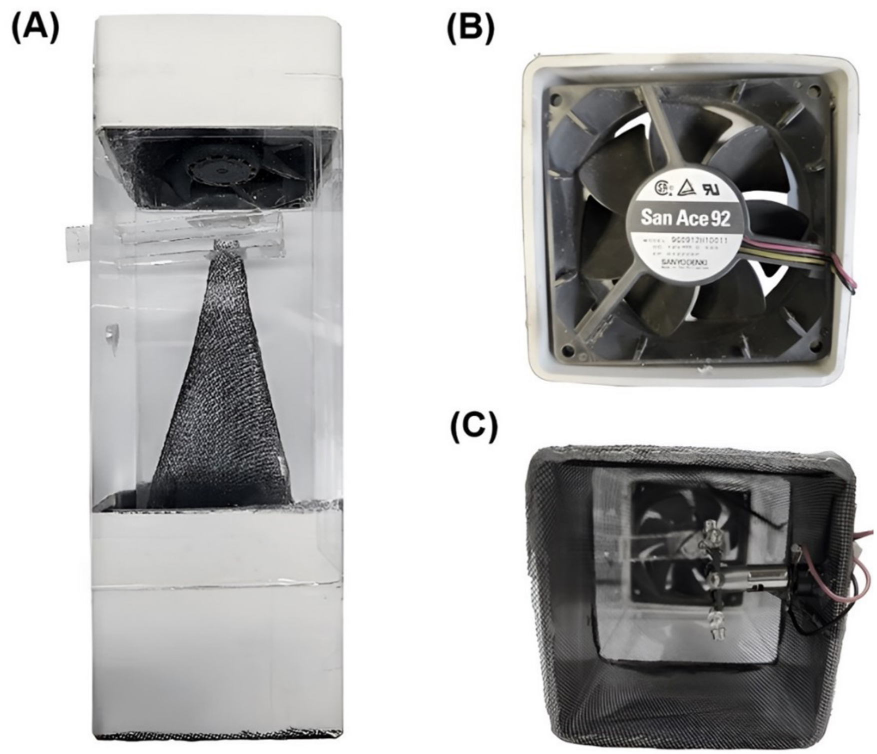

Figure 1. Trap body (A) Lateral view. (B) Outflow view (top). (C) Intake view (bottom), showing UV-LED array affixed at the bottom section of PVC.

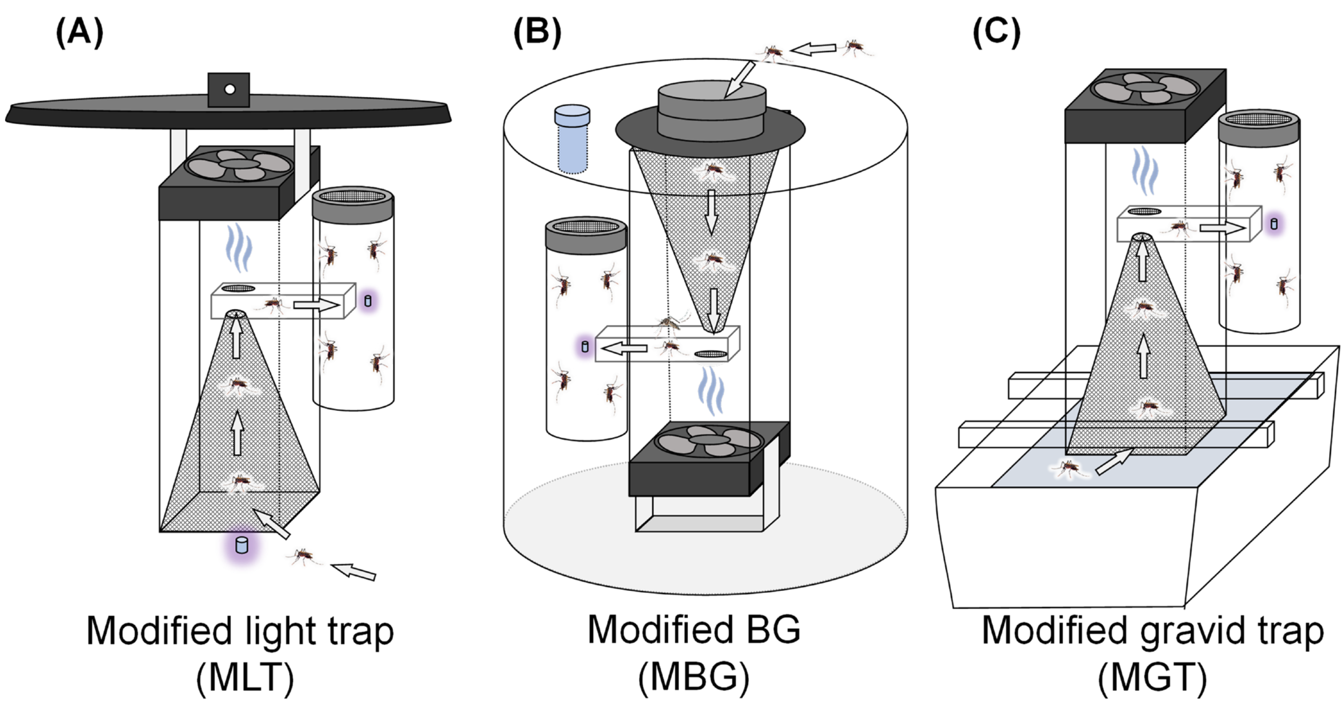

Figure 2. Diagram of modified (A) light trap, (B) BG trap, and (C) gravid trap. Arrows denote mosquito flight path.

