Brief Communication / Comunicación Breve

Mosquito (Diptera: Culicidae) species richness and abundance across a tree-height gradient: does adding CO_2 enhance the BG-Lure?

Riqueza de especies y abundancia de mosquitos (Diptera: Culicidae) en un gradiente de altitud arbórea: ¿añadir CO_2 amplifica el efecto del cebo BG?

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Abstract. Here we compare mosquito (Diptera: Culicidae) samples collected with BG-Pro traps set on alternate days with the BG-Lure (BGL) and the BG-Lure plus CO_2 (BGL+CO_2) across a tree height gradient using a fire tower in the Hoosier National Forest in Southwestern Indiana, USA. With both methods, we collected similar numbers (± SE) of mosquitoes (BGL = 1.14 ± 1.79 and BGL+CO_2 = 1.17 ± 2.56) per sampling effort unit. However, richness increased by three species when adding CO_2. These preliminary results suggest that adding CO_2 might increase the species richness of collected samples and are encouraging to pursue more detailed trials to compare the two treatments for collecting mosquitoes, considering the similar number of collected mosquitoes despite the shorter time (16 hours as opposed to 24 used in the traps with BGL only) of operation for traps where CO_2 was added.

Key words: *Culex* spp.; dry ice; Indiana; mosquito surveillance.

Resumen. Se presentan resultados de la comparación de trampas BG-Pro para mosquitos (Diptera: Culicidae) colocadas en días alternos con el cebo BG (BGL) y el cebo BG más CO_2 (BGL+CO_2) en un gradiente de altitud arbórea usando una torre de avistamiento de incendios en el Bosque Nacional Hoosier en el suroccidente de Indiana, Estados Unidos. Ambos métodos recolectaron cantidades (± EE) similares de mosquitos (BGL = 1,14 ± 1,79 and BGL+CO_2 = 1,17 ± 2,56) por unidad de muestreo. Sin embargo, se colectaron tres especies adicionales al añadir CO_2. Estos resultados preliminares sugieren que añadir CO_2 puede aumentar la riqueza de especies que se colectan y justifican hacer ensayos más detallados para comparar los dos tratamientos de recolección de mosquitos, considerando el número similar de mosquitos a pesar de que las trampas con CO_2 operaron por un tiempo más corto (16 horas) que las trampas con BGL (24 horas).

Palabras clave: *Culex* spp.; hielo seco; Indiana; vigilancia de mosquitos.

Traps are commonly used in mosquito ecology research and surveillance programs. Usually, an adult mosquito trap uses a light source, a suction fan, and volatile chemical attractants to trap mosquitoes (Silver 2007). The effectiveness of mosquito traps is

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dependent, among other factors, on the composition and concentration of the chemicals released from the attractant. Researchers have identified several chemical compounds that are highly effective in attracting adult mosquitoes to surveillance traps (Silver 2007). Several synthetic blends of compounds that mimic the naturally emitted odors of the human body are available commercially. These blends comprise volatile compounds specifically emitted by the skin or present in exhaled breath, such as lactic acid, fatty acids, and ammonia (Farajollahi *et al*. 2009). The BG-Lure is among the most common synthetic blends that imitate the odor of human sweat and is recommended to be used with BG traps (Biogents AG, Regensburg, Germany). It contains ammonia, lactic acid, hexanoic acid, and other proprietary components in specific proportions and is recommended for the use with BG traps to attract mosquitoes (Farajollahi *et al*. 2009; Degener *et al*. 2021). Similarly, carbon dioxide (CO_2) is another chemical that acts as an attractant for mosquitoes (Gillies 1980; Silver 2007). In the field, dry ice is one of the commonly used methods to generate CO_2, mimicking CO_2 emission from animal respiration. In mosquito surveillance and ecology research, researchers utilize CO_2 either alone or in combination with other lures. Various studies have shown that adding CO_2 to traps increases the efficiency and catch rates of specific mosquito species (Dormont *et al*. 2021).

