Field Evaluation of Selected Traps and Lures for Monitoring the Filarial and Arbovirus Vector, *Aedes polynesiensis* (Diptera: Culicidae), in French Polynesia

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ABSTRACT The efficacy of the BG-Sentinel (BGS) and the BG-Mosquitito (BGM) mosquito traps for sampling populations of the important filariasis and dengue vector *Aedes (Stegomyia) polynesiensis* (Marks) was evaluated in French Polynesia against human bait collections (HBC) using a modified Centers for Disease Control and Prevention backpack aspirator. Traps were baited with BG-Lure (a combination of lactic acid, ammonia, and caproic acid) or carbon dioxide plus octenol (1-octen-3-ol) known as attractants to aedine mosquitoes. Mosquito sampling was conducted on two typical islands of French Polynesia: the high, volcanic island of Moorea, and the low, coral island (atoll) of Tetiaroa. Sampling efficacy was measured in a randomized Latin Square design. Production of carbon dioxide from yeast-sugar fermentation was used as an alternative source of CO_2 because supply via dry ice, gas cylinders, or propane combustion in remote tropical islands is costly and challenging. Although the BGS trap captured the greatest number of *Ae. polynesiensis* in both island settings, catch rates of BGS or BGM baited with either lure were not significantly different from that of HBC. On Moorea, the number of collected aedes species in the BGS trap baited with either lure was significantly greater than the BGM with BG-lure. On Tetiaroa, BGM trapping was severely hampered by damage from rats, and the traps were removed from the study. Our study confirms the efficiency, comparability, and convenience of the BGS trap, a robust and safe alternative to HBC for sampling *Aedes* mosquitoes in research and surveillance efforts against filariasis and arboviruses in the South Pacific.

KEY WORDS *Aedes polynesiensis*, disease vector monitoring, BG-Sentinel, BG-Mosquitito, carbon dioxide

Pacific Island Countries are subject to severe and debilitating mosquito-borne diseases, which cause substantial mortality, morbidity, and suffering. *Aedes (Stegomyia) polynesiensis* (Marks) is the primary vector of lymphatic filariasis (LF) wherever the subperiodic form of *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae) occurs in the Polynesian region (Burkot and Ichimori 2002, Burkot et al. 2002). *Ae. polynesiensis* is also a significant vector of dengue, the most important mosquito-borne viral disease with 50–100 million cases per year worldwide (WHO 2012), and this mosquito was implicated in a Ross River Virus outbreak in the region (Gubler 1981, Miles 1984). The dog heartworm *Dirofilaria immitis* (Leidy) a filarial parasite of high veterinary importance in many tropical and temperate countries is also transmitted by *Ae. polynesiensis* (Nicolas and Scoles 1997, Russell et al. 2005). Despite sustained mass drug administration (MDA) campaigns of antifilarial prophylactic drugs over several decades, LF persists in French Polynesia and Samoa (Esterre et al. 2001, Esterre et al. 2005, Plichart et al. 2006b, Mou et al. 2009, Joseph et al. 2011). MDA alone may be insufficient for the elimination of LF in areas where *Ae. polynesiensis* is the primary vector because of its unique *W. bancrofti* transmission pattern in which transmission of stage 3 larvae becomes more efficient as the microfilariae density in the human host diminishes (Burkot et al. 2002, Lardeux et al. 2002b, Pichon 2002, Snow et al. 2006, Chambers et al. 2011). Consequently, supplemental vector control strategies have been advised to complement MDA in such areas (Esterre et al. 2001; Lardeux et al. 2002b; Burkot et al. 2006; Brelsfoard et al. 2008, 2009; Bockarie et al. 2009; Hooper et al. 2009; Chu et al. 2010). However, control of *Ae. polynesiensis* using conventional methods has been challenging because this diurnal mosquito is exophilic (Russell 2004) and uses a wide range of domestic and natural containers for larval habitat such as rat-chewed coconuts,

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leaf axils, tree holes, and crab burrows (Bonnet and Chapman 1958, Lardeux et al. 2002b). Multiple strategies have been tested for controlling *Ae. polynesiensis* (Lardeux et al. 1992, Burkot et al. 2002, Lardeux and Cheffort 2002, Lardeux et al. 2002a,b), with limited success (Burkot and Ichimori 2002, Lardeux et al. 2002a). Recently, novel vector control strategies have been investigated for the control of aedine mosquitoes including *Wolbachia*-mediated cytoplasmic incompatibility (CI) for suppression and elimination of *Ae. polynesiensis* populations by the release of sterilizing males (Sinkins 2004, Sinkins and Godfray 2004, Brelsfoard et al. 2008, Chambers et al. 2011, O'Connor et al. 2012), as well as population replacement using a disease refractory strain (Moreira et al. 2009, Walker et al. 2011).

