12-2018

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Citation Details

Peck, G. W., Castro-Llanos, F., López-Sifuentes, V. M., Vásquez, G. M., & Lindroth, E. (2018). Comparative
Analysis of Mosquito Trap Counts In the Peruvian Amazon: Effect of Trap Type and Other Covariates On
Counts and Diversity. Journal of the American Mosquito Control Association, 34(4), 291-301.

COMPARATIVE ANALYSIS OF MOSQUITO TRAP COUNTS IN THE PERUVIAN AMAZON: EFFECT OF TRAP TYPE AND OTHER COVARIATES ON COUNTS AND DIVERSITY

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ABSTRACT. Efficient detection of multiple species of adult mosquitoes in various habitats using effective traps is a crucial 1st step in any disease prevention program. Novel trap types that target tropical vectors of human diseases require field testing in the habitat of the vector–disease system in question. This paper analyzes a series of mosquito trapping studies conducted at Mapacocha, San Juan Bautista District, Loreto, Peru, during August–September 2013 and April–May 2014. Six trap configurations were evaluated in forest and rural locations. Adult mosquito counts were analyzed using full Bayesian inference of multilevel generalized linear models and posterior probability point estimates of the difference of means of the combined trap catch by trap type comparisons of all species. Light traps (Centers for Disease Control and Prevention [CDC] incandescent, white light-emitting diode [LED], and ultraviolet LED) caught greater numbers of mosquitoes compared with traps baited with yeast-generated CO_2 and Biogents Sentinel™ traps (battery powered traps without light and passive box traps). However, diversity measures (species richness, evenness, and similarity) were consistently nearly equal among trap types. Arbovirus vectors were more common in forest locations, while malaria vectors were more common near human habitations. Location had a significant effect on trap effectiveness and mosquito diversity, with traps from forest locations having greater numbers and greater species richness, compared with traps set near human habitations. The results of this study will inform mosquito surveillance trap choices in remote regions of central South America, including regions with emerging tropical diseases, such and dengue and Zika virus.

KEY WORDS   Bayesian analysis, carbon dioxide–baited trap, mosquito vectors, passive box trap, ultraviolet-light trap

INTRODUCTION

The design and analysis of mosquito monitoring programs has its part in the literature (Silver 2008), and choosing an appropriate type of trap is essential to mosquito surveillance programs (Reisen et al. 1999, Reisen and Lothrop 1999, Kline 2006). The statistical analysis and concomitant inferences from data derived from such programs continue to advance through reevaluation of traditional approaches (Ryan et al. 2004, Chaves 2010) and by using new methods (Overgaard et al. 2012, Padilla-Torres et al. 2013). For example, Ryan et al. (2004) employed spatial correlation and interpolation to examine light trap counts of adult mosquito spatial patterns and densities, assessing how the results of their statistical analysis would generalize to an independent data set. Chaves (2010) introduced to the medical entomology community statistical methods that address concerns in experimental design and data analyses, especially with respect to independence and pseudo-replication. Overgaard et al. (2012) used a log-linear model to measure sampling efficiency. Padilla-Torres et al. (2013), acknowledging that detection of mosquitoes is never perfect, implemented a set of hierarchical models of occupancy dynamics. These studies provide evidence that new methods in data analysis give deeper insights into underlying ecological processes and may inform decisions such as when to sample, how to sample, and what is the best sampling device or method.

Adult mosquito sampling programs usually follow some sort of conventional design recommendations (Reisen and Lothrop 1999, Silver 2008). However, there may be instances where the target species may be very rare or difficult to trap, resulting in a small number of adult mosquitoes collected for a given trapping period. In those cases, standard statistical analyses may produce biased results, and target species population size is grossly overestimated (Gelman et al. 2014, McElreath 2016). Bayesian inference is an alternative method of statistical inference, and is being used to analyze insect trap counts and for estimation of insect population size (Ellison 2004). In cases of small sample size, Bayesian methods are more powerful than standard statistical methods and encompass a more complete and coherent approach to representing the uncertainty associated with any estimate of mosquito population parameters (Padilla-Torres et al. 2013).

Beyond new approaches to trap count analysis, there is a need to develop and test mosquito sampling

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methods that are useful in remote locations and/or for mosquito control agencies with small budgets (Ritchie et al. 2013). Dry ice is the most commonly used source of the attractant carbon dioxide (CO_2) and has found wide use (Reeves 1953, Kline 2006, Silver 2008). However, dry ice and other sources of CO_2 are expensive, difficult to transport, and usually not available in remote areas (Smallegange et al. 2010). A yeast–sugar–water (YSW) mixture has been shown to generate sufficient CO_2 for mosquito attraction (Saitoh et al. 2004). Steiger et al. (2014) compared dry ice and YSW CO_2 sources and found that although the overall number of female mosquitoes decreased with traps using YSW, there was little effect on sample diversity (species richness) between attractant types. Cost effectiveness can also be achieved by use of non–battery powered traps for mosquito surveillance, an essential design for mosquito surveillance in remote locations without regular access to conventional batteries. Ritchie et al. (2013) showed that a passive box trap (PBT) baited with compressed tank-derived CO_2 caught approximately the same number of mosquitoes as a Centers for Disease Control and Prevention (CDC) light trap. Even greater cost effectiveness could be achieved by coupling the PBT with YSW, and such a design would be useful for mosquito control in remote locations.

