Field evaluation of two commercial mosquito traps baited with different attractants and colored lights for malaria vector surveillance in Thailand

Alongkot Ponlawat1*, Patcharee Khongtak1, Boonsong Jaichapor1, Arissara Pongsiri1 and Brian P. Evans2

Abstract

Background: Sampling for adult mosquito populations is a means of evaluating the efficacy of vector control operations. The goal of this study was to evaluate and identify the most efficacious mosquito traps and combinations of attractants for malaria vector surveillance along the Thai-Myanmar border.

Methods: In the first part of the study, the BG-Sentinel™ Trap (BGS Trap) and Centers for Disease Control and Prevention miniature light trap (CDC LT) baited with different attractants (BG-lure® and CO2) were evaluated using a Latin square experimental design. The six configurations were BGS Trap with BG-lure, BGS Trap with CO2, CDC LT with BG-lure, CDC LT with CO2, CDC LT with BG-lure plus CO2 and CDC LT with CO2. The second half of the study evaluated the impact of light color on malaria vector collections. Colors included the incandescent bulb, ultraviolet (UV) light-emitting diode (LED), green light stick, red light stick, green LED, and red LED.

Results: A total of 8638 mosquitoes consisting of 42 species were captured over 708 trap-nights. The trap types, attractants, and colored lights affected numbers of female anopheline and Anopheles minimus collected (GLM, \(P < 0.01\)). Results revealed that BGS Trap captured many anophelines but was significantly less than the CDC LT. The CDC LT, when baited with BG-lure plus CO2 captured the greatest number of anopheline females with a catch rate significantly higher than the CDC LT baited with BG-lure or CO2 alone (\(P < 0.05\)). The number of anopheline females collected from the CDC LT baited with CO2 was greater than the CDC LT baited with BG-lure (646 vs 409 females). None of the alternative lights evaluated exceeded the performance of the incandescent light bulb in terms of the numbers of anopheline and An. minimus collected.

Conclusion: We conclude that the CDC LT augmented with an incandescent light shows high potential for malaria vector surveillance when baited with CO2 and the BG-lure in combination and can be effectively used as the new gold standard technique for collecting malaria vectors in Thailand.

Keywords: Anopheles, BG sentinel, CDC light trap, Colored lights, Attractant
Background
While the international community has expedited efforts in recent years to control the spread of malaria, the disease remains endemic in 106 countries with one-fifth of the world’s population (1.2 billion people) living in areas with a known high risk of disease transmission [1]. However, within the Greater Mekong Subregion (GMS) of Southeast Asia, an actual decline in malaria incidence and deaths over the previous decade provides some degree of optimism. Regardless, serious concern remains towards the prevalence of multidrug resistance in this region. An added challenge is the presence of “border malaria” cases which has proven exceptionally difficult to monitor and yet poses as a threat to the interior of countries such as Thailand where malaria had been successfully marginalized to the borders several decades prior [2, 3]. Adding to the distress are the complex socio-political issues that have continued to plague the region over decades which only serve to stifle efforts to control the spread of the disease within the region [4]. The situation is further compounded by the fact that all six Plasmodium species associated with humans are found within the GMS and additionally, the parasite is vectored by three Anopheles species complexes (An. minimus, An. dirus and An. maculatus) with significant ecological variation to be found among and within the complexes [4]. To this end, an effective malaria surveillance program that includes monitoring for human cases, the parasites of interest, and the existence of potential or known vectors is vital to achieving effective health interventions and in evaluating possible impacts on malaria transmission.

Historically, human landing catches (HLCs) were used to survey for the existence of potential vectors of disease and to evaluate the efficacy of vector control operations. Data collected using this technique tended to provide a true sense of potential mosquito vector species and densities since the actual host was the bait. Data from HLCs could also be used to determine entomological inoculation rates, which are true estimates of the disease risk posed to humans [5–7]. However, the ethical issue of placing humans at greater risk for contracting disease, the labor-intensive aspects of the approach and the variation often found among “human attractants” justifies the need for alternative tools that are relatively safer and require minimal man-hours to operate.

To avoid human contact with mosquitoes, various devices have been developed over the years to survey for Anopheles mosquitoes [5, 8–12]. The BG Sentinel™ Trap (BGS Trap) (Biogents, A.G. Regensburg, Germany) complemented with the BG lure™ (Biogents A.G.) [13], has shown significant promise as a tool for collecting Ae. aegypti (L.) [13–16], Ae. albopictus (Skuse) [17], Culex pipiens L. [15] and Anopheles mosquitoes [18–21]. The BG-lure is a dispenser consisting of a blend of mosquito attractants (lactic acid, ammonia, and caproic acid). To date, the BGS Trap has not been field-tested in southeast Asia for anophelines and therefore no information on its efficacy in collecting malaria vectors from Thailand is available. In the current study, we evaluate the efficacy of the BGS Trap (baited with either CO2 or the BG-lure or both candidate attractants) compared with the Centers for Disease Control and Prevention (CDC) Miniature light trap Model 512 (CDC LT) (John W. Hock Company, Gainesville, FL, USA) under field conditions in Thailand.