The assessment of vector-borne disease transmission risk and the field evaluation of existing and novel vector control techniques relies strongly on the ability to estimate the size, density, distribution, and dispersal capacity of adult mosquito populations (Morrison et al. 2008). Moreover, diagnostic tools are required to assess the status of LF in countries that have transitioned to post-MDA surveillance phase or are still implementing preventive chemotherapy. The detection of microfilaria in human or vector populations is considered an important test, complementary to the monitoring of filarial antigenemia, to assess the efficiency of LF elimination programs (Ottesen 2006, Ramzy et al. 2006, Weil and Ramzy 2007). Polymerase chain reaction (PCR) assays have been developed (Rao et al. 2006) that are highly sensitive and specific for the detection of *Wb* DNA in human blood samples as well as in mosquito vectors (Williams et al. 2002, Plichart et al. 2006a, Boakye et al. 2007, Farid et al. 2007, Plichart et al. 2007). When parasite prevalence is low, large numbers of mosquitoes are required to detect any infections by xenomonitoring. Consequently, mosquito sampling methods must be sensitive in capturing mosquitoes at low densities and compatible with cost-effective implementation of multiple traps over extended geographic areas to ensure collecting sufficiently large numbers.

An effective method for sampling *Ae. polynesiensis* is therefore critical for ongoing surveillance, research, and control efforts against filariasis and arboviruses in the South Pacific. Although numerous methods to sample adult mosquitoes exist, most are unsuitable for *Ae. polynesiensis* because of their limited sensitivity for capturing adequate numbers of *Ae. polynesiensis* (Suzuki and Sone 1974, Samarawickrema et al. 1987, Samarawickrema et al. 1992, Lardeux et al. 1995, Russell et al. 2005). Among the collection techniques investigated for *Aedes* population sampling are the Fay-Prince trap, the carbon dioxide (CO_2)-baited Centers for Disease Control and Prevention (CDC) light trap (Schmaedick et al. 2008), and the CDC Backpack aspirator (Williams et al. 2006) used to sample typical *Ae. aegypti* harborage sites both indoor and around houses. The BG-Sentinel (BGS) trap (BioGents GmbH, Regensburg, Germany) has shown potential for sampling adult *Ae. polynesiensis* populations in American Samoa and French Polynesia (Schmaedick et al. 2008, Mercer et al. 2012a,b). Although the human landing collection method is variable in catch rates owing to differences in the human bait attractiveness, this method has been used for adult *Ae polynesiensis* sampling (Russell 2004, Russell et al. 2005). However, safety concerns related to the occupational risk of exposure to vector-borne diseases make human landing collections and human bait collections (HBC) undesirable for *Ae. polynesiensis* monitoring particularly in LF endemic areas or during periods of arbovirus transmission. Unlike human landing catch and HBC, which are influenced by the variable performance and attractiveness of operators and can be impractical in a variety of environments, urban and natural (Silver 2008), the BGS trap provides a standardized collection method. Collections with a CDC backpack aspirator of host-seeking mosquitoes attracted to an operator are comparable with human landing collections (Schoeler et al. 2004), but none of the previous studies described above have compared the sampling efficiency of the BGS against the HBC using the CDC backpack aspirator for collecting *Ae polynesiensis.*

The objective of this study was to evaluate the efficacy of two models of Biogents traps (BioGents GmbH, Regensburg, Germany) for monitoring populations of adult *Ae. polynesiensis* and *Aedes (Stegomyia) aegypti* (L.) mosquitoes in two typical island settings of French Polynesia. The evaluation included the BGS trap and the more recently commercialized and cheaper BGM trap with and without BG-lure (consisting of a blend of lactic acid, ammonia, and caproic acid) (Bioquip, Rancho Dominguez, CA) or carbon dioxide (CO_2) plus octenol as an attractant. CO_2, which is typically supplied via dry ice, gas cylinders, or propane combustion, is expensive and difficult to procure in often remote tropical islands settings. The use of yeast-sugar fermentation, a comparatively inexpensive and convenient source of CO_2 (Smallegange et al. 2010), was investigated in combination with octenol. The efficacy of collections from each of these trapping devices was compared with the HBC method using a CDC backpack aspirator to determine their potential as sampling alternatives.