The present study describes a comparison of an assemblage of adult mosquito trap types and the analysis of mosquito trap types on mosquito counts using linear models for Bayesian inference, with emphasis on the effect of various trap types on mosquito counts, mosquito diversity, and trap catch mosquito community similarity conducted in the Peruvian Amazon. The narrative and results of this study address a knowledge gap in the statistical analysis of adult mosquito trap counts and in the comparisons of such traps, including an analysis of the impact of environmental variables on such trapping programs.

MATERIALS AND METHODS

2013 forest site trapping study

Traps were set in a semicircular pattern in the forest approximately 200 m N of the Peruvian Army Base (centered at −3.816716 S, −73.341660 W) in Mapacocha, San Juan Bautista District, Maynas Province, Loreto State, Peru, approximately 14.5 km W–SW of the city of Iquitos. Dates of deployment were August 27, 30, 31, and September 1, 2, 3, 5, and 6, 2013. The general environment around the city of Iquitos is tropical forest, but also includes secondary forest growth, clear cuts, and scattered farms. Traps were set approximately 30 m apart, under the forest canopy at a height of 1 m, except for the Biogents Sentinel™ v1.0 trap, which was set on the ground and deployed for 8 evenings from 1800 h to 2200 h (4 h). Trap types were rotated

among trap sites twice. Four trap types were used: Biogents Sentinel mosquito trap v1.0 (BGS; BioQuip Products, Rancho Dominguez, CA; cat. no. 2880), encephalitis virus surveillance (EVS) mosquito trap with a white light-emitting diode (EVS; BioQuip cat. no. 2780), ultraviolet light trap (UV; BioQuip cat. no. 2770), and a CDC Miniature Light Trap Model 512 with incandescent light (CDC-L; John Hock Co. Gainesville, FL). A white catch bag (BioQuip cat no. 2801WW) was used for collections on all but the BGS Sentinel trap. No chemical lures were used; the only attractant was light, or in the case of the BGS, white color during daylight hours. Circular black plastic rain protectors (50 cm diameter) were installed above all traps except the BGS trap. Captured mosquitoes from all studies were transported in a cooler box with ice packs to the laboratory, anesthetized with triethylamine, and identified to species where possible using dichotomous keys (Lane 1953, Pratt 1953, Galindo et al. 1954, Consoli and Oliveira 1994).

2014 forest transect trapping study

Traps were set in the forest approximately 100 m SW of the Peruvian Army Base fence along an access road in a linear transect oriented from NW to SE (centered at −3.818799 S, −73.342394 W). Dates of deployment were April 22–26, 29, and May 2, 2014. Traps were placed 15–30 m into the virgin rainforest by clearing a 1 m path through vegetation, and traps were set 30 m apart along the transect. Traps were tested for 8 nights, deployed 1 h before sunset, and retrieved 1 h after sunrise (12 h). Four trap configurations were rotated twice through the transect. Trap configurations were as follows: CDC Miniature Light Trap Model 512 with incandescent light (CDC-L; John Hock Co. Gainesville, FL), CDC Miniature Light Trap Model 512 (without light) with YSW as a CO_2 source (CDC-Y), a BioQuip UV light trap, and a passive box trap (PBT; modeled after Ritchie et al. 2013) with a YSW jug as CO_2 source. Rain protectors were installed above all traps except the PBT. The YSW jug was modeled after Saitoh et al. (2004) using 60 g Red Star baking yeast (Lesaffre Yeast Corporation, Milwaukee, WI), 500 g table sugar, and 2 liters tap water, mixed and placed into a 3-gallon plastic water container. Preliminary tests of this system in the laboratory showed CO_2 output similar to that described in Saitoh et al. (2004) and Steiger et al. (2014) but a factor of 5 smaller than rates reported in Smallegange et al. (2010).

2014 barracks trapping study

Traps were set around the perimeter of the Peruvian Army Base (centered at −3.818406 S, −73.341494 W), placed at least 30 m apart at 4 locations that were near human gathering places but away from direct interference from ambient lighting (near main gate, outside soldiers' sleeping quarters,

an outdoor work area, and outside officers' quarters) for 4 nights, deployed 1 h before sunset and retrieved 1 h after sunrise (12 h). Two trap configurations (CDC-L and the UV) were rotated through the 4 locations on the nights of May 6, 8, 16, and 17, 2014 (on a given night 2 of each trap type were deployed). These 2 trap configurations were chosen for this 2nd round of trap deployments due to their excellent performance during the earlier forest trapping study and also because the mosquito fauna near human habitations in this area has greater densities of anthropophilic species compared with species found in the forest (Jones et al. 2004, Turell et al. 2008). Nontarget insects were enumerated for the barracks trapping study only.

Environmental covariate data for all studies were taken from records provided by the Puerto Almendras Meteorological Station (SENAMHI), Maynas Province, Loreto Department, Peru (−3.830294 S, −73.3798528 W; alt. 93 m), located approximately 4 km W of the Peruvian Army Base. Reported temperature (°C) and percentage relative humidity (%RH) were the daily average of 3 readings taken at 0700, 1300, and 1900 h. Daily precipitation data (mm) was the average of both 12-h intervals, the 1st beginning at 1900 h on the previous day and ending at 0700 h on the day of reporting, the 2nd beginning at 0700 h and ending at 1900 h on the reporting day.