The BG-Pro trap has shown effectiveness to sample medically important mosquito species in multiple and diverse ecological contexts (Degener *et al*. 2021). However, the effectiveness of such traps in tree-height gradients remains largely unexplored, despite the importance of these gradients for mosquito ecology and the transmission of several mosquito-borne pathogens (Novak *et al*. 1981; Pinto *et al*. 2009; Hendy *et al*. 2020). Here, our aim is to compare mosquito species richness and abundance from BG-Pro traps that were baited with a BG-Lure standard granular cartridge only with traps that were baited with both one kilogram of dry ice and a BG-Lure standard granular cartridge (Biogents AG, Regensburg, Germany). The dry ice was placed in the carrying bag that comes with the BG-Pro trap. To maximize species diversity in our samples, we also added the LED lights which comes with BG-Pro traps (McDermott and Mullens 2017). We conducted our study at Hickory Ridge Fire Tower in the Charles C. Deam wilderness area of Hoosier National Forest, Monroe County, Indiana, USA (Fig. 1A). The tower's coordinates are 39.0349° N, 86.3214° W (Fig. 1B). We obtained a research permit (File Code: 2610, granted on 24 March 2023) to conduct the study from the United States Department of Agriculture, which administers the study area. We configured our BG-Pro mosquito traps in the Centers for Disease Control style (Fig. 1C). Traps were set every two weeks. Traps baited with the BG-Lure were set Mondays at 5:00 PM and left running for 24 hours until retrieval on Tuesdays. Traps baited with the BG-Lure plus one kilogram of dry ice placed in the carrying bag were deployed on Tuesdays at 5:00 PM and retrieved after 16 hours at 9:00 AM on Wednesdays. The shorter duration of trap operation when CO_2 was added was done to evaluate if a similar number of mosquitoes could be collected over the time period that one kilogram of dry ice lasts in the field (Hendy *et al*. 2020). Traps were placed at different levels: Ground Level (1 m), Level 1 (8 m), Level 2 (16 m), Level 3 (23 m), and Level 4 (30 m), a gradient representative of tree-canopy height at the Hoosier National Forest (Fig. 1D). Following field collection, mosquitoes were immediately transported to the lab where all the retrieved samples were killed by keeping the samples at -20 °C for 15 minutes before undergoing taxonomic identification, which was done with a dissection scope and using taxonomic keys for the state of Indiana (Siverly 1972; Craker and Collins 2009). Voucher specimens, kept in ethanol at 100%, were deposited in the School of Public Health, Indiana University, Bloomington, IN, USA. Our study spanned from the fourth week of June (epidemiological week 26) to the third week of September (epidemiological week 38) and our total sampling effort was 70 trap-nights, split into 35 trap-nights for each treatment (BG-Lure and BG-Lure + CO_2).