We further evaluate the relative impact of colored lights on the collection of Anopheles spp. when used in conjunction with either the CDC or the BGS Trap. Ultraviolet (UV), blue, and green color are visible by insects [22–24]. Red and infrared light are invisible to most insects [24]. However, the majority of mosquito light traps are augmented with incandescent light bulbs which weakly emit the insect visible light spectra [25]. We hypothesized that insect-visible lights (UV, green) would improve the efficacy of mosquito traps by increasing the catch rate relative to invisible or weakly visible lights (incandescent and red). Data from this study may provide preliminary evidence that the incorporation of colored lights into the trap design will lead to an improved surveillance tool.

Methods
Study site
Trap comparison experiments were carried out in a malaria-endemic area located in Khun Huay, a village of over 100 non-enclosed homes, located in Mae Sot District, Tak Province in northwestern Thailand along the Thai-Myanmar border (16°43’14”N, 98°39’45”E, Fig. 1). Residents are either Thai or Karen (from Myanmar) subsistence farmers of rice, maize, and soybean and rear livestock including cattle, pigs, and goats. The region is characterized by three seasons: rainy (mid-May to mid-September), winter (mid-September to mid-February), and summer (mid-February to mid-May). The current study was carried out from June to December 2010 with mean daily temperatures ranging between 22.4 and 29.8 °C.

Experimental design
Comparative field evaluation of BGS-trap and CDC LT
Field evaluations were carried out using the BGS Trap and CDC LT. Six trap configurations were evaluated using a Latin square design as follows: (i) BGS Trap with BG-lure; (ii) BGS Trap with BG-lure plus CO2; (iii) BGS Trap with CO2; (iv) CDC LT with BG-lure; (v) CDC LT with BG-lure plus CO2; and (vi) CDC LT with CO2, respectively. Traps and attractants were operated according to manufacturers’ instructions. Note that for the
present study, the BGS Trap did not contain the BG-lure unless specified. Dry ice (1 kg per trap) placed in an insulated plastic container with a release pipe was used as the CO$_2$ source.

The village was divided into 6 cluster areas. Within each cluster area, 3 traps were operated for one particular trap configuration from 18:00 to 6:00 h with each trap in the cluster area separated by distances of at least 200 m. Therefore, for one collection night, a total of 18 traps was operated across the 6 clusters representing all 6 configurations. To account for position effects, the 3 traps for one particular configuration were rotated counterclockwise to the adjoining cluster prior to sampling the subsequent night.

Collected mosquitoes were placed on dry ice for subsequent identification. Females were morphologically identified to species [26] and parity was determined.

Efficacy evaluation of BGS trap and CDC LT augmented with different colored lights

The efficacies of the BGS Trap and CDC LT (both trap types baited with BG-lure plus CO$_2$) augmented with different light sources including light bulbs, light sticks and light-emitting diodes (LED) were evaluated for collecting anopheline mosquitoes. Four light configurations were evaluated for the BGS Trap: (i) incandescent bulb; (ii) UV LED; (iii) green light stick; and (iv) red light stick. For the CDC LT evaluation, four light configurations were tested: (i) incandescent bulb; (ii) UV LED; (iii) green LED; and (iv) red LED (Fig. 2). The 1.5-watt incandescent light (Chicago Miniature Lighting, Hackensack, NJ) was used as the control in this experiment. Colored LED light bulbs (UV, green, and red) were purchased from the local market in Thailand (Budget LED, Bangkok, Thailand). Four LED bi-pin bulbs of the same color arranged in a circular alignment were connected to each LED trap (Fig. 2). Six-inch green and red chemical light sticks rated for 12 h were used for the BGS Trap’s light source (LC Industries, Durham, NC). The light sticks were waterproof, non-toxic and non-flammable light sources emitting a 360-degree bright light.

The village was divided into two areas; one area was used to evaluate the BG Trap configurations and the other area was used for the CDC LT configurations. Each area was further divided into a set of four cluster areas. Within each cluster area, two traps, separated by approximately 200 m, were operated for one particular light configuration from 18:00 to 6:00 h. Therefore, for one collection night, 16 traps were operated across the 8 clusters representing all 8 configurations. Traps within each of the two areas (BGS Trap or CDC LT) were rotated counterclockwise after each of four collection days, comprising one replicate of the study. Six replicates were conducted. All collected samples were maintained in a cold chain (dry ice) upon transport to the field laboratory. Collected mosquitoes were separated by sex and enumerated. Collected females were morphologically identified to species and the parity rate was determined.
Physiological state determination
After identification to species, Anopheles females were dissected under a microscope and classified as either nulliparous (coiled tracheole skeins visible in the ovaries), recently blood-fed (blood in the midgut), gravid (ovarioles developed past stage II), or parous (absence of tracheolar skeins in ovaries) [27].