The commercially available BG-Sentinel trap body (Biogents, Regensburg, Germany) served as the housing for the trap body, which was operated in the down-draft position. The original BG-Sentinel fan, associated air duct, and wiring were removed and replaced with the trap body, which was held 8 cm above the inner floor of the BG trap using a steel strap and wood block (Figure 2B). The original BG intake collar was then inserted into the entrance of the trap body to preserve trap functionality. We evaluated the novel configuration of the BG trap with mixed urban container species (*Culex quinquefasciatus* Say, *Aedes albopictus* Skuse, and *Aedes aegypti* L.) in semi-field enclosure. Laboratory colonies of *Cx. quinquefasciatus*, *Ae. albopictus*, and *Ae. aegypti* were maintained in an environmental chamber (27.0 ± 0.5°C, 80.0 ± 5.0% RH, and 14:10 (L:D) h photoregime) at the Florida Medical Entomology Laboratory (FMEL) at the University of Florida, Vero Beach, Florida, USA. Mosquito larvae (~300) were reared in enamel pans (24.8 cm x 19.7 cm x 3.8 cm) containing 1.5 L of tap water and fed an equal mixture of brewer's yeast and lactalbumin on a standardized mosquito rearing schedule (Gerberg et al. 1994). Pupae were collected daily and placed in a 30 ml plastic cup at a density of 50/cup. Containers with pupae were placed into 24.0 cm x 24.0 cm x 24.0 cm mesh screen cages (BioQuip Products Inc., Rancho Dominguez, CA, USA) for adult eclosion. Female mosquitoes (5–7 days old, each species N=50, total=150) were released in a screen enclosure (103.3 cm × 104.5 cm × 207.3 cm) and each trap was operated for 14 hours (1700 to 0700). The recapture rate was compared between modified and commercially available (unaltered) BG-Sentinel traps.

The novel configuration gravid trap consisted of the trap body with supports made of square PVC tubes. The supports suspended the trap body over a black plastic wash tub (44 cm length, 34 cm width, and 17 cm depth) filled with 1.5 liters of an infusion in which oak leaves (*Quercus* spp.) had fermented for forty-eight hours (Figure 2C). The modified gravid trap was compared to the commercially available (conventional) CDC gravid trap with *Cx. quinquefasciatus* in semi-field settings. Three-day-old *Cx. quinquefasciatus* females were engorged on a live chicken according to an institutionally approved protocol (IACUC protocol 201807682) and then held in cages in the insectary for seventy-two hours. Female mosquitoes were anesthetized using carbon dioxide and examined for egg maturation under a dissection microscope. Gravid females (7 days old, N=25) were released into screened cages with either novel configuration traps or CDC gravid traps to compare the recapture rate of the two traps.

Novel configuration light traps, BG-Sentinel traps, and gravid traps were field evaluated at three locations in Indian River County, FL, USA. Trap sites included the coastal forest site and two residential suburban areas with light industry. The light trap was baited with dry ice as described above. The BG trap was baited with dry ice and BG lure, and gravid trap was baited with 1.5 liters of an oak-leaf infusion. The modified CDC light trap was hung on shepherd hooks 1.5 m above the ground, and both the modified BG trap and gravid trap were placed on the ground. The study period began on September 24 and terminated on November 14, 2019. The traps were operated for 15 hours including dusk and dawn and sampled four times weekly in Indian River County. The collected alive adult mosquitoes in the capture chamber were freeze-killed and identified under a dissecting microscope according to standard keys (Darsie and Ward 2005).

The Wilcoxon-paired test was performed using JMP Statistical Software (Version 15.0; SAS Institute Inc., Cary, NC, USA) to compare differences in mosquito recapture rates between traps across species. To compare compositions of mosquito species collected by different traps, we employed a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations based on Bray–Curtis distances, which enabled us to investigate disparities between trap types. To further understand the specific impact of each mosquito species on the observed variations between trap types (Clarke 1993), we used the SIMPER (similarity percentage) method using PAST v4.13 (Hammer et al. 2001). A chi-squared test of independence was performed using JMP Statistical Software to test for differences in the distribution of mosquito abundance collected by each trap and site. The test was considered significant at a p value of less than 0.05.