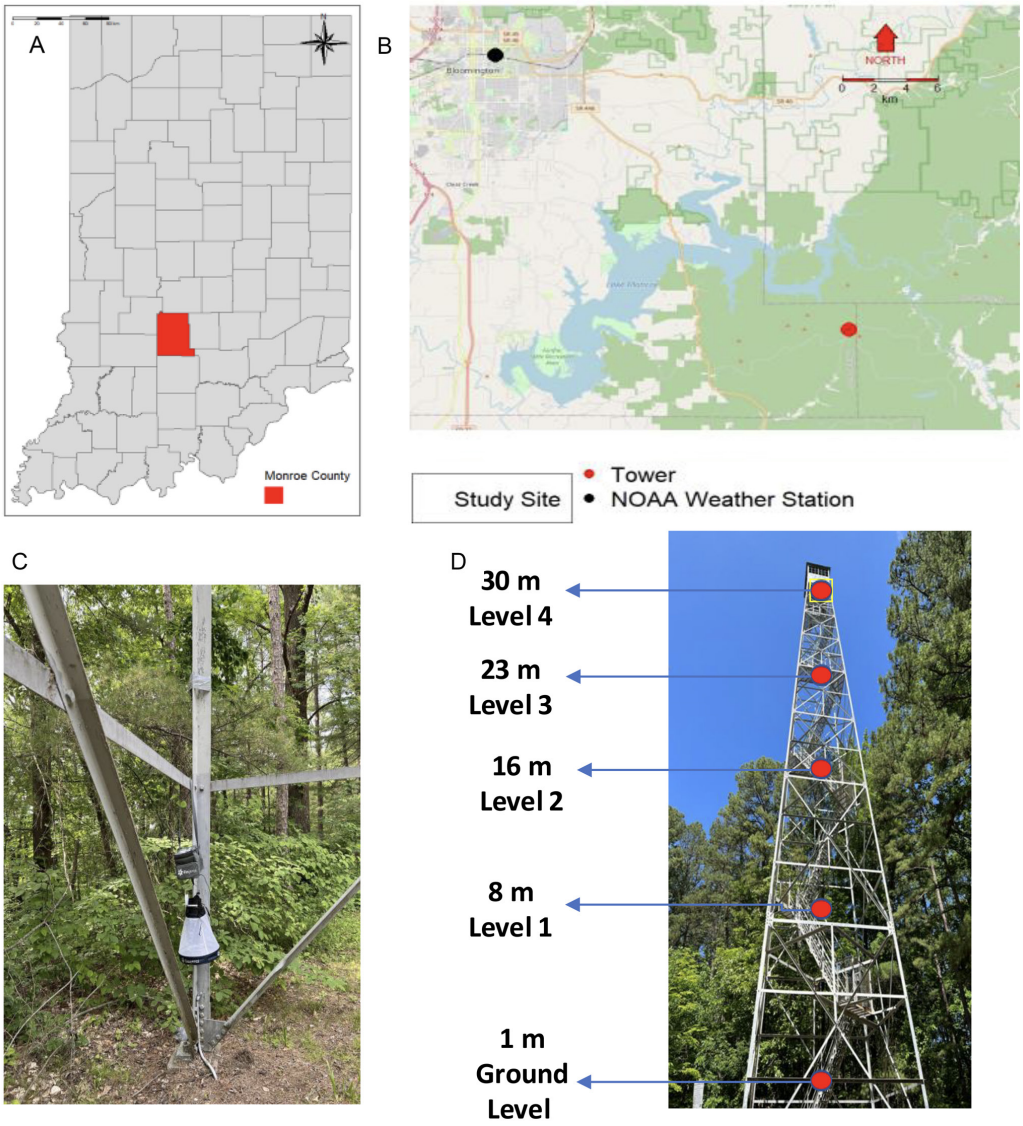

Figure 1. Study Site and Sampling Setting. **(A)** Monroe County in Indiana, USA. **(B)** Hickory Ridge Fire Tower and Nearest Weather Station within Monroe County. **(C)** BG-pro mosquito trap in CDC style. **(D)** Tower canopy height gradient. / **Figura 1.** Sitio de estudio y metodología de muestreo. **(A)** Condado Monroe, Indiana, Estados Unidos. **(B)** Torre de avistamiento de incendios y estación meteorológica más cercana dentro del Condado Monroe. **(C)** Trampa de mosquitos BG-pro configurada en estilo CDC. **(D)** Gradiente de altitud arbórea.

We tabulated the collected samples by species and tree-canopy height levels. We used a generalized linear mixed model (GLMM) with height level and sampling week as random factors, to compare the mosquito abundance, per trap-night, between the two bait treatments. In other words, the fixed effect that we tested with our GLMM was the addition of CO_2. Model diagnostics were checked, and model assumptions confirmed. All analyses were performed using the statistical software R (v 4.3.2, The R Foundation for Statistical Computing, Vienna, Austria). We used the "lme4" package for conducting GLMM and the "base" package for all other analyses using RStudio (v. 2023.9.1.494 RStudio PBC, Boston, MA) (R Core Team 2023).

We collected 81 mosquitoes over the course of the study with 70 trap-nights (Tab. 1). The average ± SE number of mosquitoes per trap-night for the two methods (with and without dry ice) were 1.14 ± 1.79 and 1.17 ± 2.56, respectively. The total number of samples collected with dry ice and without dry ice was 40 and 41, respectively. The GLMM parameter estimate for comparing mosquito abundance with the two bait treatments (BG-Lure vs BG-Lure + CO_2) was not significantly different from zero (parameter estimate = 0.29, SE = 0.51, z value =0.57, p-value = 0.56), meaning the number of mosquitoes was similar in the two bait treatments. According to the GLMM there was more variability between height levels (level height $\widehat{var} = 0.73$) than through time (sampling week $\widehat{var} = 0.62$).

Table 1. Vertical distribution of mosquito species across five tree-height levels at Hickory Ridge fire tower, Monroe County, Indiana, USA. Ground Level= 1 m, Level 1 = 8 m, Level 2 = 16 m, Level 3 = 23 m, Level 4 =30 m. Total indicates the total number of samples for each species. (BGL = BG-Lure, CO_2 is dry ice). / **Tabla 1.** Distribución vertical de especies de mosquitos en cinco niveles del dozel en la torre de avistamientos de incendios de Hickory Ridge, Condado Monroe, Indiana, Estados Unidos. Nivel base (Ground Level) = 1 m, Nivel (Level) 1 = 8 m, Nivel (Level) 2 = 16 m, Nivel (Level 3) = 23 m, Nivel (Level) 4 =30 m. Total indica el número total de muestras de cada especie. (BGL = cebo BG, CO_2 es hielo seco).

Height Levels	**Ground Level**		**Level 1**		**Level 2**		**Level 3**		**Level 4**		**Total**	
Species	**BGL**	**BGL & CO_2**	**BGL**	**BGL & CO_2**	**BGL**	**BGL & CO_2**	**BGL**	**BGL & CO_2**	**BGL**	**BGL & CO_2**	**BGL**	**BGL & CO_2**
Aedes triseriatus	4	4	1	3	0	0	0	1	0	0	5	8
Aedes trivittatus	0	2	0	1	2	0	0	0	0	1	2	4
Aedes vexans	0	4	1	2	1	2	0	1	0	0	2	9
Anopheles barberi	0	1	0	0	0	0	0	0	0	0	0	1
Anopheles punctipennis	1	1	0	0	0	0	0	0	0	0	1	1
Anopheles quadrimaculatus	0	1	0	0	0	0	0	0	0	0	0	1
Coquillettidia perturbans	0	1	0	1	0	2	0	0	0	0	0	4
Culex erraticus	2	3	0	0	0	0	1	0	0	0	3	3
Culex pipiens/restuans	4	2	7	3	6	1	4	0	0	0	21	6
Culex territans	0	1	1	0	2	0	1	0	0	0	4	1
Uranotaenia sapphirina	0	1	1	0	0	0	0	0	0	0	1	1
Morphologically damaged samples	1	1	1	0	0	0	0	0	0	0	2	1
Total	**12**	**22**	**12**	**10**	**11**	**5**	**6**	**2**	**0**	**1**	**41**	**40**