Materials and Methods

Sampling Devices. In this study BGS and BGM traps were evaluated against the HBC using a battery powered modified CDC backpack aspirator (model 1412, John W. Hock Company, Gainesville, FL) collection. HBC was used with a two-person operating team involving a static human volunteer acting as bait and an aspirator operator, both wearing long trousers and shirts for protection from mosquito bites. The CDC backpack aspirator was used to capture approaching mosquitoes attracted to human bait similar to human landing collections. HBC were conducted for 15 min (average suction airflow 13.0 m/s) for each 24-h collection period.

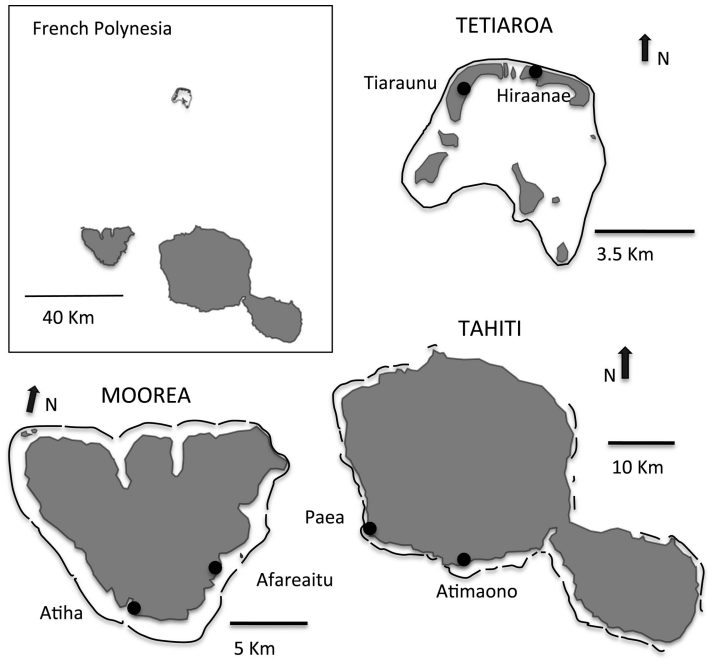

Fig. 1. Map of study sites on Tahiti, Moorea, and Tetiaroa –French Polynesia.

The BGS trap was developed primarily to collect adult *Ae. aegypti* (Kröckel et al. 2006, Williams et al. 2006, Maciel-de-Freitas et al. 2007) and *Aedes albopictus* (Skuse) (Farajollahi et al. 2009). This sampling device was also shown to collect adult *Ae. polynesiensis* quite effectively (Schmaedick et al. 2008, Mercer et al. 2012a,b, Hapairai et al. 2013).

The BGM trap offers a combination of visual cues and a blend of chemical attractants released using an airflow mimicking convection currents created by the human body (similar to the BGS) in a more compact and light design. A significant difference of the BGM trap is that mosquitoes must first pass through the fan blades before being captured in the net.

To prevent ant infestation, BGS and BGM were suspended from a tree branch 20–30 cm above the ground, as *Ae. polynesiensis* flies low and has a propensity to bite around the ankles. Engine grease was applied on the strings used for trap suspension and on the CO_2 tubing to prevent predation of collected mosquitoes by ants.

All traps and the CDC backpack aspirator were powered using 12-V, 20Ah (Fullriver, Guang Zhou City, China) batteries, charged (Oz-charge, Fairfield, Australia) in parallel in groups of two at 24-h intervals. The suction power of all sampling devices was measured at start and end of each mosquito collection using a wind meter (model 3000, Kestrel, Champlain, NY). Batteries powering BGS and BGM traps were placed on the ground next to the traps and replaced after each 24-h collection period. Collected mosquitoes were identified to species using a microscope (LEICA-EZ4D) and species keys (Belkin 1962) before male and female specimen were counted for calculation of sex ratio.