RESULTS

During preliminary optimization tests, the light traps were compared in updraft and downdraft configurations under field settings. In total, 3,899 adult mosquitoes consisting of eight genera and twelve species were collected over six nights. Species of *Culex* (*Cx. nigripalpus* Theobald and *Cx. iolambdis* Dyar), *Aedes* (*Ae. taeniorhynchus* Wied., *Ae. albopictus*, and *Ae. infirmatus* Dyar & Knab), *Anopheles* (*An. crucians* Wied. and *An. atropos* Dyar & Knab), *Deinocerites* (*De. cancer* Theobald), *Psorophora* (*Ps. columbiae* Dyar & Knab), *Mansonia* (*Ma. titillans* Walker), *Wyeomyia* (*Wy. vanduzeei* Dyar & Knab), and *Uranotaenia* (*Ur. lowii* Theobald) were sampled by the traps, combined. The most abundant species captured by both updraft and downdraft traps were *Cx. nigripalpus*, *Cx. iolambdis*, *Ae. taeniorhynchus*, *An. crucians*, and *De. cancer*, comprising 99.1% of all mosquitoes. Updraft traps collected greater number of females of those species (129.4%) than downdraft traps although these differences were not significant (Figure 3A).

In the three species settings comprising 50 individuals each from *Cx. quinquefasciatus*, *Ae. albopictus*, and *Ae. aegypti*, a total of 150 females were released, and the recapture rate was compared between novel configuration and commercially available (unaltered) BG-Sentinel traps. The total recapture rate was slightly higher with the modified BG trap (56.5%) than with the unmodified trap (53.0%) (Figure 3B), however, the effect was not consistent for individual species. The modified BG traps captured significantly greater numbers of *Cx. quinquefasciatus* (X^2=4.7440; df=1; *P*=0.0294) and *Ae. albopictus* (X^2=5.3976; df=1; *P*=0.0202) females than unmodified BG-Sentinel trap, however, the recapture rate of *Ae. aegypti* was greater for the unmodified BG-Sentinel trap (97.0%) (X^2=5.6000; df=1; *P*=0.0180) compared to the modified BG trap (74.0%). Gravid *Cx. quinquefasciatus* (N=25) were released into screened cages with either modified or unmodified CDC gravid traps to compare the recapture rate of the two traps. The recapture rate was higher with the unmodified CDC gravid traps (68.0%) than with the modified gravid trap (45.1%), although the difference was not statistically significant (*P*=0.0545) (Figure 3C).

