Determinants of Male *Aedes aegypti* and *Aedes polynesiensis* (Diptera: Culicidae) Response to Sound: Efficacy and Considerations for Use of Sound Traps in the Field

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ABSTRACT   Understanding the mating competitiveness of male mosquitoes in field settings is essential to programs relying on the mass release of modified male mosquitoes, yet studies on male ecology have been hampered by the lack of a convenient trapping method. An existing promising method makes use of the innate attraction of males to female flight tones. Here, we present laboratory, greenhouse, and field experiments on the efficacy of sound traps for the collection of *Aedes aegypti* (L.) and *Aedes polynesiensis* Marks, and laboratory experiments with *Ae. aegypti* on the effects of male age, size, and mating status on responsiveness to a range of frequencies. Age and mating status influenced the overall responsiveness to sound, whereas male size did not. There were no interactions between these factors and sound frequency. A Centers for Disease Control and Prevention miniature light trap modified to produce a tone of 465 Hz collected 76.2% of *Ae. aegypti* males in laboratory cages, and 49.7% of males in a greenhouse enclosure. In two sets of experiments in laboratory cages, 50.8 and 46.5% of male *Ae. polynesiensis* were captured with a trap producing a tone of 440 Hz. In the field, CDC miniature light traps or BG-Sentinel traps fitted with a portable speaker producing tones of 440 or 465 Hz captured significantly more male *Ae. polynesiensis* when placed near a male swarm than did traps that did not produce sound. When the trap was placed at a distance of 16.5 m from the nearest swarm, there was no significant difference in the number of males caught between control and sound-producing traps. The numbers of *Ae. aegypti* males captured were low under all circumstances in the field.

KEY WORDS   sound trap, swarms, males, mosquitoes, *Aedes*

The need to study male mosquito behavior and life history in field settings has intensified in recent years as a result of novel control techniques that rely on genetic or biological modifications of male mosquitoes having reached field trial stages (Harris et al. 2011, Hoffmann et al. 2011, James et al. 2011). Insight into the survival, dispersal, and mating behavior of males is particularly relevant to techniques, whereby successful implementation depends on the ability of released males to mate competitively with wild-type females to suppress populations (Benedict and Robinson 2003, Reisen 2004, Howell and Knols 2009). In addition, knowledge of mating behavior and its effects on gene flow is paramount to techniques that rely on the spread of transgenes through mosquito populations (Scott et al. 2002, Ferguson et al. 2005). Despite this need, the behavior of males under field conditions remains understudied.

A consequence of the emphasis on field studies of female mosquito behavior is that most of the current sampling methods are designed with the ability to trap either host-seeking or oviposition-ready female mosquitoes. However, developing adequate tools to monitor free-living male mosquitoes is a pressing issue. Notable exceptions to the current state of the art are the use of floral-baited traps, designed to attract mosquitoes of both sexes and all gonotrophic stages (Foster 2008), and sound traps (Belton 1994); the latter appear to have fallen out of favor in part as a result of their inability to collect female mosquitoes (Silver 2008). Sound traps function by evoking a male's behavioral response to an acoustic tone, mimicking the fundamental frequency of a conspecific female's wing beat (Belton 1994, Gibson and Russell 2006, Cator et al. 2009).

A number of field trials of sound traps have been performed showing the feasibility of the approach, starting with the work of Kahn and Offenhauser (1949), who succesfully attracted male *Anopheles albimanus* Wiedemann in Cuba to a sound trap. In a later study, an average of 249.6 male *Culex tritaeniorhychus* Giles per day were captured using a trap consisting of a speaker, producing a 400 Hz tone, sandwiched between two polystyrene foam boards that were painted black and covered in glue. With a variation of this trap, an average of 5.5 male *Aedes albopictus* (Skuse) per

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day were captured (Ikeshoji and Ogawa 1988). Using a cylinder design that consisted of a tripod, the legs of which were wrapped in black cloth to serve as a swarm marker, and a rolled up polyethylene sheet in which a speaker was placed, *Cx. tritaeniorhynchus*, *Culex tarsalis* Coquillett, and *Culex quinquefasciatus* Say males were all successfully captured in field settings (Ikeshoji et al. 1985, 1987). The greatest number of *Mansonia* spp. (870) and *Ae. albopictus* (a mean of 17.8) males per trapping event were captured in cylinder sound traps that added dry ice and a caged guinea pig to the trap design (Kanda et al. 1987).