Data analysis
All statistical analyses were performed using IBM SPSS statistical software, version 23. Total number of Anopheles and An. minimus Theobald females captured from each trap configuration were pooled by night (either 2 or 3 traps/trap configuration/night) for statistical analysis. Generalized linear model (GLM) with negative binomial error and log link function was used to analyze effects of trap configurations, different attractants and colored lights on the numbers of mosquitoes collected in both experiments. The dispersion coefficients were estimated using maximum likelihood estimation. Parameter coefficients were tested using Wald Chi-square. Incident rate ratios (IRR) of BGS Trap, tested attractants, and colored lights were calculated relative to CDC LT with CO₂ and incandescent light as the reference. The values of IRR greater or lower than 1 indicate higher or lower trapping performance relative to the reference one. The hypothesis was tested using the Chi-square ($\chi^2$). The physiological state (parity rate) was determined and calculated as a percentage of parous females from the total mosquitoes dissected. The Chi-square ($\chi^2$) test was used to statistically analyze the physiological state data.

Results
Comparative field evaluation of BGS-trap and CDC LT
A total of 42 mosquito species (6079 female mosquitoes) was collected. From the collections spanning 324 trap-nights, the predominant species were Cx. vishnui Theobald (28.66%), An. minimus (25.86%) and Cx. quinquefasciatus Say (12.22%). The BGS Trap (with CO₂, BG-lure, or both) and CDC LT (with CO₂, BG-lure, or both) collected 1676 (27.57%) and 4403 (72.43%) female mosquitoes, respectively (Table 1). The BGS Trap collected a total of 30 mosquito species while the CDC LT collected 38 mosquito species. The predominant species collected by the BGS Trap were Cx. quinquefasciatus ($n=685$), Cx. vishnui ($n=354$), An. minimus ($n=241$), An. vagus Doenitz ($n=143$) and Ae. albopictus ($n=88$). Species most commonly found in the CDC LT included An. minimus ($n=1340$), Cx. vishnui ($n=1388$), Ae. vexans (Meigen) ($n=395$), An. sawadwongporni Rattanarithikul & Green ($n=162$), An. vagus ($n=162$), Cx. gelidus Theobald ($n=157$), Ae. vigilax (Skuse) ($n=144$) and An. splendidus Koidzumi ($n=119$). Notably, the CDC LT trapped more species and greater numbers of anthropophilic malaria vectors such as An. minimus, An. vagus, An. sawadwongporni and An. splendidus than the BGS-Trap (Table 1).

Statistical analysis revealed that trap type and attractants affected numbers of female anopheline and An. minimus collections (Table 2). The performance of the BGS Trap for collecting female anopheline and An. minimus was significantly lower than CDC LT (female anopheline: $\chi^2 = 60.207$, df = 1, $P < 0.0001$; An. minimus: $\chi^2 = 71.333$, df = 1, $P < 0.0001$) (Table 2). The combination of BG-lure and CO₂ as the trap attractants tended
to enhance anopheline and *An. minimus* collections (IRR = 1.20 and 1.57) albeit not significantly. The addition of BG-lure to the gold standard technique for malaria vector collection (CDC LT with CO₂) increases the effectiveness of sampling for *Anopheles* (IRR = 1.72, *P* = 0.041) and *An. minimus* (IRR = 1.99, *P* = 0.013) (Table 3). Interestingly, comparison of efficacies (*Anopheles* collection) between CDC LT baited with either CO₂ or BG-lure alone revealed no statistically significant differences (IRR = 0.67, *P* = 0.135). The current field study shows that the BG-lure, when used in concert with CO₂, can significantly enhance *Anopheles* and *An. minimus* CDC LT collections relative to the CDC LT with just CO₂.

Within the BGS Trap collections, we found that augmentation with CO₂ can improve the efficacy of the trap for malaria vector collections. The number of *Anopheles* collected by BGS Traps augmented with CO₂ was significantly higher than BGS Trap baited only with BG-lure (*χ²* = 34.554, *df* = 1, *P* < 0.0001, Table 3). However, the efficacy of BGS-Trap baited with CO₂ was not significantly different from the trap baited with BG-lure plus CO₂ for *Anopheles* capture (*P* = 0.92). For *An. minimus* collections, adding BG-lure into the BGS Trap baited with CO₂ significantly improved the trap efficacy (IRR = 1.55, *P* = 0.046, Table 3).