Statistical analysis

Bayesian Estimation Supersedes the *t*-test (BEST) package (Kruschke 2013, Kruschke and Meredith 2017) was used as an alternative to *t*-tests, producing posterior estimates for trap group means and their differences. Priors were minimally informative, e.g., normal priors with large standard deviation for the associated mean. Convergence and fit were assessed with *R-hat* and *n.eff* metrics. Values of *R-hat* <1.10 met the convergence criteria, while *n.eff* values >10,000 were considered necessary for estimation of 95% highest density intervals (HDI).

R statistical software was used for statistical computing (R Core Team 2017). RStudio (RStudio Programming Team 2017) was used as an integrated development environment for statistical analysis with R, and the RStan package (Stan Development Team 2017) was used as an R language interface for performing inference and posterior analysis for Stan language programs. Scripts to guide model construction, evaluation, and parameter comparisons were derived following McElreath (2016), and model evaluations were performed using R scripts modified from templates outlined in the rethinking R package (http://xcelab.net/rm/software/). Stan script settings were as follows: iter = 3,000; warmup = 1,000; and chains = 4. The following diagnostics were performed on all Stan analyses outputs: trace plots were checked for stationarity and good mixing and *n.eff* was >10,000 for the estimated number of indepen-

dent samples generated; to ensure convergence of the Markov chains, and to ensure that *R-hat* = 1.00 from above (Gelman and Hill 2007, Gelman et al. 2014). We built 3 multilevel models with a Poisson likelihood, using a log-link to equate the expected outcome parameter, λ, with a series of linear models: model 1 included terms for trap, site, date, precipitation, temperature, and percentage relative humidity; model 2 included terms for trap, site, and date; while model 3 used trap as its only term (although all models included an intercept term). Precipitation, temperature, and percentage relative humidity were standardized (subtracting the mean and dividing by the standard deviation) before analysis. Each term in the above models had its own parameter, and to reduce the chance of overfitting, regularizing (flat) priors on all model parameters were used. Since small sample size increases the influence of the prior distribution on statistical inference, we placed lower thresholds on the analysis of counts. For the forest data sets, taxa with fewer than 32 total mosquitoes (*n*) counted were not analyzed, while taxa with fewer than 8 total mosquitoes counted were not analyzed for the Peruvian Army Base data set.

We ranked competing models by ordering their respective Watanabe–Akaike information criterion (WAIC) values and *w*, the Akaike weight for each model, an estimate of the probability that the model will make the best predictions on new data depending on the set of models considered (Burnham and Anderson 2011). We consider the WAIC to be the expected deviance of a model on future data, and we consider Akaike weights analogous to posterior probabilities of models, conditional on expected future data (McElreath 2016). For species diversity analysis we followed the distinctions between entropy, indices, and diversity outlined by Jost (2006, 2007). The R package Vegetarian (Charney 2015) was used to compute species diversity indices (α, β, γ) and community overlap indices with uncertainty estimates for trap count data sets using standard methods.

RESULTS

Overall trends

The combined mosquito trap catch reported here is 5,363 individuals, including 7 genera and 28 species. Per species trap catch rates for the 2013 forest sites ranged from 0 to 50.38 (*Culex* (*Culex*) *coronator* Dyar and Knab; BGS) per trapping period. The 2013 combined overall species–trap catch rates (mean [SE], *n* = number of trap nights) per period in decreasing magnitude were as follows: UV (159.13 [24.63], *n* = 8) > CDC-L (97.88 [15.76]) > BGS (89.50 [26.61]) > EVS (33.63 [5.19]). Per species trap catch rates for the 2014 forest sites ranged from 0 to 22.00 (*Aedeomyia squamipennis* (Lynch Arribalzaga); UV) per trapping period. The 2014 trap (mean [SE]) per period trap catch rates in decreasing
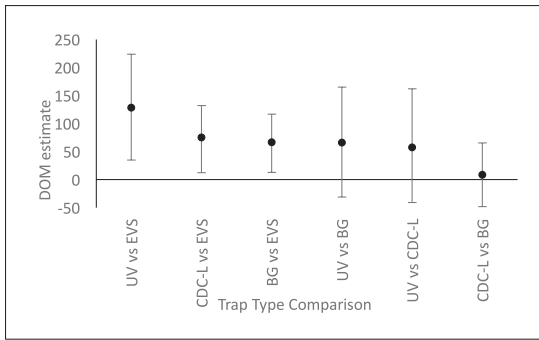
Fig. 1. Posterior probability point estimates of the difference of mosquito catch rate means (difference of means, DOM) of all species combined trap catch by trap type comparison for 2013 forest sites. Error bars are 95% highest density interval (HDI), $n = 8$ trap periods. Trap comparisons where the HDI does not include 0 are significantly different. Trap legend: UV = BioQuip ultraviolet light trap, CDC-L = Centers for Disease Control and Prevention light trap, BGS = BioGents Sentinel, EVS = BioQuip EVS trap.
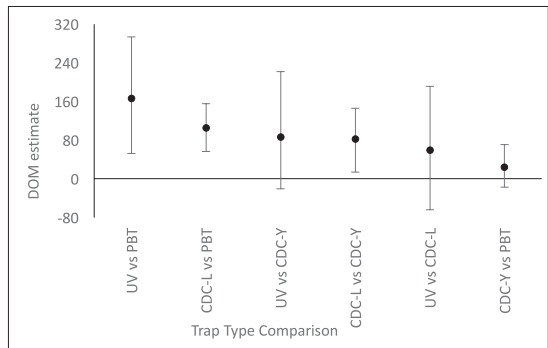