Sampling Routines. The relative efficiency of each collection method was measured in six different locations on three different islands (Fig. 1). Evaluations of the BGS and BGM traps were first conducted with and without BG-Lure (BioGents GmbH, Regensburg, Germany) under both low and high mosquito densities on the island of Tahiti (Fig. 1) using a randomized 4 by 4 Latin Square experimental design to account for location effects. Treatments consisted of one of each BGS, BGS+BG-lure (BGS+L), BGM, or BGM+BG-lure (BGM+L) traps, rotated daily among stations. Collections on the grounds of the ILM Medical Entomology Research Laboratory (17° 43′49.00 S 149° 34′39.00 W) in a relatively low mosquito density area were replicated twice. These collections were conducted from 22 to 26 August 2011 and again from 29 August 2011 to 1 September 2011. Principal breeding containers included rock holes and leaf axils of *Hibiscus tiliaceus* (L.). Collections under high mosquito density were conducted in Atimaono (17° 46′03.00 S 149° 26′58.00 W) in an abandoned coconut grove [*Cocos nucifera* (L.)]. Plants in the understory consisted of *Hibiscus tiliaceus* and *Solanum torvum* (O.P. Swartz). The main breeding containers were ratchewed coconuts, abundant on the ground and domestic water containers. These collections were conducted on 21 and 22 September and 3 and 4 October, 2011.

On Moorea, trap evaluation used a randomized 5 by 5 Latin Square experimental design. Treatments consisted of one of each BGS, BGS+L, BGM, BGM +L, and BGS or BGM plus CO_2 and octenol (BGS+C/O, BGM+C/O, respectively) against the HBC, with each treatment rotated daily among stations. Trap evaluation in Moorea was done by replicating the Latin

Table 1. Carbon dioxide avg flow rate (ml/min) produced under semi-field conditions by different yeast-sugar solutions

Sugar (g)	Avg CO_2 production (ml/min ± SD)	
	1 h	24 h
600	100.67 ± 5.85	42.04 ± 4.03
700	104.93 ± 13.60	40.40 ± 8.17
800	92.71 ± 33.59	35.27 ± 7.05
900	91.31 ± 26.66	39.59 ± 4.49

Averages are based on measurements taken at either 1 or 24 h after mixing the 35 g yeast with various quantities of sugar in 2.5 liters of tap water. Measurements were done outdoor in a shaded area, during the day.

Square experiment from 7 to 12 November 2011, in valleys of the Afareaitu (17° 32′04.00″ S 149° 47′53.00″) and Atiha (17° 33′15.00″ S 149° 49′27.00″) districts on the windward side of the island (Fig. 1). The main breeding containers included both natural (tree holes, rat-chewed coconuts, etc.) and domestic (tires, small plastic containers, tanks, etc.) containers. Both valleys include a small residential area, with most human dwellings distributed from the shoreline inward. This area has been extensively surveyed for LF.

On Tetiaroa, trap evaluation was conducted by replicating the Latin Square experiment from 23rd to 26th November 2011 on Tiaraunu (16° 58′23.00″ S 149° 33′56.00″) and Hiraanae (16° 58′25.00″ S 149° 33′17.00″), 2 of the 13 islets (motu) composing this atoll (Fig. 1). The main water-holding containers found were rat-chewed coconut shells, which were abundant on the ground.

Carbon Dioxide Production. CO_2 was generated by mixing dry instant yeast—*Sacchoromyces cerevisiae* (Fermipan red, Casteggio Lieviti srl, Casteggio, Italy), powdered sugar (Chelsea, Auckland, New Zealand), and tap water in 5-liter plastic bottles. Yeast-produced CO_2 was delivered to the trap CO_2 intake using a silicone tubing connection. The average volume of CO_2 produced from a range of sugar concentrations was first estimated (Smallegange et al. 2010) before conducting mosquito sampling tests in semifield conditions. Smallegange et al. (2010) suggested that 35 g of yeast in 2.5 liters of tap water produced the most carbon dioxide. In the present experiment, the same amount of yeast was mixed with either 600, 700, 800, or 900 g of sugar. CO_2 yield was measured 1 h after mixing and again 24 h later.

Geographic and Climatic Measurements. Climatic data were recorded using an automated weather station (Hobo U30 model, Onset Computer Corporation, Inc., Pocasset, MA). GPS location and elevation was measured with a Garmin 78S model (Garmin International, Inc., Olathe, KS).

Xenomonitoring. DNA from pools of *Ae. polynesiensis* females collected in valleys of the Afareaitu and Atiha districts was extracted using a modification of the Qiagen DNeasy kit protocol (Qiagen, Hilden, Germany). qPCR assays were performed using the *Wb*-LDR primers (Rao et al. 2006) and the SYBR Green fluorescence dye (Bio-Rad, Hercules, CA) with melting curve analysis. PCR reactions were run on a Bio-Rad I-Cycler (model 170-8731, Bio-Rad) using the protocol described by Chambers et al. (2009).