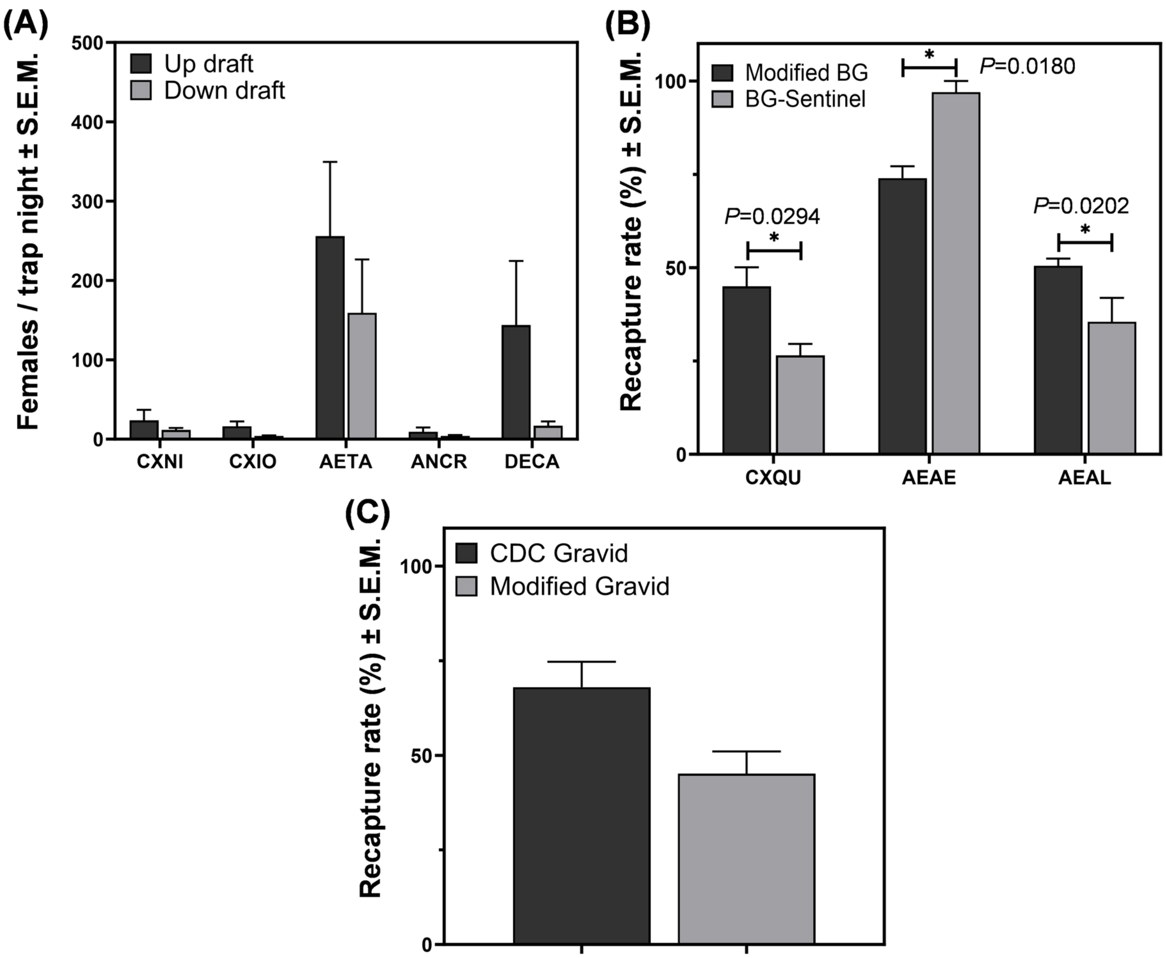

Figure 3. Modified trap configuration validation. (A) Capture rate for the most common nuisance and vector species between up- and downdraft light traps baited with dry ice. (B) Recapture rate (%) for the three nuisance and vector species (N=50) between BG-Sentinel and modified BG traps baited with dry ice in a screened enclosure. (C) Recapture rate (%) for *Culex quinquefasciatus* (N=25) between CDC gravid and modified gravid traps baited with infusion in a screened enclosure. Mosquito species abbreviations: CXNI, *Culex nigripalpus*; CXIO, *Culex iolambdis*; AETA, *Aedes taeniorhynchus*; ANCR, *Anopheles crucians*; DECA, *Deinocerites cancer*; CXQU, *Culex quinquefasciatus*; AEAE, *Aedes aegypti*; AEAL, *Aedes albopictus*. Asterisk denotes statistical significance.

In total, 15,567 adult mosquitoes consisting of eight genera and 28 species were collected by the combined trapping methods and sites during field evaluation. The distinctive design of the mosquito traps and capture chambers (Figure 2) prevented mosquitoes from being damaged by the blade fan and maintained their viability throughout the sampling period. Of the mosquitoes collected, 98.9% (N=15,390) were female and 1.1% (N=177) were male. The PERMANOVA yielded significant results for the comparison between the mosquito community comprising the mosquitoes collected by different traps (F=20.58; P<0.0001) (Figure 4). In a pairwise comparison, the mosquito compositions between traps demonstrated significant differences across sites (Figure 4), except for the comparison between light traps and BG traps (P=0.1555) only in suburban-1. The subsequent SIMPER analysis of the mosquito community highlighted that *Cx. nigripalpus* (average dissimilarity: 39.6), *De. cancer* (13.1), and *Cx. iolambdis* (6.9) were the predominant species responsible for the observed dissimilarities. Mosquito species that predominantly contribute to mosquito compositions are provided in Table S1. The mosquito composition collected varied across different collection sites. *Culex nigripalpus* and *De. cancer*, for example, dominated overall collections constituting 37.7 and 30.5% of total mosquito collections in the coastal forest, (Table 1). *Culex nigripalpus* was the most collected species in both suburban sites, constituting 66.9 and 67.7% of each overall collection, followed by *Ae. taeniorhynchus* (13.1%) and *Ma. titillans* (6.5%) (Tables 2 and 3). *Culex nigripalpus* and *Cx. iolambdis* were the most common *Culex* spp. from combined traps in the coastal forest, while *Cx. nigripalpus* and *Cx. quinquefasciatus* were the most common species in suburban sites. *Aedes taeniorhynchus* was the most common *Aedes* species collected among all three sites. The secondary dominant *Aedes* spp. was *Ae. infirmatus* in coastal forest and suburban-1, and *Ae. sollicitans* Walker in suburban-2. *Anopheles crucians* was the most collected *Anopheles* species in coastal forest, while *An. quadrimaculatus* Say in suburban sites was more common. The chi-square test of independence, which assessed the effectiveness of the different traps in capturing mosquitoes, revealed significant variation (P<0.0001) in the distributions of overall mosquito numbers across the three sites (Tables 1-3).