Fig. 2. Posterior probability point estimates of the difference of mosquito catch rate means (DOM) of all species combined trap catch by trap type comparison for 2014 forest sites. Error bars are 95% highest density interval (HDI), $n = 7$ trap periods. Trap legend: UV = BioQuip ultraviolet light trap, CDC-L = Centers for Disease Control and Prevention light trap, CDC-Y = centers for disease control trap, light removed, and yeast jug added, PBT = passive box trap.

magnitude were as follows: UV (39.03 [28.59], $n = 7$) > CDC-L (24.16 [16.43]) > CDC-Y (7.41 [5.63]) > PBT (1.41 [1.19]). Per species trap catch rates for the 2014 army barracks sites ranged from 0 to 5.75 (*Anopheles darlingi* Root; UV) per trapping period, with the UV having the highest catch rate (7.14 [3.78], $n = 4$), while the CDC-L exhibited a lower catch rate (2.50 [2.60]). Comparing these 2 trap types across dates and locations, the UV LED trap had the highest catch rates, and the CDC-L trap had the 2nd highest trap catch rates, and both these traps had similar catch rates in the 2 forest sites (2013 and 2014). Far fewer mosquitoes were caught near the Peruvian army barracks when compared with the forest sites (catch rates in the forest sites were >10 times the barracks catch rates for similar traps). There were numerous nontarget insects caught in the army barracks trapping study. The ratios of mosquitoes to nontarget insects were 1:46 for the CDC-L, 1:194 for the UV, and 1:115 overall.

BEST analysis

Using BEST as an alternative to *t*-tests, we produced posterior estimates for trap group means and their differences. Posterior probability point estimates of the difference of means (DOM) of combined trap catch by trap type comparison of all species for 2013 and 2014 forest sites revealed significant differences (95% HDI does not include 0) for 3 of the 6 possible comparisons (Figs. 1 and 2). Posterior probability DOM point estimate for 2014 army barracks sites of combined trap catch by trap type of all species (CDC-L vs. UV trap) was not significant (DOM = 8.39, 95% HDI [–3.23, 20.05], $n = 4$ trap nights).

Bayesian Hierarchical Poisson Regression

2013 forest sites: Thirteen taxonomic groups were evaluated (Table 1). In 7 cases the Akaike weights (*w*) are reported as unity (1.00) for 1 of the 3 hierarchical models, and 6 of those 7 cases are unity for model 1 (model 1 terms include: trap, site, date, precipitation, temperature, and %RH). Thus, the environmental covariates increased model predictive probability greatly for this data set. The 7th case was *Cx*. (*Culex*) *coronator*, where $w = 1.0$ for model 2 (model 2 terms include: trap, site, and date). In 9 cases model 1 was the top ranked model (largest *w* value), while models 2 and 3 were ranked 1st twice. Of special interest were those cases where the trap term alone gave the best predictive probability (model 3): *Culex* (*Melanoconion*) *theobaldi* Lutz (*w* = 0.74) and *Culex* (*Mel*.) *portesi* Senevet and Abonnenc (*w* = 0.98).

2014 forest sites: Eleven taxonomic groups were evaluated (Table 2). In 6 cases the Akaike weight (*w*) was unity, and 4 of those 6 were model 1, while the other 2 of the 6 were model 3. *Culex* (*Cux*.) *coronator*, with $w = 0.99$ for model 3, was very near unity, suggesting that trap type alone, independent of date, location, and environmental covariate, was the most important predictor of trap catch for this species. Six of the eleven taxa evaluated had model 3 ranking highest, including the "All mosquito sp." group and the "All *Culex* (*Cux*.) spp. L." group. *Uranotaenia* (*Uranotaenia*) spp. counts were analyzed where the trap term alone gave the best predictive probability (model 3).

2014 army barracks sites: Seven taxonomic groups were evaluated (Table 3). *Uranotaenia.* (*Ura*.) spp. Lynch Arribalzaga analysis provided the 1 *w* value of unity. For the other 6 taxa model 1 was top ranked, while model 2 ranked 1st for the "All

Table 1. Hierarchical model analysis of mosquito counts from the Forest sites in August–September 2013.