Age grading and blood meal status identification took place for a total of 2477 *Anopheles* females (Table 4). Almost all of the females were found to be in the empty stage (99.1%). Notably, nearly half of the samples captured by the BGS Trap baited with BG-lure were recently blood-fed specimens (41.5%, *n* = 17). Parity rates of *Anopheles* mosquitoes collected by BGS-Trap and

| Trap type | Tested attractant | Trap-night | *Anopheles* | *An. minimus* | Aedes | Culex | Othersa |
|-----------|------------------|------------|-------------|---------------|-------|-------|---------|
| Exp. I    | CDC LT           | CO₂        | 54          | 646           | 412   | 347   | 673     | 24      |
|           | BG-lure + CO₂    | 54         | 973         | 693           | 207   | 999   | 11      | 25      |
|           | BG-lure          | 54         | 409         | 230           | 11    | 82    | 7       |
| BGS Trap  | CO₂              | 54         | 204         | 90            | 39    | 457   | 11      |
|           | BG-lure + CO₂    | 54         | 204         | 132           | 60    | 375   | 16      |
|           | BG-lure          | 54         | 41          | 15            | 16    | 248   | 5       |
| Exp. II   | CDC LT           | Incandescent | 48         | 585           | 189   | 2     | 25      | 48      |
|           | UV LED           | 48         | 448         | 127           | 10    | 23    | 20      |
|           | Green LED        | 48         | 298         | 79            | 6     | 5     | 21      |
|           | Red LED          | 48         | 410         | 168           | 6     | 6     | 11      |
| BGS Trap  | Incandescent     | 48         | 96          | 34            | 49    | 24    | 31      |
|           | UV LED           | 48         | 81          | 12            | 10    | 41    | 73      |
|           | Green light stick| 48         | 43          | 5             | 27    | 13    | 29      |
|           | Red light stick  | 48         | 27          | 3             | 36    | 26    | 27      |

Others include mosquitoes in 5 genera (*Armigeres*, *Downsiomyia*, *Lutzia*, *Uranotaenia* and *Mansonia*)

### Table 2

The efficacy of the BGS trap and different attractants relative to the CDC LT and CO₂ for collecting anophelines and *An. minimus* collected by BGS Traps augmented with CO₂ was significantly higher than BGS Trap baited only with BG-lure (*χ²* = 34.554, *df* = 1, *P* < 0.0001, Table 3). However, the efficacy of BGS-Trap baited with CO₂ was not significantly different from the trap baited with BG-lure plus CO₂ for *Anopheles* capture (*P* = 0.92). For *An. minimus* collections, adding BG-lure into the BGS Trap baited with CO₂ significantly improved the trap efficacy (IRR = 1.55, *P* = 0.046, Table 3).

Age grading and blood meal status identification took place for a total of 2477 *Anopheles* females (Table 4). Almost all of the females were found to be in the empty stage (99.1%). Notably, nearly half of the samples captured by the BGS Trap baited with BG-lure were recently blood-fed specimens (41.5%, *n* = 17). Parity rates of *Anopheles* mosquitoes collected by BGS-Trap and

### Abbreviations:
- IRR: estimated incident rate ratio
- CI: confidence interval
- *P*-value: based on maximum likelihood estimation of GLM with negative binomial error and log link function

| Treatment | IRR | 95% CI | *χ²* | *P*-value |
|-----------|-----|--------|------|-----------|
| *♀* Anopheles | CDC light trap (reference) | 0.19 | 0.13–0.29 | 60.21 | < 0.0001 |
| | BG-sentinel | 0.19 | 0.13–0.29 | 60.21 | < 0.0001 |
| | CO₂ (reference) | 0.38 | 0.23–0.63 | 13.80 | < 0.0001 |
| | BG-lure + CO₂ | 1.20 | 0.74–1.97 | 0.54 | 0.462 |
| | BG-lure | 0.38 | 0.23–0.63 | 13.80 | < 0.0001 |
| *♀* *An. minimus* | CDC light trap (reference) | 0.15 | 0.10–0.23 | 71.33 | < 0.0001 |
| | BG-sentinel | 0.15 | 0.10–0.23 | 71.33 | < 0.0001 |
| | CO₂ (reference) | 0.36 | 0.21–0.61 | 13.93 | 0.002 |
| | BG-lure + CO₂ | 1.57 | 0.94–2.61 | 2.98 | 0.084 |
| | BG-lure | 0.36 | 0.21–0.61 | 13.93 | 0.002 |

Abbreviations: IRR estimated incident rate ratio, CI confidence interval, corresponding *P*-value based on maximum likelihood estimation of GLM with negative binomial error and log link function
CDC LT baited with different attractants ranged from 31.7 to 44.1%. No significant differences could be detected ($\chi^2 = 5.79, P = 0.33$, Table 4).

**Efficacy evaluation of BGS trap and CDC LT augmented with different colored lights**

For over 384 trap-nights, a total of 2557 female mosquitoes were captured representing 41 species in 11 genera including *Anopheles*, *Armigeres*, *Culex*, *Aedes*, *Bothaella*, *Lorrainea*, *Downsiomyia*, *Malaya*, *Mansonia*, *Uranotaenia* and *Heizmannia* (Table 1). *Anopheles* spp. were the most abundant (77.7% of the total collected mosquitoes, $n = 1988$). Seventeen *Anopheles* species were collected of which *An. minimus* was predominant ($n = 1490$). The BGS-Trap (all configurations) and CDC LT (all configurations) trapped 633 and 1924 mosquito females, respectively (Table 1). The main species collected by the BGS Trap were *An. minimus* ($n = 164$), *Ar. subalbatus* (Coquillett) ($n = 137$), *Ae. albopictus* ($n = 100$), *Cx. vishnui* ($n = 85$) and *An. peditaeniatus* (Leicester) ($n = 55$) while the common species collected by CDC LT were *An. minimus* ($n = 1326$), *An. peditaeniatus* ($n = 276$), *Ar. subalbatus* ($n = 89$) and *An. tessellatus* Theobald ($n = 44$). All totaled, a greater number of anophelines was attracted to the CDC LT configurations than the BGS Trap configurations.