In the South Pacific region, *Aedes polynesiensis* (Marks) is the primary vector of the diurnal subperiodic form of *Wuchereria bancrofti* Cobbold, a causative agent of lymphatic filariasis. A challenge to the elimination of lymphatic filariasis in the South Pacific region is the efficiency with which *Ae. polynesiensis* becomes infected when feeding on low microfilaremic blood. This efficiency has been posited as a cause for rebounds in lymphatic filariasis prevalence after cessation of previous mass drug administration (MDA) programs, and justifies the inclusion of vector control in elimination programs (Burkot and Ichimori 2002). However, this day-active species is difficult to control using traditional methods, owing to its tendency to rest and bite outdoors and use of unobtrusive natural and artificial containers as larval development sites. A version of the sterile insect technique that is based on *Wolbachia*-induced cytoplasmic incompatibility (Laven 1967), resulting in karyogamy failure and embryonic arrest when a female mates with a male harboring a different *Wolbachia* type than her own (Werren 1997), has been proposed as a potential vector control tool to be integrated with MDA programs aimed at eliminating filariasis (Brelsfoard et al. 2008). The mating competitiveness of males from a strain in which a different *Wolbachia* type has been introgressed has been studied in both laboratory and field cages (Brelsfoard et al. 2008, Chambers et al. 2011), but field studies on male *Ae. polynesiensis* ecology and fitness have been stymied by the low catch numbers of males using traditional female-based traps (Schmaedick et al. 2008, Mercer et al. 2012). Successful adaptation of a sound-trap design could facilitate such studies in the future.

Here, we report on an attempt to transfer this technology from *Aedes aegypti* (L.), which we include as a baseline because it has been the subject of many prior studies on male responses to female wing beat frequencies (Roth 1948, Wishart and Riordan 1959, Ikeshoji 1985, Cator et al. 2009), to *Ae. polynesiensis*. Additionally, we report on laboratory studies on effects of *Ae. aegypti* male age and physiological state on their response to sound, and consider how such effects may skew field collections.

Materials and Methods

Mosquito Stocks and Rearing Procedures. For laboratory experiments, *Ae. aegypti* (Waco strain; originally colonized by G. Craig and K. Blank, and maintained at the University of Kentucky since 1998) and *Ae. polynesiensis* (APS strain; colonized from American Samoan wild-type mosquitoes in 2010 by A. Koppel) males were used. A suspension of liver powder (MP Biomedicals LLC, Solon, OH) in water (6 g/liter) was used to hatch eggs overnight. Larvae were reared at a density of 300 per 21 by 21 by 7.5-cm plastic pan (Pactive, Lake Forest, IL), unless specified otherwise. Pans held 500 ml of aged water and received 200 mg of liver powder on the first and third day of larval development. Adults were maintained with *ad libitum* access to a 10% sucrose solution. The rearing room has a photoperiod of 16:8 L:D h and is kept at ≈29°C and 73% relative humidity (RH). Blood feeding occurred by allowing mosquitoes access to mice for 20 min when required for experiments (Institutional Animal Care and Use Committee 00905A2005).

Responses of Individual Males. Three experiments using *Ae. aegypti* males were performed by assaying individual males. Males were transferred gently by mouth aspirator to a small plastic Dixie cup (Dixie Consumer Products, Atlanta, GA) covered with netting. Individual males were then presented with a generated tone by placing a speaker (JBuds, JLab Audio, Oceanside, CA) on top of the netting and playing a tone from an "iPod" mp3-player (Apple, Cupertino, CA) for 10 s. A response was scored as positive if the male flew to the speaker and pressed his terminalia to the netting as if copulating (Duhrkopf and Hartberg 1992). The tones used in all experiments were generated using Audacity (http://audacity.sourceforge.net) and consisted of a fundamental frequency (e.g., 465 Hz) and its second and third harmonic; each additional harmonic attenuated by 5 dB to mimic a harmonic stack of a female mosquitoes' flight tone (Cator et al. 2010). The earbud speaker produced a sound pressure level of ≈57.8 dB at 2 cm distance (Sinometer Instruments, Shenzhen, China).