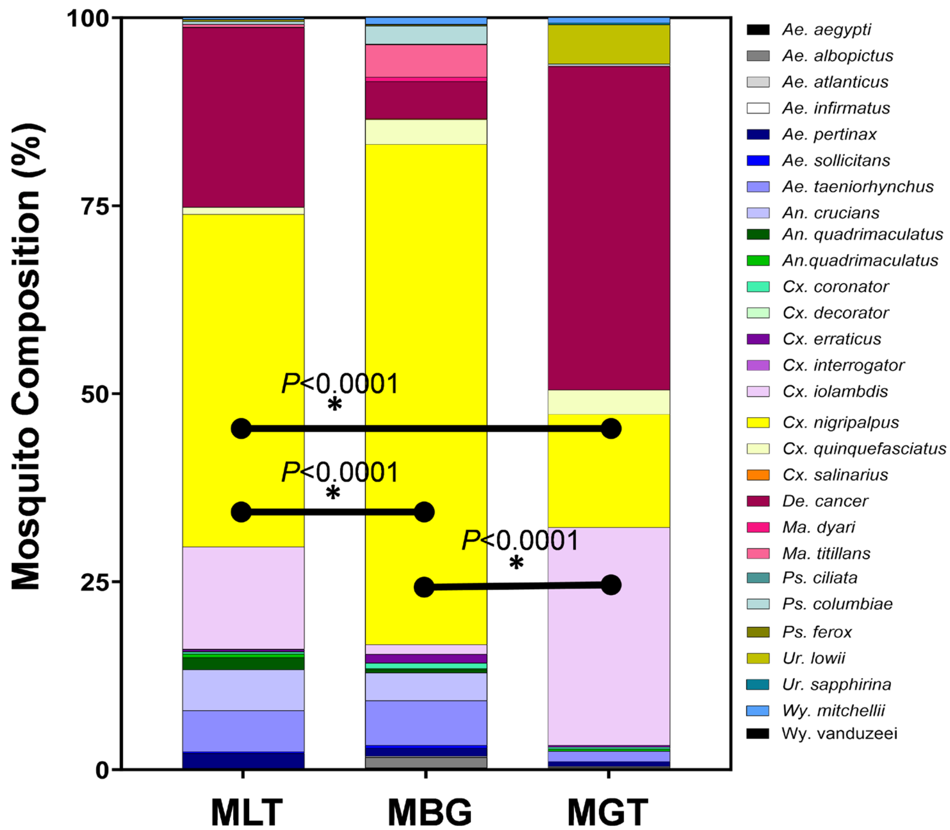

Figure 4. Mosquito composition captured during field deployment of three different modified traps at (A) coastal forest, (B) suburban-1, and (C) suburban-2 sites in Indian River County, Florida, 2019. MLT indicates modified light traps baited with dry ice and UV-LED array bulbs. MBG indicates modified BG trap baited with dry ice and BG lure. MGT indicates modified gravid trap baited with 1.5 liters of an infusion.

Table 1. Trap Index for female mosquitoes collected by three different modified traps at a coastal forest in Indian River County, Florida, 2019. MLT indicates modified light traps baited with dry ice and UV-LED array bulbs. MBG indicates modified BG trap baited with dry ice and BG lure. MGT indicates modified gravid trap baited with 1.5 liters of an infusion.

Mosquito species	Collecting method			Statistical outcomes	
	MLT (TI)	MBG (TI)	MGT (TI)	X^2	*P*
Ae. aegypti	0.03	0.03	-	1.011	0.6031
Ae. albopictus	-	0.07	-	4.046	0.1323
Ae. atlanticus	-	-	-	-	-
Ae. infirmatus	0.10	-	-	6.138	0.0465*
Ae. pertinax	8.07	0.67	0.17	26.433	0.0001*
Ae. sollicitans	-	-	-	-	-
Ae. taeniorhynchus	13.73	1.47	0.07	47.224	<0.0001*
An. crucians	19.03	2.33	-	65.274	<0.0001*
An. quadrimaculatus	3.67	0.17	-	46.574	<0.0001*
Cx. coronator	0.43	0.07	0.03	5.331	0.0696
Cx. declarator	0.23	-	-	6.136	0.0465*
Cx. erraticus	0.70	0.27	0.03	15.449	0.0004*
Cx. interrogator	0.03	-	-	2.000	0.3679
Cx. iolambdis	55.13	0.97	8.03	54.786	<0.0001*
Cx. nigripalpus	124.57	15.37	0.43	64.974	<0.0001*
Cx. quinquefasciatus	0.27	0.03	0.07	5.228	0.0732
Cx. salinarius	0.03	0.03	-	1.011	0.6031
De. cancer	97.47	3.97	12.37	54.907	<0.0001*
Ma. dyari	0.07	0.03	-	2.046	0.3595
Ma. titillans	0.07	0.03	-	2.046	0.3595
Ps. ciliata	-	-	-	-	-
Ps. columbiae	0.10	0.13	-	3.983	0.1365
Ps. ferox	0.07	0.10	-	2.070	0.3553
Ur. lowii	0.27	0.03	0.33	7.357	0.0253*
Ur. sapphirina	0.03	-	0.03	1.011	0.6031
Wy. mitchellii	0.73	0.40	0.07	12.361	0.0021*
Wy. vanduzeei	0.03	-	-	2.000	0.3679
Total	324.87	26.17	21.63	1092.097	< 0.0001*
Total species found	23	19	11		

Trap Index (TI): Average number of female mosquitoes per trap night
Asterisks: Significant differences as determined by chi-squared test.

Table 2. Trap Index for female mosquitoes collected by three different modified traps at a suburban-1 in Indian River County, Florida, 2019. MLT indicates modified light traps baited with dry ice and UV-LED array bulbs. MBG indicates modified BG trap baited with dry ice and BG lure. MGT indicates modified gravid trap baited with 1.5 liters of an infusion.