The trap type, and different colored lights significantly influenced the number of female anophelines (GLM, $\chi^2 = 130.261, df = 1, P < 0.0001$ and $\chi^2 = 13.082, df = 3, 0.004$, respectively) and the number of *An. minimus* collected (GLM, $\chi^2 = 120.678, df = 1, P < 0.0001$ and $\chi^2 = 12.237, df = 3, 0.007$, respectively). Significantly lower numbers of female anophelines and *An. minimus* were collected by the BGS Trap relative to the CDC LT (Table 5). None of the colored lights tested could exceed the performance of incandescent bulbs as conventional light sources for CDC LT (Table 5). An overview of the results showed the order of trap efficiency as incandescent $>$ UV $>$ green $>$ red. As expected, the number of female anopheline (IRR = 0.50, $P = 0.004$) and *An. minimus* (IRR = 0.43, $P = 0.002$) collected were significantly lower when traps were augmented with red light (Table 5).

**Table 3** The efficacy of different attractants (for each trap type) relative to the CO$_2$ for collecting anophelines and *An. minimus*

| Treatment | IRR  | 95% CI  | $\chi^2$ | P-value |
|-----------|------|---------|----------|---------|
| CDC LT    |      |         |          |         |
| ♀ Anopheine CO$_2$ (reference) | 1.72 | 1.02–2.88 | 4.19 | 0.041 |
| BG-lure + CO$_2$ | 0.67 | 0.40–1.13 | 2.23 | 0.135 |
| BG-lure | 1.99 | 1.16–3.41 | 6.19 | 0.013 |
| BG-lure | 0.63 | 0.36–1.09 | 2.74 | 0.098 |
| ♀ *An. minimus* CO$_2$ (reference) | 1.02 | 0.66–1.60 | 0.01 | 0.917 |
| BG-lure + CO$_2$ | 0.19 | 0.11–0.33 | 34.55 | < 0.0001 |
| BG-lure | 1.55 | 1.01–2.39 | 4.00 | 0.046 |
| BG-lure | 0.18 | 0.09–0.34 | 27.65 | < 0.0001 |
| BGS-Trap |      |         |          |         |
| ♀ Anopheine CO$_2$ (reference) | 1.02 | 0.66–1.60 | 0.01 | 0.917 |
| BG-lure + CO$_2$ | 0.19 | 0.11–0.33 | 34.55 | < 0.0001 |
| BG-lure | 1.55 | 1.01–2.39 | 4.00 | 0.046 |
| BG-lure | 0.18 | 0.09–0.34 | 27.65 | < 0.0001 |

**Abbreviations:** IRR estimated incident rate ratio, CI confidence interval, corresponding P-value based on maximum likelihood estimation of GLM with negative binomial error and log link function

**Table 4** Percentage of female *Anopheles* (actual number) in different physiological conditions collected from each trap configuration and the parity rate for each trap configuration

| Physiological condition | BGS-Trap | CDC LT | Grand total |
|-------------------------|----------|--------|-------------|
|                         | BG-lure  | BG-lure + CO$_2$ | CO$_2$ |
|                         | BG-lure  | BG-lure + CO$_2$ | CO$_2$ |
|                         | BG-lure  | BG-lure + CO$_2$ | CO$_2$ |
|                         | BG-lure  | BG-lure + CO$_2$ | CO$_2$ |
| Nulliparous             | 19.5 (8) | 55.4 (113) | 63.7 (130) |
| Parous                  | 31.7 (13) | 44.1 (90) | 35.8 (73) |
| Gravid                  | 7.3 (3) | 0.5 (1) | 0.5 (1) |
| Recently blood-fed      | 41.5 (17) | 0 (0) | 0 (0) |
| Total                   | 41 | 204 | 204 |
| Parity rate (%)         | 31.7 | 44.1 | 35.8 |

Anopheles spp. were collected of which *An. minimus* was predominant ($n = 1490$). The BGS-Trap (all configurations) and CDC LT (all configurations) trapped 633 and 1924 mosquito females, respectively (Table 1). The main species collected by the BGS Trap were *An. minimus* ($n = 164$), *Ar. subalbatus* (Coquillett) ($n = 137$), *Ae. albopictus* ($n = 100$), *Cx. vishnui* ($n = 85$) and *An. peditaeniatus* (Leicester) ($n = 55$) while the common species collected by CDC LT were *An. minimus* ($n = 1326$), *An. peditaeniatus* ($n = 276$), *Ar. subalbatus* ($n = 89$) and *An. tessellatus* Theobald ($n = 44$). All totaled, a greater number of anophelines was attracted to the CDC LT configurations than the BGS Trap configurations.