Data Analysis. Comparisons between numbers of captured mosquitoes were transformed as $\mathrm{Log}_{10}(x + 1)$ to correct for lack of normality and unequal variances in the raw data. Treatments were compared with each other using analysis of variance (ANOVA) and mean separation by the Tukey multiple comparison test. Statistical analysis was done using GraphPad Prism version 5.0 (GraphPad Software Inc., LA Jolla, CA). The likelihood ratio, G-test for goodness-of-fit was used to compare male:female ratio of the BGS+L and HBC, which measured the departure from a 1:1 expected ratio. Estimates of LF prevalence through xenomonitoring was calculated using the PoolScreen (v. 2.02) software (Department of Biostatistics and Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL), which provided maximum likelihood estimates (MLE) with 95% CIs based on likelihood ratio method.

Ethics Approval. This study was conducted under biosafety ethics approval number SPHTMRS-2011–2, Institutional Biosafety Committee, James Cook University.

Results

Carbon Dioxide Production. The mix generating the most CO_2 after 1 h was 35 g yeast + 700 g sugar in 2.5 liters of water (Table 1) with an average of 104.93 ml/min (SD ±13.60 ml/min). Consequently, this yeast–sugar solution was used in traps in combination with octenol. Yeast production of CO_2 decreased on average by 61% after 24 h regardless of the sugar quantity used.

Trap Comparison. The average climatic conditions recorded during the study are presented in Table 2. A cumulative total of 424 male and 4,574 female *Ae. polynesiensis* were collected by all collection methods on Tahiti, Moorea, and Tetiaroa (Table 3). Furthermore, a cumulative total of 418 male and 157 female *Ae. aegypti* (L.) were collected on Tahiti and Moorea but not Tetiaroa where *Ae. aegypti* is absent (uninhabited study site). In total, 45 male and 65 female *Culex quinquefasciatus* Say were collected on Tahiti and

Table 2. Average climatic conditions measured in Paea, Afareaitu, and Tetiaroa during the study period (Aug. to Nov., 2011)

Island	Location	Sampling period (d)	Temp (°C)	RH (%)	Wind speed (m/s)	Gust speed (m/s)	Wind direction (degrees)	Insolation (W/m)	Precipitation (mm/d)
Tahiti	Paea	4	23.39	84.74	0.44	0.88	121.89	123.59	8.76
Moorea	Afareaitu	5	25.01	75.97	0.82	1.37	220.34	96.78	0
Tetiaroa	Onetahi	3	25.88	84.93	5.02	8.29	77.26	133.26	14.15

Table 3. Mean *Ae. polynesiensis* and *Ae. aegypti* mosquitoes (mean ± SD) captured per treatment on three islands

Island	Treatment[a,b]	N[c]	*Ae. polynesiensis*		*Ae. aegypti*	
			♂	♀	♂	♀
Tahiti	BGS+L	12	1.83 ± 2.95a[d]	15.5 ± 33.48a	0.58 ± 0.90a	0.0 ± 0.0a
	BGS	12	2.08 ± 1.83a	9.67 ± 25.39a	1.58 ± 3.68a	0.42 ± 0.51a
	BGM+L	12	1.42 ± 2.07a	5.50 ± 6.07a	1.17 ± 1.47a	0.08 ± 0.29a
	BGM	12	0.75 ± 1.22a	3.00 ± 3.22a	0.58 ± 1.24a	0.25 ± 0.62a
Moorea	BGS+L	10	4 ± 3.46a	35.2 ± 24.91ab	13.00 ± 18.90a	4.90 ± 8.27a
	BGS+C/O	10	10.4 ± 12.20a	95.70 ± 129.06ab	8.00 ± 13.03a	3.80 ± 5.33a
	BGM+L	10	2.8 ± 2.82a	9.40 ± 6.15c	6.30 ± 9.83a	1.6 ± 2.17a
	BGM+C/O	10	2.00 ± 1.89a	15.90 ± 21.54b	5.7 ± 11.90a	3.1 ± 7.41a
	HBC	10	3.70 ± 7.35a	35.80 ± 34.7b	4.10 ± 7.17a	1.40 ± 1.71a
Tetiaroa	BGS+L	6	12.00 ± 10.55ab	228.67 ± 292.82a	NR[e]	NR
	BGS+C/O	6	5.17 ± 4.67bc	102.33 ± 76.79a	NR	NR
	HBC	6	0.67 ± 0.82c	33.67 ± 13.38a	NR	NR

[a] Collection periods for BGS and BGM treatments were 24-h and 15 min for HBC.