Mosquito species	Collecting method			Statistical outcomes	
	MLT (TI)	MBG (TI)	MGT (TI)	X^2	P
Ae. aegypti	0.10	0.13	-	3.178	0.2042
Ae. albopictus	0.10	0.27	0.07	5.513	0.0635
Ae. atlanticus	-	0.03	0.03	1.011	0.6031
Ae. infirmatus	0.40	0.10	-	5.259	0.0721
Ae. pertinax	0.10	0.13	0.03	1.082	0.5820
Ae. sollicitans	-	-	-	-	-
Ae. taeniorhynchus	7.37	2.27	0.30	15.350	0.0005*
An. crucians	1.83	0.33	-	27.502	<0.0001*
An. quadrimaculatus	2.93	0.17	-	32.487	<0.0001*
Cx. coronator	0.43	0.47	-	10.998	0.0041*
Cx. declarator	-	0.03	-	2.000	0.3679
Cx. erraticus	0.10	0.23	-	2.069	0.3554
Cx. interrogator	0.03	-	-	2.000	0.3679
Cx. iolambdis	0.10	0.07	0.60	0.365	0.8331
Cx. nigripalpus	27.83	20.80	2.27	33.637	<0.0001*
Cx. quinquefasciatus	1.90	2.23	0.40	9.334	0.0094*
Cx. salinarius	-	-	-	-	-
De. cancer	0.03	-	0.50	1.012	0.6030
Ma. dyari	0.07	0.07	-	2.070	0.3553
Ma. titillans	0.23	0.20	-	5.062	0.0796
Ps. ciliate	-	-	-	-	-
Ps. columbiae	0.27	0.37	-	7.227	0.0270*
Ps. ferox	-	0.03	-	2.000	0.3679
Ur. lowii	-	-	0.07	4.046	0.1323
Ur. sapphirina	-	-	0.03	2.000	0.3679
Wy. mitchellii	-	-	-	-	-
Wy. vanduzeei	-	-	-	-	-
Total	43.83	27.93	4.30	683.409	< 0.0001*
Total species found	17	18	10		

Trap Index (TI): Average number of female mosquitoes per trap night
Asterisks: Significant differences as determined by chi-squared test.

Table 3. Trap Index for female mosquitoes collected by three different modified traps at a suburban-2 in Indian River County, Florida, 2019. MLT indicates modified light traps baited with dry ice and UV-LED array bulbs. MBG indicates modified BG trap baited with dry ice and BG lure. MGT indicates modified gravid trap baited with 1.5 liters of an infusion.

Mosquito species	Collecting method			Statistical outcomes	
	MLT (TI)	MBG (TI)	MGT (TI)	X^2	*P*
Ae. aegypti	-	0.07	-	4.047	0.1322
Ae. albopictus	-	0.79	0.03	26.843	<0.0001*
Ae. atlanticus	-	-	-	-	-
Ae. infirmatus	-	-	-	-	-
Ae. pertinax	-	0.07	-	4.047	0.1322
Ae. sollicitans	1.03	0.31	-	9.325	0.0094*
Ae. taeniorhynchus	1.17	1.07	0.03	15.667	0.0004*
An. crucians	1.34	0.34	-	15.154	0.0001*
An. quadrimaculatus	2.1	0.1	0.1	17.932	0.0001*
Cx. coronator	0.21	0.07	0.03	5.146	0.0763
Cx. declarator	0.03	-	-	2.000	0.3679
Cx. erraticus	0.24	0.45	0.03	6.402	0.0407*
Cx. interrogator	0.07	-	-	4.047	0.1322
Cx. iolambdis	0.34	-	0.03	5.581	0.0614
Cx. nigripalpus	29.17	18.1	1.86	37.632	<0.0001*
Cx. quinquefasciatus	1.76	0.41	0.52	2.011	0.3659
Cx. salinarius	-	0.03	-	2.000	0.3679
De. cancer	0.03	0.03	-	1.012	0.6030
Ma. dyari	0.07	0.38	-	11.773	0.0028*
Ma. titillans	1.31	3.38	0.03	41.128	<0.0001*
Ps. ciliata	-	0.03	-	2.000	0.3679
Ps. columbiae	1.17	1.52	0.07	9.308	0.0095*
Ps. ferox	-	-	-	-	-
Ur. lowii	0.62	-	1.21	13.108	0.0014*
Ur. sapphirina	0.07	-	-	4.047	0.1322
Wy. mitchellii	0.24	0.31	0.14	0.685	0.7100
Wy. vanduzeei	-	-	-	-	-
Total	39.63	26.57	3.97	631.975	<0.0001*
Total species found	18	18	12		

Trap Index (TI): Average number of female mosquitoes per trap night
Asterisks: Significant differences as determined by chi-squared test.