Taxonomic group	n	Model[1]	WAIC	w
All mosquito spp.	3,041	1	2,060.4	0.56
All *Culex* spp.	2,477	1	1,664.6	1.0
Aedeomyia (*Ady*.) *squamipennis*	91	1	213.9	0.87
Aedes (*Och*.) *serratus*	236	1	395.1	1.00
Culex (*Cux*.) *coronator*	785	2	682.9	1.0
Culex (*Mel*.) *theobaldi*	62	3	134.6	0.74
Culex (*Mel*.) *vomerifer*	156	1	176.0	1.0
Culex (*Cux*.) *declarator/mollis*	39	1	114.6	1.0
Culex (*Mel*.) *gnomatus*	183	1	235.2	1.0
Culex (*Me*.) *pedroi*	380	2	375.7	0.81
Culex (*Mel*.) *portesi*	167	3	227.0	0.98
Mansonia (*Man*.) spp.[2]	116	1	222.8	0.68
Uranotaenia (*Ura*.) spp.	53	1	128.8	1.00

[1] Models were ranked using Akaike weight (*w*), among other information criteria and their associated error estimates. See "Materials and Methods" for details of analysis and outputs. Model legend: 1) terms used: trap, site, date, precipitation, temperature, and % RH; 2) terms used: trap, site, date; 3) term used: trap.
[2] All *Mansonia* species combined (*flaveola, indubitans/titillans,* and spp.).

Culex (*Cux*.) spp." group, and model 3 ranked 1st for *Culex* (*Cux*.) *coronator* (*w* = 0.91) and *Mansonia* (*Mansonia*) *indubitans* Dyar and Shannon/*titillans* Walker (*w* = 0.65).

Diversity analysis

2013 forest sites: In terms of total species sampled (*n*) and total mosquitoes captured (*N*), a hierarchy emerged: UV (*n* = 25, *N* = 1,273) > CDC-L (*n* = 21, *N* = 783) > EVS (*n* = 21, *N* = 269) > BGS (*n* = 18, *N* = 716). In terms of total species sampled and total mosquitoes captured, the UV trap was superior, with the CDC-L and EVS capturing equal numbers of species but greater numbers of mosquitoes sampled for the CDC-L compared with the EVS. While the BGS drew in the smallest number of species, it trapped mosquitoes in comparatively large numbers. Trends in α diversity (within a trap type) between the EVS, UV, and CDC-L traps were roughly equal (Table 4), while the BGS trap showed markedly smaller diversities. Diversity calculations for the overall data set revealed some difference between α

(average diversity among the 4 trap types) and γ (global diversity for entire data set) when calculated across all trap types (Table 4, right columns). The degree of community overlap (similarity (SE); Table 5) ranged from 0.700 (0.019) for the CDC-L/BGS to 0.959 (0.012) for the CDC-L/EVS. The most striking pattern within the 2013 forest similarity table are the relatively low values for the BGS trap compared with the other trap types.

2014 forest sites: In terms of total species sampled (*n*) and total mosquitoes captured (*N*), a hierarchy emerged: UV (*n* = 23, *N* = 1,249) > CDC-L (*n* = 23, *N* = 773) > CDC-Y (*n* = 15, *N* = 237) > PBT (*n* = 9, *N* = 45). Trends in α diversity (within a trap type) between the UV and CDC-L traps were roughly equal (Table 4), while the CDC-Y trap showed intermediate α diversity values and the PBT showed markedly smaller α diversities. Diversity calculations for the overall data set revealed some difference between α (average diversity among the 4 trap types) and γ (global diversity for entire data set) when calculated across all trap types (Table 4, right columns), with β values somewhat greater than 1.

Table 2. Hierarchical model analysis of mosquito counts from the Forest sites in April–May 2014.

Taxonomic group	n	Model[1]	WAIC	w
All mosquito spp.	2,304	3	3,052.2	1.00
All *Culex* spp.	1,427	3	2,247.9	1.00
Adedeomyia (*Ayd*.) *squamipennis*	252	1	463.7	1.00
Aedes (*Och*.) *serratus*	123	2	188.4	0.96
Anopheles (*Ano*.) *mattogrossensis*	94	1	463.3	1.00
Coquillettidia (*Rhy*.) *venezuelensis*	51	1	142.4	1.00
Culex (*Cux*.) *coronator*	163	3	344.8	0.99
Culex (*Cux*.) *declarator/mollis*	109	3	278.3	0.77
Culex (*Mel*.) *portesi*	98	3	234.5	0.97
Mansonia (*Man*.) *indubitans/titillans*	118	1	214.8	1.00
Uranotaenia (*Ura*.) spp.	155	3	394.5	0.87

[1] Models were ranked using Akaike weight (*w*), among other information criteria and their associated error estimates. See "Materials and Methods" for details of analysis and outputs. Model legend: 1) terms used: trap, site, date, precipitation, temperature, and % RH, 2) terms used: trap, site, date; 3) term used: trap.

Table 3. Hierarchical model analysis of mosquito counts from the adjacent army base sites in April–May 2014.

Taxonomic group	n	Model[1]	WAIC	w
All mosquito spp.	135	1	109.8	0.76
All *Culex* spp.	73	2	99.7	0.77
Aedeomyia (*Ady*.) *squamipennis*	11	1	35.2	0.50
Anopheles (*Nys*.) *darlingi*		1	36.7	0.91
Culex (*Cux*.) *coronator*	32	3	64.7	0.91
Mansonia (*Man*.) *indubitans/titillans*	8	3	33.5	0.65
Uranotaenia (*Ura*.) spp.[2]	14	1	34.7	1.00

[1] Models were ranked using Akaike weight (*w*), among other information criteria and their associated error estimates. See "Materials and Methods" for details of analysis and outputs. Model legend: 1) terms used: trap, site, date, precipitation, temperature, and % RH; 2) terms used: trap, site, date; 3) term used: trap.