[b] BGS, BG-Sentinel trap alone; BGS+L, BG-Sentinel trap with BG-lure; BGS+C/O, BG-Sentinel trap with CO_2 plus octenol; BGM, BG-Mosquitito trap alone; BGM+L, BG-Mosquitito trap with BG-lure; BGM+C/O, BG-Mosquitito trap with CO_2 plus octenol; HBC, human bait collection using a CDC backpack aspirator.

[c] *N* is the total number of days sampled for each treatment.

[d] For each species on a given island, means in the same column followed by the same letter are not significantly different (Tukey's multiple comparison test; $P = 0.05$ on Log_{10} $(x + 1)$ transformed trap catches).

[e] NR, not relevant; *Ae. aegypti* is absent from Tetiaroa.

Moorea. *Cx. quinquefasciatus* and *Culex annulirostris* (Skuse) were observed on Tetiaroa, but no specimens were collected by any of the traps or collection methods tested on this atoll. Also collected in Tahiti were 3 male and 13 female *Wyeomyia (Wyeomyia) mitchellii* (Theobald), the bromeliad mosquito (Marie and Bossin 2013). During the entire study, suction velocity at end of collection period was at least 70% of the starting velocity.

On Tahiti, BGS traps with or without BG-Lure collected more male and female *Ae. polynesiensis* and *Ae. aegypti* than their BGM counterparts. Although BGS+L collected more female *Ae. polynesiensis* on average, the differences between treatments were not significant for either females ($F = 0.221$; df = 3; $P = 0.881$) or males ($F = 1.312$; df = 3; $P = 0.282$). Although traps were placed in areas less suitable for *Ae. aegypti*, (forest and coconut grove), low numbers of this species were collected in both BGS and BGM traps. There were no significant differences between treatments for *Ae. aegypti* males ($F = 0.568$; df = 3; $P = 0.638$) or females ($F = 2.567$; df = 3; $P = 0.066$).

The average number of females collected with the HBC method was 33.67 (SD ±13.38) mosquitoes in Tetiaroa and 35.8 (SD ±34.70) mosquitoes in Moorea, with most females collected on the fly. The average number of females collected with BGS and BGM traps in the respective islands was not significantly different from HBC (Table 3).

On Moorea, collections were conducted close to human dwellings near densely forested areas to increase the chance of sampling both aedine species. As in Tahiti, BGS traps with or without BG-Lure collected more male and female *Ae. polynesiensis* and *Ae. aegypti* mosquitoes than their BGM counterparts. For *Ae. polynesiensis*, BGS+L or BGS+C/O collected significantly more females than BGM+L ($F = 4.676$; df = 4; $P = 0.003$). ANOVA and mean separation by the Tukey multiple comparison test showed no significant differences. For *Ae. polynesiensis* males, collecting methods did not differ ($F = 1.321$; df = 4; $P = 0.276$). Trapping differences were also not significant for *Ae. aegypti* on Moorea for males ($F = 0.660$; df = 4; $P = 0.622$) or females ($F = 0.575$; df = 4; $P = 0.682$).

The trapping study in Tetiaroa was designed to follow the same experimental protocol as in Moorea, comparing Biogents traps with HBC. However, on the first day of experiment, the electrical wires connecting the Biogents traps to the batteries were destroyed by rats in three of the four BGM traps (75%) and one of the four BGS traps (25%), thus preventing a complete 24-h period collection sample. Rat-induced damages of BGS traps were prevented by placing the battery and electrical wires inside the trap. No such solution was applicable to the lighter conical design of BGM traps. After three consecutive days of sampling, 5 out of the 12 BGM traps (41%) had undergone electrical wire damage by rats. Consequently, the BGM traps had to be removed from the Tetiaroa study and the experiment adjusted to a randomized 3 by 3 Latin Square (BGS+L, BGS+C/O, and HBC). BGS+L collected more *Ae. polynesiensis* males and females than BGS+C/O or HBC. Male collections were significantly greater with BGS+L than with the HBC sampling method ($F = 4.233$; df = 2; $P = 0.034$). For females, the observed difference was not significant ($F = 1.748$; df = 2; $P = 0.207$).