To investigate the effect of male age on responsiveness to different frequencies, males were separated from females as pupae and allowed to eclose in test tubes half-filled with water and plugged with cotton. Emerged males from one cohort were transferred to male-only cages with access to sugar and held until used in an assay. Because exact time of emergence was not scored, males used in the experiments were considered to be 1–2, 3–4, or 6–7 d old. Per age group, 30 males were tested, each male being tested once at three different frequencies (440, 465, and 490 Hz) with a ≈20-min period between subsequent exposures. The order in which the sounds were played for the males was rotated (six possible orders, five males per sequence). Experiments were performed between 0830–0930 hours in the morning.

To test the response of males according to mating status to different frequencies, males from one cohort were either kept without females, as described above, or in a cage at a 1:1 sex ratio, for 5–6 d. Thirty males of each mating status were tested at three different frequencies, as described above. Experiments were performed between 1930–2100 hours.

To investigate whether the adult size of males affects their response to particular frequencies, mosqui-

toes were reared at high or low (i.e., 500 or 100 first instar larvae per pan, respectively) density. Males were kept without access to females and tested 7 d after emergence between 1930–2100 hours. Per size class, 20 males were tested against one of five frequencies (350, 400, 450, 500, and 550 Hz), for a total of 200 males. After the assay, males were knocked down with chloroform and transferred to a polymerase chain reaction (PCR) tube and frozen, to allow for subsequent wing measurements. Of each male, the left wing was removed using forceps, mounted on a slide, and photographed at 10× magnification using a dissection microscope (MZFLIII, Leica, Bannockburn, IL) and attached camera (LH037290, Olympus, Center Valley, PA). Wing lengths were measured from alular notch to distal edge, excluding the fringe, using ImageJ (Abramoff et al. 2004) and calibrated by comparison to a stage-micrometer photographed at the same magnification. Similar individual assays were attempted with *Ae. polynesiensis*, but not pursued owing to their limited response to generated tones, consisting occasionally (below 450 Hz) of a brief orientation to the sound source, never curling their terminalia up against the netting.

Efficacy of Sound Traps in Confined Enclosures. To test the efficacy of a sound trap design for both *Ae. aegypti* and *Ae. polynesiensis*, laboratory cage (both species) and mesocosm assays (*Ae. aegypti*) were performed. The traps consisted of the downdraft fan and motor and collection bag and cup of a Center for Disease Control and Prevention (CDC) miniature light trap (John W. Hock Company, Gainesville, FL) laid flat on their side. At the mouth of the fan, a small computer speaker (S120, Logitech, Newark, CA) was placed at an angle of 45° so that sound was projected into the trap. The speaker produced a sound pressure level of ≈93 dB at 2-cm distance. A frequency of 465 Hz was used for *Ae. aegypti.* For *Ae. polynesiensis*, 440 Hz was used, based on preliminary experiments with individual males mentioned above and a promising initial sound trap test; however, we did not test whether this is the optimal frequency for this species. The laboratory cages used were 58 by 58 by 58 cm (BioQuip, Rancho Cominguez, CA). In each assay, a control and a treatment trap were placed at opposite diagonal sides inside the cage, and rotated between replicates. The control was similar to the treatment, except for not having a speaker (*Ae. aegypti* [N = 3], *Ae. polynesiensis* experiment 1 [N = 3]), or having a speaker that did not produce sound (*Ae. polynesiensis* experiment 2 [N = 4]).

Assays consisted of 38–60 1-wk-old males, kept separated from females, released into the cage with traps running and a tone being generated for 1 h between 0800 and 1000 hours. At the conclusion of the hour, trap bags were frozen and numbers caught in control and treatment traps tallied. The same sound trap and control were tested for *Ae. aegypti* in a greenhouse enclosure. The enclosure was a 4 by 3.5 by 2.2-m wooden structure enclosed in lumite mesh, with entry through a vestibule. Temperature was maintained at 28°C, and overhead lights maintained a photoperiod of 16:8 L:D h cycle with sharp transitions. Two potted

ferns (*Nephrolepis exaltata* (L.) Schott) were placed in the center of the enclosure. A full description of the mesocosm will be presented elsewhere (Mains et al., in preparation). Three replicates were performed; in each, 100 males were released into the mesocosm the afternoon before the trial to allow males to acclimate. Resting boxes consisted of dark-green plastic flowerpots turned sideways; sugar was provided in these resting boxes in the form of a small glass vial filled with a 10% sucrose solution, stoppered with a dental wick. The following day, both traps and sound were turned on between 1530 and 1630 hours, and the number of males caught in each trap tallied afterwards.