The dominant mosquito species identified in all samples (n=27), *i.e.*, from both bait treatments (BG-Lure only and BG-Lure + CO_2) were mosquitoes that can either be *Culex pipiens* L., 1758 or *Culex restuans* Theobald, 1901 which we refer as *Cx. pipien/restuans*, and that were morphologically undistinguishable as key morphological characters, such as the presence of white scales in the scutum, to separate *Cx. restuans* from *Cx. pipiens* were missing (Harrington and Poulson 2008). We also collected the following seven species with both bait treatments: *Aedes triseriatus* (Say, 1823), *Aedes trivittatus* (Coquillett, 1902), *Aedes vexans* (Meigen, 1830), *Anopheles punctipennis* (Say, 1823), *Culex erraticus* (Dyar and Knab, 1906), *Culex territans* Walker, 1856 and *Uranotaenia sapphirina* (Osten Sacken, 1868). When we added dry ice we were able to also collect three additional species: *Anopheles barberi* Coquillett, 1903 *Anopheles quadrimaculatus* Say, 1824 and *Coquillettidia perturbans* (Walker, 1856).

The dominant mosquito species in traps baited with BG-Lure only was *Cx. pipiens*/*restuans* which accounted to 51% (n = 21) of the samples, followed by *Ae. triseriatus* at 12% (n = 5), and *Cx. territans* at 10% (n = 4). In contrast, the dominant species in the BG-Lure + CO_2 treatment were *Ae. vexans* constituting 23% of the collected mosquitoes (n = 9), followed by *Ae. triseriatus* at 20% (n = 8), and *Culex pipiens*/*restuans* at 15% (n = 6).

Table 1 also provides a detailed summary of the vertical stratification of all collected mosquito species. Mosquitoes were more abundant in traps with the added dry ice at the Ground Level (1 m, n = 22.55%), followed by Level 1 (8 m, n = 10.25%), Level 2 (16 m, n = 5.13%), Level 3 (23 m, n = 2.5%), and Level 4 (30 m, n = 1.3%). In contrast, the abundance without dry ice was more even across the two lowest levels of the tree-canopy height gradient, which include Ground Level (1 m, n = 12.29%) and Level 1 (8 m, n = 12.29%), followed by Level 2 (16 m, n = 11.27%), and Level 3 (23 m, n = 6.15%). Notably, no mosquitoes were found at Level 4 (30 m, n=0) without dry ice. Using dry ice, we collected 22 female mosquitoes (55%) and 17 male mosquitoes (43%), with one damaged sample lacking morphological characters for sex identification. Without dry ice, we observed 29 male mosquitoes (71%) and 12 female mosquitoes (29%).

The inclusion of dry ice as an additional attractant resulted in increased species richness, but per trap-effort unit of sampling abundance was similar. Limitations of this study include its short duration, which only considered one mosquito season. We did not observe the synergistic effects of the BG-Lure and CO_2 in terms of catch rates as reported in previous studies (Roiz *et al*. 2016) probably because traps with CO_2 were deployed for a shorter time that traps with BG-Lure alone. This result however makes the point that CO_2 addition leads to a similar number of collected mosquitoes over a shorter period of trap operation.

In summary, our study revealed no significant difference in overall mosquito abundance between the two trapping methods. However, the choice of lure influenced the dominant species *i.e*., *Culex* spp. with the BG-Lure only method and *Ae. vexans* and *Ae. triseriatus* with the addition of dry ice. Despite observing higher species richness with dry ice as reported by previous studies (Farajollahi *et al.* 2009), we consider that optimal lure combination may vary depending on the targeted species. It is essential for practitioners to customize their lure combinations to better target species of interest. We recommend extended experiments for a comprehensive comparison able to offer recommendations about sampling specific species, for maximizing the richness of species sampled and finding the ideal time period for trap operation in the field.

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Author Contributions

SK: Conceived of the research, and performed the analyses. **SK, MGA:** Collected the data. **SK, CSB:** Wrote the manuscript first draft. **SK, MGA, CSB, LFC:** Contributed to manuscript writing and approved the submitted version.

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