Male:Female Ratio. *Ae. polynesiensis* male:female ratios were calculated for Tahiti, Moorea, and Tetiaroa (Table 4). Ratios were female biased in all locations where BGS+L and HBC were tested. The G-test for goodness-of-fit established that female biases were significant for all locations except Paea, Tahiti. Comparatively *Ae. aegypti* male:female ratios were male biased for both BGS+L and HBC (Table 4). This male bias was significant at both sampled locations for the BGS+L. Calculation of G-test comparing BGS+L with

Table 4. G-test for goodness of fit of male:female ratio for *Ae. polynesiensis* and *Ae. aegypti* comparing BGS+L with HBC at various locations on Tahiti and Moorea

Species	Island	Location	Treatment	N[a]	Avg male:female ratio	G-test	*P* value
Ae. polynesiensis	Tahiti	Paea	BGS+L	8	0.83	0.18	0.67
		Atimaono	BGS+L	4	0.07	168.86	<0.001
	Moorea	Afareaitu	BGS+L	5	0.08	172.08	<0.001
		Atiha	BGS+L	5	0.15	233.02	<0.001
		Afareaitu	HBC	5	0.06	237.25	<0.001
		Atiha	HBC	5	0.12	533.66	<0.001
	Tetiaroa	Tiaraunu	BGS+L	3	0.06	1052.84	<0.001
		Hiraanea	BGS+L	3	0.04	379.35	<0.001
Ae. aegypti	Moorea	Afareaitu	BGS+L	5	3.42	26.63	<0.001
		Atiha	BGS+L	5	2.17	117.18	<0.001
		Afareaitu	HBC	5	3.50	0.19	0.66
		Atiha	HBC	5	1.50	1.08	0.30

[a] *N* is the number of days sampled per treatment at each location.

HBC was not possible in Tahiti, as the later treatment was not performed.

Xenomonitoring. In total, 107 pools of mosquitoes were analyzed by PCR: 66 pools were from Atiha (n = 1,256) and 41 pools from Afareaitu (n = 735). In Atiha, two pools originating from the same trapping station were PCR positive for *W. bancrofti.* In Afareaitu, three pools from two different sampling stations were PCR positive for *W. bancrofti.* LF transmission prevalence was estimated for Atiha and Afareaitu at 0.17% (0.02–0.59%) and 0.435% (0.08–1.26%), respectively, with a 95% CI. There was no significant difference in the number of positive pools collected using either trap.

Discussion

This study provides a comparative examination of three sampling devices and methods for collecting adult *Ae. polynesiensis* in typical island settings of French Polynesia. On both high, volcanic (Moorea) and low, coral (Tetiaroa) island settings, the BGS trap performed very well, demonstrating it is a suitable alternative to the HBC using the CDC backpack aspirator. On Moorea, the BGS and BGM traps with either attractant collected numbers of male and female *Ae.* polynesiensis similar to the HBC. On Tetiaroa, the BGS with either attractant collected more mosquitoes than the HBC. Although the HBC allowed the collection of both *Ae. polynesiensis* females and males mosquitoes attracted to the host, the BGS+L collected more male *Ae. polynesiensis* in Tetiaroa than the HBC. This is of particular importance in the ongoing evaluation effort of novel vector control strategies (e.g., sterile and incompatible insect techniques), where reliable estimates of the (male) population is pivotal to control success (Benedict and Robinson 2003, Ferguson et al. 2005).

Large-scale surveillance and sampling in remote islands of French Polynesia requires efficient and logistically manageable sampling devices and attractants. HBC, like human landing collections, are labor and time intensive. By comparison, several BGS traps can be set at different nearby locations in the time required for a single HBC. In addition, a single operator can only do one backpack aspirator collection at a defined time of the day while sets of traps deployed across even distant locations can simultaneously sample mosquitoes during a 24-h cycle. Collection of *Aedes* mosquitoes is best achieved when they are most active. For *Ae. polynesiensis,* the peaks of host-seeking and blood-feeding activity are usually in the early morning (from dawn to 0930 hours) and late afternoon (from 1500 hours to dusk) (Jachowski 1954). Logistical constraints may make the HBC difficult to achieve during such narrow time periods, potentially leading to additional sampling biases. Moreover, attractiveness of the human bait will vary from operator to operator thus further increasing the HBC sampling bias. By comparison, BGS traps offer a standardized design, limiting trap-to-trap sampling variations and thus more consistent sampling outcomes. Collection cycles of 24 h allow operators to set traps at any time of the day, encompassing an entire diurnal cycle of *Aedes* activity.

