Human Landing Collections Can Be Replaced by an Effective Mosquito Trap

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Introduction

Human landing collections (HLC) were the gold standard in the Singapore Armed Forces for monitoring populations of *Anopheles* and other vector mosquito populations, but ethical concerns regarding exposure of collectors to mosquito-borne diseases have led to the abandonment of this sampling method. In the search for an alternative to HLCs, the BG-Pro trap was compared with HLCs to gauge its effectiveness as a replacement. The objectives of this study were to 1) determine the operational reliability of the traps in a heavily forested tropical environment; 2) evaluate and compare the effectiveness of the BG-Pro traps to Human Landing Catch (HLC); and compare the traps efficacy when used in two different configurations.

Methods and Materials

Collections were made in Singapore at 4 military training sites in two locations: Western Training Area (WTA) and Tekong Island (TI) (Figure 1). Collection sites A11 and A27 were located in the WTA and N03 and S29 were on TI. All sites were heavily forested and served previously as human landing count (HLC) surveillance sites, where *Anopheles* malaria vectors had been collected. The study was conducted over 25 days between December 2020 and February 2021. Human landing collections were conducted between 1900 and 0400 by a pair of collectors who exposed their legs and captured mosquitoes in glass vials as they landed. BG-Pro traps were set up in the standard and inverted orientations using stainless steel trap stands that provided protection from rain (Figures 2a, 2b). BG-Pro traps were operated between 1900 and 0700.

Two trap configurations were used in this study because previous research showed larger collections of *Anopheles* mosquitoes could be obtained using the trap in the inverted position (Hiscox et al, 2014; Batista et al, 2017). BG-Pro traps were baited with CO_2 from gas cylinders and Mbita-5 (MB5) lure (ammonia (2.5%), lactic acid (85%), tetradecanoic acid (0.00025%) and 3-methyl-1-butanol (0.000001%)(Mukabana et al, 2012). Using a 3x3 Latin square design, the 3 collection methods were rotated among 3 sub-locations at each site daily over a 9-day period. All mosquitoes collected were sent to the Entomology team (Singapore Armed Forces Environmental Public Health) for counting and identification.

Figure 1 Location of collection sites in Singapore

Figure 2a - BG-Pro in standard configuration
Figure 2b - BG-Pro in inverted postion

Culex sitiens	3430	*Aedes albopictus*	431
Cx. quinquefasciatus.	1226	*Ae. amesii*	150
Cx. vishnu	934	*Ae. malayensis*	12
Cx. pseudovishnu	825	*Ae. vexans.*	4
Cx. tritaeniorhynchus	708	*Ae. collessi*	2
Cx. spp.	1653		
		Mansonia bonnae.	384
Anopheles fragilis	824	*Ma. uniformis.*	70
An. sineensis.	402		
An. epiroticus.	178	*Coquillitidia crassipes*	1047
An. tessellatus	312		
An. barbirostris	2	*Armigeres spp.*	250
An. spp	742	*Verrallina spp.*	474

Table 1. Major species collected

Results

A total of 14060 mosquitoes representing 24 species in 9 genera were identified. The most commonly collected mosquitoes are shown in Table 1. The BG-Pro traps collected significantly more mosquitoes over the course of the study than the HLC (Figure 3). Traps also collected a higher number of species per night. The inverted traps had mean of 9.8 species per night followed by the standard configuration with 8.3 and HLC with a mean of 6 different species (Figure 4). The maximum number of species collected were 15, 13, and 11 for the BG-Pro inverted, BG-pro standard , and HLC, respectively. Among the 4 collection sites, the 2 located on Tekong Island (S29 and N03) had the highest mean mosquito diversity. In all locations, the inverted trap collected the widest variety of species (Figure 4).

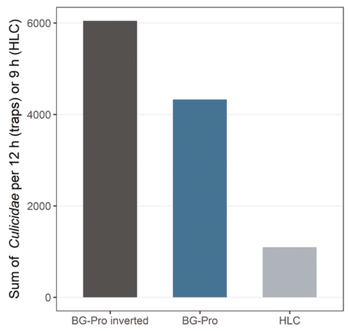

Figure 3 Total mosquitoes collected by collection method.