The modified light traps yielded collections of 12,250 (78.7%) mosquitoes, comprising 26 species spanning eight genera. *Culex interrogator* Dyar & Knab and *Wy. vanduzeei* were only collected in light traps (Figure 4). The most abundant species captured in light traps included *Cx. nigripalpus* (N= 5,418: 44.2%), *De. cancer* (N=2,926: 23.9%), *Cx. iolambdis* (N=1,667: 13.6%), *Ae. taeniorhynchus* (N=667: 5.4%), and *An. crucians* (N=665: 5.4%). Excluding *Deinocerites*, the light traps captured larger quantities of *Culex*, *Aedes*, and *Anopheles* species, with *Ae. aegypti* (N= 4: 0.03%) and *Ae. albopictus* (N=3: 0.02%) being found in minimal proportions.

The modified BG traps yielded collections of 2,420 (15.5%) mosquitoes, comprising 25 species spanning eight genera. The most commonly collected species were *Cx. nigripalpus* (N=1,610: 66.5%), *Ae. taeniorhynchus* (N=143: 5.9%), *De. cancer* (N=120: 5.0%), *Ma. titillans* (N=105: 4.3%), and *An. crucians* (N=90: 3.7%) (Figure 4). Furthermore, the BG traps captured *Culex* (N=1,770: 73.1%), *Aedes* (N=222: 9.2%), and *Mansonia* (N=119: 4.9%) species in greater proportions of the total collected specimens. Notably, *Ae. albopictus* (N=33: 1.4%) and *Ae. pertinax* Grabham (N=26: 1.1%), constituted larger numbers compared to findings from the light traps. *Psorophora ciliata* Fabr. was solely captured by BG traps (Figure 4).

The gravid traps captured 897 (5.8%) mosquitoes, representing 16 species across eight genera. The gravid traps yielded the most abundant species, which comprised *De. cancer* (N=386: 43.0%), *Cx. iolambdis* (N=260: 29.0%), *Cx. nigripalpus* (N=135: 15.1%), *Ur. Lowii* (N=47: 5.2%), and *Cx. quinquefasciatus* (N=29: 3.2%) (Figure 4). *Culex* species accounted for the highest proportion (N=428: 47.7%) of total mosquito collections. Relatively, very low numbers of *Anopheles* (N=3: 0.33%) species were captured in the gravid traps.

DISCUSSION

Our results indicated that up- and downdraft orientation of the light trap yielded equivalent mosquito abundance and composition (Figure 3A). However, there is at least one advantage of the updraft position over the downdraft position, in that the updraft trap funnel is less likely to become clogged by falling objects (leaves, berries, beetles, etc.). In contrast, insects and fallen leaves are quickly drawn into downdraft traps due to the fan suction and gravitational pull. Previous studies showed that updraft design enhanced trapping effectiveness for some mosquito species, including *An. albimanus* (Rupp and Jobbins 1969, Wilton and Fay 1972, Kline 1999). Interestingly, *An. crucians* and *An. atropos* were exclusively captured in our updraft traps, suggesting that traps targeting malaria vectors may benefit from this orientation.

Our finding of significantly higher overall recapture rates of *Cx. quinquefasciatus* and *Ae. albopictus* (Figure 3B) suggests that the modification of the BG trap (Figure 2B) does not compromise the efficacy of the commercial trap, which is highly effective in collecting diurnal active mosquitoes (e.g., *Aedes*) (Geier et al. 2006, Maciel-de-Freitas et al. 2006). Although crepuscular or nocturnal active mosquitoes (e.g., *Culex*, *Anopheles*) are relatively less attracted to conventional BG-Sentinel traps compared to UV light-baited traps (Mwanga et al. 2019), our minor modifications of the BG-Sentinel trap resulted in an increase in the number of *Culex* females recaptured, which is important for surveillance of zoonotic viruses (e.g., West Nile virus, St. Louis encephalitis virus) transmitted by these mosquitoes. Recapture rate of the modified BG traps for *Ae. aegypti* females were lower compared to conventional BG-Sentinel traps, which may indicate that the modification negatively impacts release of attractants (BG lure or carbon dioxide). However, this speculative explanation was not tested (Logan et al. 2008). The nearly equivalent recapture rate of gravid females in the novel configuration gravid trap compared to the conventional CDC gravid trap (Figure 3C) suggests that the modifications of the gravid trap are more robust to changes than the BG trap. Additionally, the robust shell of our capture chamber (Figure 2C) provided protection for the collected mosquitoes, preventing physical damage during the stages of removal from the trap, transportation, and placement in a freezer for termination. Overall, our findings underscore the advantages of trap modifications in capturing specific mosquito species and improving surveillance effectiveness, contributing to more efficient vector control strategies.