The trap type, and different colored lights significantly influenced the number of female anophelines (GLM, $\chi^2 = 130.261, df = 1, P < 0.0001$ and $\chi^2 = 13.082, df = 3, 0.004$, respectively) and the number of *An. minimus* collected (GLM, $\chi^2 = 120.678, df = 1, P < 0.0001$ and $\chi^2 = 12.237, df = 3, 0.007$, respectively). Significantly lower numbers of female anophelines and *An. minimus* were collected by the BGS Trap relative to the CDC LT (Table 5). None of the colored lights tested could exceed the performance of incandescent bulbs as conventional light sources for CDC LT (Table 5). An overview of the results showed the order of trap efficiency as incandescent $>$ UV $>$ green $>$ red. As expected, the number of female anopheline (IRR = 0.50, $P = 0.004$) and *An. minimus* (IRR = 0.43, $P = 0.002$) collected were significantly lower when traps were augmented with red light (Table 5).

Within the CDC LT group, significantly fewer anophelines were collected when green (IRR = 0.56, $P = 0.001$)
and red lights (IRR = 0.56, P = 0.001) were used as light sources when compared to the numbers collected by the control (incandescent light). Significantly fewer *An. minimus* were collected when green (IRR = 0.55, P = 0.001,) and red lights (IRR = 0.57, P = 0.002) were used as light sources when compared to the numbers collected by the control (Table 6). However, analysis of the results demonstrated lower catch rates of anopheline females (IRR = 0.74) and *An. minimus* (IRR = 0.81) in the UV light relative to the incandescent light, although no significant differences were statistically detected.

A similar significant effect of different light colors was observed within the BGS Trap configurations. No significant differences were detected in UV light augmented traps compared to the control (P > 0.05, Table 6). However, significantly lower capture numbers of malaria vectors in green and red colored lights relative to the incandescent light were detected (P < 0.001).

### Table 5 Efficacy of BGS trap and different colored lights relative to the CDC LT and the incandescent light for capturing anophelines and *An. minimus* females

| Treatment                          | IRR     | 95% CI     | χ²   | P-value |
|------------------------------------|---------|------------|------|---------|
| *♀* Anopheline                     |         |            |      |         |
| CDC light trap (reference)         |         |            |      |         |
| BG-sentinel                        | 0.13    | 0.09–0.19  | 130.26| < 0.0001|
| Incandescent (reference)           | 0.80    | 0.50–1.29  | 0.83 | 0.363   |
| UV (10–400 nm wavelength)          | 0.48    | 0.30–0.77  | 9.12 | 0.003   |
| Green (490–570 nm wavelength)      | 0.50    | 0.31–0.81  | 8.09 | 0.004   |
| Red (620–780 nm wavelength)        |         |            |      |         |
| *♀* *An. minimus*                  |         |            |      |         |
| CDC light trap (reference)         | 0.11    | 0.08–0.17  | 120.68| < 0.0001|
| BG-sentinel                        | 0.81    | 0.48–1.37  | 0.61 | 0.436   |
| Incandescent (reference)           | 0.51    | 0.30–0.87  | 6.14 | 0.013   |
| UV (10–400 nm wavelength)          | 0.43    | 0.25–0.74  | 9.37 | 0.002   |
| Green (490–570 nm wavelength)      |         |            |      |         |
| Red (620–780 nm wavelength)        |         |            |      |         |

*Abbreviations*: IRR estimated incident rate ratio, CI confidence interval, corresponding P-value based on maximum likelihood estimation of GLM with negative binomial error and log link function

### Table 6 Efficacy of different colored lights (for each trap type) relative to the incandescent light for collecting anophelines and *An. minimus*