The BGS trap has been tested with CO_2 in many studies. Carbon dioxide typically supplied via compressed gas cylinders (Kawada et al. 2007) or using the semicontrolled sublimation of dry ice (Farajollahi et al. 2009, Bhalala et al. 2010) is known to increase catch rates in traps. However, the supply of CO_2 via dry ice, gas tanks or propane combustion is expensive and often difficult to procure in tropical, often remote, Pacific islands settings. The use of yeast-sugar fermentation (Smallegange et al. 2010), investigated here in combination with octenol, was a comparatively inexpensive and rather convenient alternative source of CO_2, the components (yeast, sugar, and plastic bottles) being cheap and available locally. The CO_2 produced under semifield conditions 1 h after mixing the optimal yeast:sugar proportion (104.93 ± 13.60 ml/min; mean ± SD) was lower than the flow rate typically used with mosquito traps but within the flow rate recommended by the manufacturer for BGS (either 70 or 175 ml/min flow rates depending on the type of Biogents nozzle CO_2 flow restrictor used). However, after 24 h of yeast-sugar fermentation, the CO_2 flow rate from yeast generation had dropped below the minimum recommended flow rate for BG traps (70 ml/min), indicating that yeast-generated CO_2 may not be sufficiently reliable for 24-h collections under the

conditions observed in South Pacific tropical island settings. Another important drawback of using yeast-fermentation as a source of carbon dioxide is that the seasonal fluctuation of the daily average temperature will likely affect the CO_2 flow rate, leading to possible biases in mosquito catch rates, which might prevent the use of this method for comparative long-term temporal studies. Turner et al. (2011) recently identified that 2-butanone, a volatile odorant, can trigger a response in *Aedes* mosquitoes that is indistinguishable from that elicited by CO_2. This discovery warrants the comparative evaluation of 2-butanone formulations versus CO_2 in BGS traps, as this compound might provide a compact and economical lure highly suited for vector research and surveillance in remote locations. One other observed shortcoming of using the CO_2 plus octenol mix compared with the BG-lure was the number of nontarget insect species trapped. A large number of *Drosophila* and Noctuidae were collected thus considerably increasing the sorting effort before mosquito species identification could be done.

The setting time and handling ease of BGS versus BGM traps were similar. Although the retail price of the BGM trap is significantly lower than the BGS trap, the quality of mosquitoes collected with BGM traps in this study was generally poor. Wings and legs were usually broken from the thorax, and most specimens had lost their scales, thus making species identification more difficult and therefore time-consuming. Moreover, on all three islands, the BGS traps collected more mosquitoes than their BGM counterparts. Finally, the light, low cost BGM trap design did not cope comparatively as well as the BGS with the rather harsh environmental conditions typically found on low, coral islands settings like Tetiaroa.

The number of *Ae. aegypti* males collected in Moorea was greater than that of females (male bias) for BGS+L and HBC. This is consistent with other studies (Williams et al. 2006, Ball and Ritchie 2010). By comparison, male:female ratios of *Ae. polynesiensis* collected in Tahiti and Moorea using BGS+L or HBC showed an opposite trend, with significantly more females collected than males. Similar observations were made previously with adult *Ae. polynesiensis* collections using BGS (Mercer et al. 2012a,b, Hapairai et al. 2013). Although differential trophic preferences between *Ae. aegypti* and *Ae. polynesiensis* might play a role, the basis for such differences in male:female collection between the two species remains unknown.

The increasing use of BGS traps for measuring changes in *Ae. polynesiensis* population size and structure (age, sex ratio) or to monitor disease transmission warrants further studies to better characterize trap biases and better define sample size requirements for reliable population density estimates.

We conclude that, understanding the population size, structure, and dynamics of *Ae. polynesiensis* is important for monitoring the impact of control strategies. Our results demonstrate that the BGS trap with either lure is as effective as human bait collections for sampling aedine mosquitoes in typical island settings of the South Pacific. With comparatively lower catch rates and logistical issues encountered, the BGM trap may not be suited for all Pacific island conditions. Although convenient and inexpensive, the use of yeast generated inconsistent CO_2 levels under the conditions tested. Our study confirms the efficiency and convenience of the BGS trap, a robust and safe alternative to human landing/biting collection for sampling *Aedes* mosquitoes in research and surveillance efforts for filariasis and dengue in the South Pacific.

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