[2] All *Uranotaenia* combined (*geometrica, pulcherrima,* spp.).

The degree of community overlap (similarity) ranged from 0.552 (0.061) for the PBT/UV to 0.972 (0.006) for the CDC-L/UV (Table 5). General patterns within the 2014 forest similarity table are the relatively low values for traps being compared with the PBT and CDC-Y traps and the overall similarity was less in 2014 compared with 2013.

2014 army barracks sites: In terms of total species sampled (*n*) and total mosquitoes captured (*N*), the UV (*n* = 14, *N* = 100) was greater than the CDC-L (*n* = 8, *N* = 35). The α diversity (within a trap type) for the UV was greater than the CDC-L traps (Table 4). Diversity for the overall data set showed distinct differences in α (average diversity among the 4 trap types) and γ (global diversity for entire data set) when comparing the UV and CDC-L traps (Table 4, right columns), with β values slightly greater than 1. The degree of community overlap (similarity) for the UV/CDC-L community comparison was lower for traps set in the barracks compared with the forest (Table 5).

Supplemental materials—Raw count data, R scripts, and environmental meta-data are available from GWP (geopeck@pdx.edu or gwpeck5@gmail.com).

DISCUSSION

The present study describes the analysis of mosquito trap type on mosquito counts using linear models for Bayesian inference, with emphasis on the effect of various trap types on mosquito counts, and an analysis of mosquito diversity and trap catch mosquito community similarity in the Peruvian Amazon. Early studies provided some of the 1st results of mosquito trapping efforts in and around Iquitos, Loreto, e.g.,: Morales-Ayala (1971) found 20 species in the Iquitos area, while Need et al. (1993) found 25. A later 3-year study by Pecor et al. (2000) found a total of 16 genera and 96 species from traps placed in Iquitos and up to 40 km outside the city. Centers for Disease Control and Prevention (CDC) light traps (baited with dry ice) and human bait collections were used in the Pecor study. A series of complementary studies (Pecor et al. 2000, Jones et al. 2004, Turell et al. 2008, Reinbold-Wasson et al. 2012) was conducted from September 1996 to October 1997 in and around Iquitos, with dry ice traps being compared to human landing catches. It is noteworthy that both Need et al. (1993) and Jones et al. (2004) conducted part of their surveillance in a

Table 4. Diversity index analysis by location/date and trap type using the R package Vegetarian. *n* = number of mosquito taxa detected; *N* = number of mosquitoes sampled. Diversity measure types: alpha (α) denotes the diversity within a single sample, beta (β) denotes differences in diversity between samples, and gamma (γ) denotes the total diversity over the set of samples. Shannon diversity measures (*S*). Standard errors for parameter estimates (parentheses) are bootstrap calculations after 1,000 iterations.

| Diversity by location | Trap type (α) | | | | All traps | | |
	BGS	EVS	UV	CDC-L	α	β	γ
Forest (2013)							
n	18	21	25	21		25	
N	716	269	1,273	783		3,041	
S	5.27 (0.26)	11.77 (0.67)	11.34 (0.31)	10.94 (0.37)	9.36 (0.20)	1.17 (0.01)	10.91 (0.24)
Forest (2014)							
n	15	23	9	23		28	
N	237	1,249	45	773		2,304	
S	8.50 (0.47)	10.25 (0.31)	6.55 (0.66)	10.94 (0.39)	8.89 (0.29)	1.37 (0.04)	12.16 (0.34)
Barracks (2014)							
n			14	8		14	
N			100	35		135	
S			8.87 (0.72)	4.34 (0.74)	6.20 (0.58)	1.27 (0.06)	7.87 (0.64)

Table 5. Similarity matrix analysis by location/date and trap type using the R package Vegetarian. Standard errors for parameter estimates (parentheses) are bootstrap calculations after 1,000 iterations. In general, as the similarity index value approaches 1.0, there is increasing community overlap between trap types.

	BGS	EVS	UV	CDC-Y	PBT	CDC-L
Forest 2013						
EVS	0.784 (0.025)					
UV	0.768 (0.016)	0.948 (0.012)				
CDC-L	0.700 (0.019)	0.959 (0.012)	0.953 (0.008)			
Forest 2014						
UV				0.708 (0.023)		
PBT			0.552 (0.061)	0.726 (0.052)		
CDC-L			0.972 (0.006)	0.664 (0.025)	0.577 (0.060)	
Barracks 2014						
UV						0.656 (0.072)

forested area approximately 400 m NE of Puerto Almendras, a small town that is approximately 1.4 km W of the present study site, and that Need et al. collected on site at the Peruvian Army Base cited above. There are many mosquito vectors and vector-borne diseases in the Iquitos area, including selected *Aedes* and *Culex* species that carry arboviruses (Turell et al. 2008), and anophelines that carry *Plasmodium* species (Reinbold-Wasson et al. 2012).