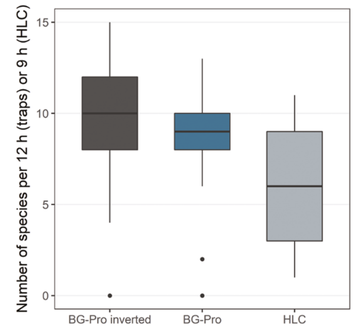

Figure 4 Species diversity by collection method

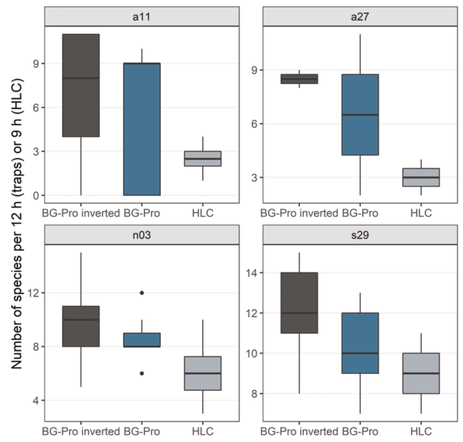

Figure 5 Species diversity by collection location

Looking at mean catch rates for total *Anopheles* spp., the inverted BG-Pro, the BG-pro, and HLC collected an average of 34.6, 23.1 and 2.4 *Anopheles* per night (Figure 6). For all *Culex*, catch rates per night were also higher in both trap types (inverted 138.4, standard 110) compared to HLC (28) (Figure 7). For the important vector species, The inverted trap also had the highest mean catch rates: *An. sinensis* (inverted 9.16, standard 2.6, HLC 3.52); *An. eprioticus* (inverted 3.96, standard 0.68, HLC 2.0); *Aedes albopictus* (inverted 2.92, standard 1.24, HLC 0.36); and *Cx. quinquefasciatus* (inverted 17.72, standard 7.48, HLC 0.00). *Aedes aegypti* was not collected in this study, most likely due to the nature and location of the collection sites. The HLC outperformed the standard BG-Pro configuration for *An. sinensis* and *An. epiroticus* while the standard configuration was better for *Ae. albopictus* and *Cx. quinquefasciatus* (Figure 8).

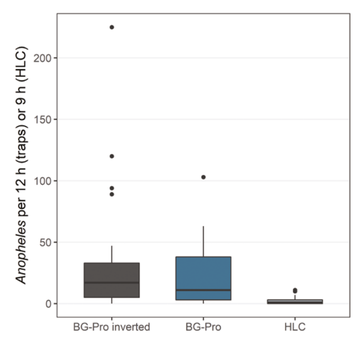

Figure 6 Total *Anopheles* mosquitoes collected from all locations

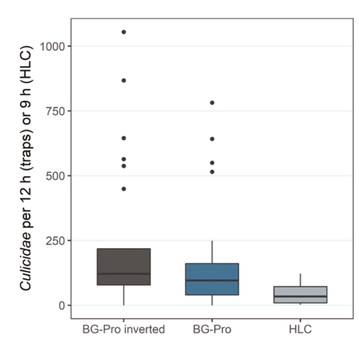

Figure 7 Total *Culex* mosquitoes collected from all locations

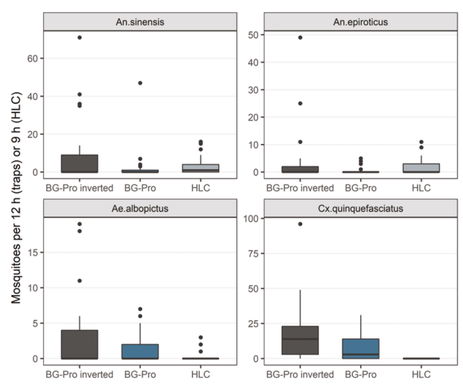

Figure 8 Major vector species collected by collection method

Summary

Both of the trap configurations outperformed the HLC in total number of mosquitoes, vector species collected as well as total species diversity. This finding provides compelling evidence for a decision to replace HLCs with more effective traps. Four of the 5 main vector species occuring in Singapore, *Aedes albopictus*, *Anopheles epiroticus*, An. *sinensis* and *Cx. quinquefasciatus*, were collected by all methods. The inverted BG-Pro was the most effective sampling method. This may reflect mosquito behaviour in a forest setting where they tend to rest on vertical surfaces, or under broad leaves. Inverted traps will therefore tend to catch more mosquitoes flying low and seeking to rest under cover. One issue encountered with trap collected mosquitoes was that a higher percentage could not be acurately identified to species level compared to the HLC collections. Mosquitoes from the HLCs, which were collected and stored individually, were in generally better condition. However, given the much higher numbers collected by the traps, there was still a greater number of identifiable specimens in the trap collections.

Conclusions

BG-Pro traps are effective at catching mosquitoes in heavily forested areas and are robust enough to withstand adverse tropical weather. The traps catch vector mosquitoes of interest as well as providing a good picture of species diversity. Using BG-Pro traps as a substitute for human landing collections eliminates the ethical concerns of disease exposure risk to field personnel, eliminates collector bias, frees up personnel for other tasks, and provides a time saving, standardized and reproducible collection method.