During our field trial, the modified light traps had a notably higher overall collection of mosquitoes encompassing a broader range of species than other trap types, irrespective of the specific sites. This underscores the suitability of light traps for inclusion in comprehensive mosquito surveillance programs. For example, our results showed that the light traps exhibited notably elevated mosquito capture rates, surpassing those of the BG traps and gravid traps by factors of 5.1 ($P<0.0001$) and 13.7 ($P<0.0001$) respectively. Species compositions consisted of eight genera and 28 species across sites consisting of 92.9% (26/28) in light trap, 89.3% (25/28) in the BG trap, and 57.1% (16/28) in the gravid trap of the total number of mosquito species identified across sites (Figure 4 and Table 1-3). Previous studies found that mosquito traps baited with UV light and carbon dioxide, primarily visual and olfactory stimuli, increased overall trap effectiveness compared to other trap types (Silver 2007, Li et al. 2016). *Culex* were the dominant species in the light traps, comprising 59.4% of the entire collection. Particularly, *Cx. nigripalpus* and *Cx. iolambdis* were the most common *Culex* species from light traps. The low abundance of *Cx. quinquefasciatus* at the coastal forest area (Table 1) in comparison to the two suburban sites (Tables 2 and 3) agrees with previous findings associating this mosquito with (sub)urban locales in Florida (O'Meara and Evans 1983). We successfully obtained mosquito specimens from the capture chamber in excellent condition without any physical damage that was commonly reported in previous studies (Gama et al. 2013, Rodrigues et al. 2014). For example, the wing patterns of *Anopheles* species are crucial for mosquito identification but vulnerable to damage when mosquitoes pass through fan blades within traps. However, from a total collection of 924 *Anopheles* mosquitoes, representing three distinct species, we successfully identified all specimens based on the well-preserved patterns of dark and pale spots on their wings. This potential advantage enables more precise species identifications during mosquito surveillance.

The modified BG traps demonstrated greater effectiveness in collecting *Aedes* species, including a significant increase in *Ae. albopictus* (11 times) and *Ae. aegypti* (1.75 times) when compared to light traps (Figure 4 and Table 1-3). The modified BG trap also captured eight different *Culex* species, with *Cx. nigripalpus* being the most abundant. *Mansonia* and *Psorophora* mosquitoes were collected 2.2 and 1.3 times more in our modified BG-Sentinel trap when compared to those by light traps. Similarly, previously published studies found a considerably higher proportion of *Mansonia and Psorophora* species collected from conventional BG-Sentinel trap (Batista et al. 2018, Hendy et al. 2020) likely due to their association with mammalian hosts (Edman 1971, dos Santos Silva et al. 2012). While total numbers were low, 47% of the mosquitoes collected in the modified gravid traps belonged to the *Culex* genus (Table 1-3). This finding is significant as *Culex* species are known to act as vectors for various diseases affecting birds, humans, and other animals (Farajollahi et al. 2011). We found a relatively lower number of mosquitoes collected in gravid traps, compared to light or BG traps. It is likely that the oak leaf infusion used in our field evaluation was not very attractive to gravid *Culex* females and contributed to low overall numbers of mosquitoes collected by gravid traps.

Mosquito traps are not only a tool for use in collecting diverse mosquito species but also in estimating the risk associated with vector-borne diseases and developing effective strategies to protect against deadly pathogens. The present study showed that the novel mosquito trap configuration results in robust vector mosquito capture (and recapture) rates. Diverse species and important vector groups were effectively sampled in semi-field and field evaluations, indicating that the novel configuration preserves trap efficacy.

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AUTHOR CONTRIBUTIONS

D.K., T.J.B., and N.D.B-C. executed the experimental plans. D.K. and N.D.B-C. prepared conducted experiments and wrote the manuscript. All authors approved the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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Table S1. SIMPER (similarity percentage) analysis was performed to identify the species that predominantly contributed to the observed variations when comparing different traps.

Mosquito species	Av. dissim	Contrib. %	Cumulative %
Ae. aegypti	0.3085	0.3644	99.44
Ae. albopictus	0.8738	1.0320	95.26
Ae. atlanticus	0.0469	0.0554	99.90
Ae. infirmatus	0.1298	0.1532	99.59
Ae. pertinax	0.9441	1.1150	94.22
Ae. sollicitans	0.6237	0.7365	98.58
Ae. taeniorhynchus	4.7880	5.6540	75.97
An. crucians	4.1740	4.9290	80.90
An. quadrimaculatus	2.0900	2.4690	89.39
Cx. coronator	0.7948	0.9386	96.19
Cx. decorator	0.0480	0.0567	99.85
Cx. erraticus	0.6966	0.8226	97.85
Cx. interrogator	0.0369	0.0436	99.95
Cx. iolambdis	6.8890	8.1350	70.31
Cx. nigripalpus	39.5800	46.7400	46.74
Cx. quinquefasciatus	2.8270	3.3380	84.24
Cx. salinarius	0.0259	0.0305	99.98
De. cancer	13.0700	15.4400	62.18
Ma. dyari	0.4144	0.4893	99.07
Ma. titillans	2.2710	2.6820	86.92
Ps. ciliata	0.0141	0.0167	99.99
Ps. columbiae	1.6890	1.9950	91.38
Ps. ferox	0.0616	0.0727	99.79
Ur. lowii	1.4630	1.7280	93.11
Ur. sapphirina	0.1088	0.1285	99.72
Wy. mitchellii	0.7029	0.8301	97.02
Wy. vanduzeei	0.0043	0.0051	100.00