To our knowledge, this is the 1st study to investigate this particular assemblage of trap types simultaneously in forested areas around and within a military barracks in the Peruvian Amazon. While mosquito surveillance and trap evaluations have been conducted in this region of Peru in the past (Rogers et al. 1993, Jones et al. 2004), 2 of the traps (PBT and CDC-Y) evaluated in the present study are novel (Bhalala and Arias 2009, Ritchie et al. 2013, Steiger et al. 2014). The high species diversity observed in the present study was reported in previous studies (Need et al. 1993, Pecor et al. 2000) and is to be expected, since this region of the world has one of the highest reported mosquito diversities (Rueda 2008). Ultraviolet light is highly attractive to a wide variety of insects, including biting flies, and is a common tool for insect collection efforts (Silver 2008). In this study, light, especially UV light, was more attractive than CO_2 produced through a YSW system. The relatively lower diversity values for army barracks were probably due to its distance from the contiguous forest (100 m), many interfering lights, and other factors.

The BGS trap has been evaluated extensively in many regions of the world, including Australia (Williams et al. 2014), eastern North America (Meeraus et al. 2008), China (Li et al. 2016), Central Africa (Schmied et al. 2008), and South America (Hiwat et al. 2011). However, this study is the 1st instance of a nonchemically baited BGS trap compared against standard and novel trap types. Hiwat et al. (2011) found that the BGS trap (with a CO_2 source) was effective for sampling *An. darlingi* (2 to 6 per trap night) and *Culex* spp. (39 to 46 per trap night). This contrasts with the present study,

where the average BGS *Culex* spp. catch per 4-h trapping period was 73, with 69% of that catch identified as *Culex*. (*Cux.*) *coronator*, a species native to South and Central America, but one that is invading and spreading within North America (Connelly et al. 2016). Obenauer et al. (2014) caught 25/20 species in suburban/sylvatic sites in Florida, using a 3-h trap period (0800 h to 1100 h). These rates of species richness are slightly larger than the present study (18 species detected); however, Obenauer et al. (2014) achieved these rates using a BGS baited with ammonia and CO_2, while the present study used none. Farajollahi et al. (2014) used unbaited BGS and CDC light traps in New Jersey. Species richness was low in both cases (4 and 5, respectively). However, adding a lure and CO_2 to the BGS and CDC light trap increased trap catch species richness (13 and 6, respectively). The comparatively higher species diversity observed in the present study is most likely explained by location effects: New Jersey has far fewer mosquito species than the Peruvian Amazon. In the present study, the UV trap caught 1.8 times (44% more) as many total mosquitoes as the BGS.

Ritchie et al. (2013) tested the PBT against the CDC light trap in 2 locations: Smithfield Waste Disposal Facility near Cairns, Australia, and Graves Swamp, a cypress oak–cabbage palm depression swamp located in Indian River County, Florida. The Centers for Disease Control model 512 light trap (John W. Hock, Gainesville, FL) was used as the "control" trap in all field trials. In the 1st trial, the PBT received carbon dioxide from 1 kg of dry ice, released from an insulated cooler via a tube extending from the top of the cooler into the passive trap interior. In the next 4 trials, PBTs received 250–500 ml/min CO_2 from a compressed cylinder. Examination of field trials from wooded sites revealed that the large PBT collected fewer mosquitoes than the CDC light trap (average ratio of PBT catch to CDC catch was ~0.5). This same ratio calculation in the present study yielded 0.18, a 64% reductive difference. Thus, the most likely reason for this difference was the CO_2 source: the solid dry ice

and compressed tank provided a greater amount of the CO_2 attractant than the YSW mixture (8 to 20 times more). In the present study the PBT/CDC light trap species ratio was 0.4, whereas the average species ratio per trial (PBT/CDC) for Ritchie et al. (2013) was $\sim$1.8 ($n = 5$ trial data tables). As with the catch ratio, we attribute this larger species ratio to the greater amount of CO_2 being released. This large PBT/CDC ratio also suggests that the PBT was slightly better at sampling the mosquito community diversity.

Using CDC light traps supplied with CO_2 from a yeast–sugar–water (YSW) mixture, Saitoh et al. (2004) was able to catch *Aedes* (*Stg.*) *albopictus* (Skuse), *Ae.* (*Stg.*) *japonicus* Theobald, and *Cx. pipiens* L. in residential neighborhoods in Japan. The number of mosquitoes collected using YSW-based CO_2 was about half that of traps baited with dry ice, but it was always greater than collection rates in unbaited traps. One likely reason for this difference was the rate of CO_2 production: a 1 kg chunk of dry ice (typical mass for 1 trap night) produced 12 times more CO_2 than the YSW mixture for 1 trap. Saitoh et al. (2004) had a per trap night overall catch rate of 12.3, with 6 species detected (0.5 species per trap night), whereas the present study had a per trap night overall catch rate of 6.4, with 9 species detected (1.3 species per trap night). This difference in overall catch rate hinges on high densities of *Culex pipiens* in Saitoh et al. (2004); the catch rates are nearly equal when it is removed. The difference in species detected per trap night (a 62% difference) was most likely a function of location and the higher diversity of mosquitoes in the Peruvian Amazon compared with Japan.