| Treatment     | IRR     | 95% CI     | χ²   | P-value |
|---------------|---------|------------|------|---------|
| *♀* Anopheline|         |            |      |         |
| CDC LT        |         |            |      |         |
| Incandescent  | 0.74    | 0.53–1.03  | 3.11 | 0.078   |
| UV (10–400 nm wavelength) | 0.56    | 0.40–0.79  | 10.99| 0.001   |
| Green (490–570 nm wavelength)    | 0.56    | 0.40–0.78  | 11.54| 0.001   |
| Red (620–780 nm wavelength)      |         |            |      |         |
| *♀* *An. minimus*                 |         |            |      |         |
| CDC LT        | 0.81    | 0.56–1.15  | 1.42 | 0.233   |
| Incandescent  | 0.55    | 0.38–0.79  | 10.38| 0.001   |
| UV (10–400 nm wavelength)          | 0.57    | 0.40–0.82  | 9.27 | 0.002   |
| Green (490–570 nm wavelength)      |         |            |      |         |
| Red (620–780 nm wavelength)        |         |            |      |         |
| BGS Trap      |         |            |      |         |
| Incandescent  | 0.66    | 0.38–1.13  | 2.28 | 0.131   |
| UV (10–400 nm wavelength)          | 0.27    | 0.14–0.51  | 16.4 | < 0.0001|
| Green (490–570 nm wavelength)      | 0.31    | 0.16–0.58  | 13.05| < 0.0001|
| Red (620–780 nm wavelength)        |         |            |      |         |
| *♀* *An. minimus*                  |         |            |      |         |
| Incandescent  | 0.54    | 0.28–1.04  | 3.43 | 0.064   |
| UV (10–400 nm wavelength)          | 0.22    | 0.10–0.48  | 14.35| < 0.0001|
| Green (490–570 nm wavelength)      | 0.17    | 0.07–0.40  | 16.60| < 0.0001|

*Abbreviations*: IRR estimated incident rate ratio, CI confidence interval, corresponding P-value based on maximum likelihood estimation of GLM with negative binomial error and log link function
The physiological stage and blood meal status were determined for 1988 female *Anopheles* collected from the eight trap configurations. Most of the collected females were in the empty stage (98.3%). Only 1.0–3.1% of adults collected from these traps contained blood. Parity rates of *Anopheles* mosquitoes collected by BGS-Trap and CDC LT baited with different colored-lights did not vary significantly, ranging from 37.2–55.6% ($\chi^2 = 3.65, P = 0.82$) (Table 7).

### Discussion

The present study represents the first field evaluation of the BGS Trap for capturing malaria vectors in Thailand and southeast Asia. Our findings demonstrated that the BGS Trap captured a variety of mosquitoes (30 species) during the night including important malaria vectors. However, results from this field study are similar to previous studies in which the CDC LT trapped significantly more mosquitoes and anopheline females than the BGS Trap and proved that mosquito species composition depends on trap type [18, 28, 29]. Moreover, important anthropophilic malaria vectors were caught by CDC LT. In addition to the design of the trap, trap height tends to play an important role in mosquito collections [30, 31].

Findings from the present study confirmed that CDC LT, when baited with BG-lure and CO$_2$, yields higher numbers of anophelines and *An. minimus* than other trap configurations. Our results parallel a similar environmental chamber study which found that the combination of the BG-lure and CO$_2$ proved to be most effective at attracting *An. gambiae* [21]. Surprisingly, our results demonstrated no significant differences in anopheline catch numbers between the CDC LT baited with CO$_2$ and the CDC LT baited with the BG-lure alone. Our findings revealed that the BG-lure can be used as a substitute for CO$_2$ as the attractant for CDC LT in malaria-centric areas and in study sites where viable CO$_2$ sources present a challenge. Our research has shown an equal proportion of parous females in each trap configuration suggesting that physiological stage does not make *Anopheles* prone to one particular trap.

The second objective of this study was to evaluate the efficacy of the BGS Trap and CDC LT when baited with different colored lights for capturing malaria vectors. Findings from the current study show the CDC LT (baited with BG-lure and CO$_2$) augmented with an incandescent light is a favorable tool for malaria vector surveillance in Thailand and can be applied in other countries. Interestingly, not all insect visible lights attract *Anopheles* mosquitoes. Our results show that colored lights and trap type have no significant impact on the collections of different physiological stages of female *Anopheles* (both parous and nulliparous). The CDC LT augmented with an incandescent light bulb, BG-lure and CO$_2$ is most effective and reliable in collecting anophelines and can be effectively used as the new gold standard technique to collect malaria vectors.

### Conclusion

The CDC LT augmented with an incandescent light bulb, BG-lure and CO$_2$ is most effective and reliable in collecting anophelines and can be effectively used as the new gold standard technique to collect malaria vectors. The BG-lure can be substituted for CO$_2$ as an attractant in CDC LT in hard-to-reach malaria sites where CO$_2$ sources are scarce. The BGS Trap can collect a variety of night-time feeding mosquitoes including important malaria vectors; however, the number of *Anopheles* collected is significantly lower than the CDC LT. The data presented here will assist researchers in selecting the most appropriate malaria vector surveillance tools.

### Abbreviations

- BGS: BG Sentinel™ trap
- CDC LT: Centers for disease control and prevention miniature light trap
- GLM: Generalized linear model
- GMS: Greater Mekong Subregion
- HLC: Human landing counts
- IRR: Incident rate ratios
- LED: Light-emitting diodes

### Acknowledgements

We acknowledge the excellent support of villagers in the study site. This work was financially supported by the Military Infectious Disease Research Program – Identification and Control of Insect Vectors of Infectious Diseases (Task Area U). The views expressed herein are the private views of the authors and do not reflect the official views of the Department of the Army, Department of Defense, or the U.S. Government.