Yeast–sugar–water CO_2 generation systems have been tested in equatorial Africa. Smallegange et al. (2010) used Mosquito Magnet-X counter flow geometry traps baited with YSW-produced CO_2 and reported catching significantly more mosquitoes than unbaited traps (up to 34 h after mixing the ingredients) and also significantly more than traps baited with industrial CO_2, both in the laboratory and in the semi-field. The results suggested that, at least for this assemblage of African species, YSW-produced CO_2 can effectively replace industrial CO_2 for sampling of *Anopheles, Culex,* and *Mansonia* spp. The present study caught 9 (PBT) and 15 (CDC-Y) species, including *Culex* and *Mansonia* spp.

Steiger et al. (2014) investigated the effectiveness of YSW-generated CO_2 as a method for sampling mosquitoes through a series of replicated field trials in 3 different vegetation types: rainforest, mangrove forest, and dry forest. They compared mosquito capture rates and community composition between standard dry ice–baited and YSW-baited traps. The catch rates at the rainforest site were nearly equal for the dry ice–baited and YSW-baited traps ($\sim$15 per trap period), while the dry ice–baited trap catch rates were much larger in the mangrove ($\sim$300 per trap

period) and dry forest ($\sim$30 per trap night) compared with the YSW-baited traps in the mangrove ($\sim$90 per trap period) and dry forest ($\sim$7 per trap night) sites. Comparing Steiger et al. with the present study, the dry forest trap catch rate was nearly equal to our YSW-baited CDC trap catch rate ($\sim$34 per trap night), while the dry forest YSW-baited trap catch rate was similar to our YSW-baited CDC trap catch rate ($\sim$6 per trap night). The species richness for the YSW-bait at the dry forest site ($\sim$3 species per trap period) was nearly equal to the species richness for our CDC-Y configuration ($\sim$2 species per trap period).

Various LED-baited mosquito trap configurations have been evaluated in the previous literature. Burkett et al. (1998) evaluated the attractiveness of various colored LEDs and incandescent lights for mosquitoes in Florida. In the 1st 2 trials, no significant differences were observed in the total number of mosquitoes captured in modified CDC traps with 6 different colored LED light sources (including blue and standard incandescent) in either the CO_2-baited or unbaited traps. However, differences were observed in the response of individual species to specific light colors. In a trial with 6 colors of LED, plus incandescent and no light, overall CDC trap catch was greatest with the standard white broad-spectrum incandescent light, followed by blue, green, orange, yellow, red, no light control, and infrared, respectively. Rogers et al. (1993) evaluated chemical light sticks in the peri-Iquitos area and had mixed trapping results depending on species ($n = 36$), with yellow and green light being most attractive overall. In the present study, the UV was the most attractive across all mosquito species in all 3 trials (2013 and 2014 forest sites, and 2014 barracks site), followed by the standard white incandescent bulb and the white LED in the EVS trap.

Anopheles darlingi was 1st detected in Loreto on the border between Peru and Brazil in 1933, but it was detected in the Iquitos area in 1984 and has fluctuated in density and distribution since (Fernandez et al. 2014). Starting in the mid-1990s it became a major malaria vector in the peri-Iquitos area (Reinbold-Wasson et al. 2012). We observed it actively host seeking in high numbers every evening during all 3 studies, and it continues to infect Peruvian Army personnel seasonally at this site. Trapping methods for *An. darlingi* in the Amazon basin have been reviewed (Lima et al. 2014); however, no previous Amazon basin studies have tested a UV trap for *Anopheles* surveillance. The relatively high catch rate in this study indicates that a UV trap may find use as a surrogate for human landing counts to estimate local *An. darlingi* population density, once a robust linear relationship between the 2 surveillance techniques has been established.

To our knowledge, this is the 1st study to use a Bayesian approach to model counts of mosquitoes from an assortment of trap types. Indeed, the vast

majority of studies involving the analysis of mosquito trap counts and the methodological guidance for mosquito sampling have used, or suggest the use of, frequentist methods of statistical analysis (Service 1993, Southwood and Henderson 2000, Silver 2008). However, Bayesian approaches are finding use in studies of mosquito population dynamics (Reiczigel et al. 2010, Overgaard et al. 2012, Villela et al. 2015). We chose the Bayesian approach for 3 general reasons (Link and Barker 2010): 1) Simplicity: the methods extend in a straightforward manner to more complex data and models; 2) Exactness: Bayes' approach provides sensible methods of analysis without the generalizations of frequentist statistics, even when dealing with small sample sizes and limited data; and 3) coherency: Bayesian inference is a self-consistent and qualitatively simple system of reasoning.

The present study demonstrates that light traps, especially UV-based light traps, are very effective at sampling diverse assemblages of mosquito communities in a representative equatorial rainforest ecosystem. It also suggests that a yeast-based system of CO_2 production may be a useful alternative for conventional dry ice in remote locations. This study also represents one of the 1st applications of Bayesian modelling applied to mosquito count data sets and is a departure from the traditional frequentist methods of analysis and comparison. New approaches to the analysis of mosquito trap counts may lead to new insights on the spatio-temporal dynamics of mosquito populations, and mosquito control agencies using these techniques may sharpen the precision of their control efforts.

ACKNOWLEDGMENTS

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