### Table 7 Percentage of Anopheles female (actual number) in different physiological conditions captured from 8 different colored-light trap configurations and the parity rate for each trap configuration

| Physiological condition | BGS-Traps | CDC LT | Grand total |
|-------------------------|-----------|--------|-------------|
|                         | Incandescent | UV LED | Green LED | Red LED | Incandescent | UV LED | Green LED | Red LED | Incandescent | UV LED | Green LED | Red LED |
| Nulliparous             | 55.2 (53)  | 49.4 (40) | 62.8 (27) | 44.4 (12) | 51.8 (303) | 51.8 (232) | 55.7 (166) | 54.9 (225) | 53.2 (1058) |
| Parous                  | 43.8 (42)  | 48.2 (39) | 37.2 (16) | 55.6 (15) | 46.5 (272) | 45.1 (202) | 43.3 (129) | 44.2 (181) | 45.1 (896) |
| Gravid                  | 0 (0)      | 0 (0)   | 0 (0)     | 0 (0)     | 0 (0)      | 0 (0)   | 0 (0)     | 0 (0)     | 0 (0)      |
| Recently blood-fed      | 1.0 (1)    | 2.5 (2) | 0 (0)     | 0 (0)     | 1.7 (10)   | 3.1 (14) | 1.0 (3)   | 1.0 (4)   | 1.7 (34)   |
| Total                   | 96         | 81     | 43        | 27        | 585       | 448     | 298       | 410       | 1988       |
| Parity rate (%)         | 43.8       | 48.2   | 37.2      | 55.6      | 46.5      | 45.1    | 43.3      | 44.2      | 44.2       |

*Ponlawat et al. Parasites & Vectors (2017) 10:378*
Funding
This work was financially supported by the Military Infectious Disease Research Program – Identification and Control of Insect Vectors of Infectious Diseases (Task Area U). The funding agency played no role in the design or implementation of the study, analysis or interpretation of the data, or the preparation and submission of the manuscript.

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article.

Authors’ contributions
APL and BPE designed the experiments. APL, PK and BI performed the field experiments. BI identified mosquito samples. APS analyzed the data. APL and BPE wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1 Vector Biology and Control Section, Department of Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand.
2 Armed Forces Pest Management Board, Silver Spring, MD, USA.

Received: 5 May 2017 Accepted: 28 July 2017
Published online: 07 August 2017

References
1. Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F, et al. A research agenda to underpin malaria eradication. PLoS Med. 2011;8(1):e1000406.
2. Delacollette C, D’Souza C, Christophel E, Thimasarn K, Abdur R, Bell D, et al. Malaria trends and challenges in the greater Mekong subregion. Southeast Asian J Trop Med Public Health. 2009;40(4):674–91.
3. Cui L, Yan G, Sattabongkot J, Cao Y, Chen B, Chen X, et al. Malaria in the endemic area in western Thailand. J Med Entomol. 2004;41(2):151–67.
4. Delacollete C, D’Souza C, Christophel E, Thimasarn K, Abdur R, Bell D, et al. Malaria trends and challenges in the greater Mekong subregion. Southeast Asian J Trop Med Public Health. 2009;40(4):674–91.
5. Cui L, Yan G, Sattabongkot J, Cao Y, Chen B, Chen X, et al. Malaria in the greater Mekong subregion: heterogeneity and complexity. Acta Trop. 2012;121(3):227–39.
6. Parker DM, Carrara VI, Pukrittayakamee S, McGready R, Nosten FH. Malaria ecology along the Thailand-Myanmar border. Malar J. 2015;14:388.
7. Service MW. Mosquito ecology: field sampling methods. 2nd ed. London: Elsevier; 1999.
8. Mbogo CE. Sampling techniques for adult Afrotropical malaria vectors and their reliability in the estimation of entomological inoculation rate. Tanzan Health Res Bull. 2005;7(3):17–24.
9. Kilama M, Smith DL, Hutchinson R, Kigozi R, Yeka A, Lavoy G, et al. Estimating the annual entomological inoculation rate for Plasmodium falciparum transmitted by Anopheles gambiae s.s. using three sampling methods in three sites in Uganda. Malar J. 2014;13:111.
10. Sithiprasasna R, Jaichapor B, Chanaimongkol S, Khongtak P, Khoungtak P, Lealsrivattanakul T, Tiang-Trong S, et al. Evaluation of candidate traps as tools for conducting surveillance for Anopheles mosquitoes in a malaria-endemic area in western Thailand. J Med Entomol. 2004;41(2):151–7.
11. Harris C, Khinoda J, Lwetoijera D, Dongus S, Devine G, Majumber S. A simple and efficient tool for trapping gravid Anopheles at breeding sites. Parasit Vectors. 2011;4:125.
12. Hiwat H, De Rijk M, Andriessen R, Koennaadt CJ, Takken W. Evaluation of methods for sampling the malaria vector Anopheles darlingi (Diptera, Culicidae) in Suriname and the relation with its biting behavior. J Med Entomol. 2011;48(5):1039–46.