Efficacy of Sound Traps in a Field Setting. Testing the efficacy of this sound trap for the purposes of collecting male *Ae. polynesiensis* in its natural habitat, in addition to its usefulness for collecting male *Ae. aegypti*, was facilitated by the discovery of *Ae. polynesiensis* stationary swarms and their swarm markers in American Samoa (Tuten et al. 2013). The following experiments were performed near a mango tree [*Mangifera indica* (L.)], where male swarms were consistently observed in the late afternoon. Three experiments, each consisting of five replicates, were performed using the CDC-light trap version of the sound trap (Fig. 1A). In the first experiment, both control and treatment sound trap were placed at the base of the shaded side of the tree, ≈50 cm apart, angled at 90°, facing inward. The generated tone, played with an mp3-player (SanDisk, Malpita, CA) and a portable mp3-speaker (Altec Lansing, Milford, PA) had a fundamental frequency of 440 Hz. Traps were run for an hour between 1645 and 1800 hours, after which trap bags were frozen, and trapped mosquitoes were subsequently tallied according to species and sex. Whereas this experiment tested whether males could be drawn out of a swarm to a sound trap, the second experiment tested whether a sound trap is efficacious for *Ae. polynesiensis* at greater distances. A breadfruit tree (*Artocarpus altilis* [Parkinson] Fosberg) that stood ≈16.5 m from the aforementioned mango tree and clear of other vegetation was observed to be consistently devoid of male swarms. The control and treatment trap were placed at this tree, and replicates otherwise performed in the same manner. For the third field experiment, traps were placed by the mango tree, but now produced a tone of 465 Hz, to see if a greater number of *Ae. aegypti* males would be attracted at this frequency. A fourth experiment was performed to test the efficacy of a BG-Sentinel trap (Biogents AG, Regensburg, Germany) that incorporated a portable speaker and mp3-player (Fig. 1B); these used a frequency of 440 Hz and were set by the same mango tree. A chemical lure was not used. In all experiments, position of the control and treatment traps was switched between replicates.

Statistical Analysis. Individual responses were analyzed with generalized linear models (GLM) with binomial distributions and probit link functions in JMP nine (SAS Institute, Cary, NC). Terms included the frequency of the tone, the order in which males were subject to different frequencies, and depending on the

Fig. 1. (A) View from above of the miniature CDC-light trap version of the sound trap as used in the field; (B) picture of a BG trap with portable speaker, placed by a swarm site.

experiment: male age, size, and mating status. Additionally data were analyzed for any interactions among terms. To analyze the sound trap assays, the counts of mosquitoes caught in the control or treatment (i.e., nonresponders were not included in the analysis) were used. GLMs with binomial distributions indicated no differences between replicates. Counts of replicates were therefore pooled together and tested for significance with exact binomial tests.

Results

Lab Assay: Responses of Individual Males. The responses of *Ae. aegpyti* males to sound depended on male age ($\chi^2 = 25.04$; $P < 0.0001$), but not the frequency of the tone, the order in which males were subject to different tones, or any interactions between these factors (Fig. 2). There was a significant difference between the response of mated and unmated males to generated tones (Fig. 3), with unmated males having a stronger response; the final model included
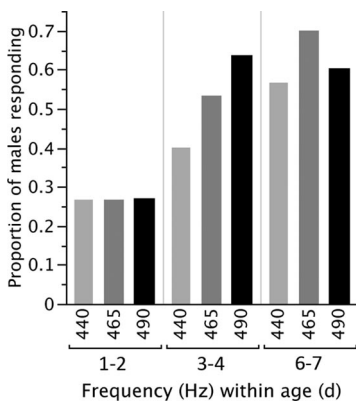

Fig. 2. Proportion of individualized male *Ae. aegypti* responding to tones of different frequencies in cups, according to age (1–2, 3–4, and 6–7 d old).

only mating status ($\chi^2 = 55.56$; $P < 0.0001$), and not the frequency of the tone, the order in which males were subject to the tones, or any interactions. Males reared at high and low density had significantly different wing lengths ($t = 31.44$; $P < 0.0001$). However, male size did not affect responsiveness to generated tones (Fig. 4); the final model included frequency ($\chi^2 = 53.66$; $P < 0.0001$), but not male size (regardless of whether wing length or larval density was used as proxy) or any interactions.

Efficacy of Sound Traps in Confined Enclosures. In laboratory cage assays, 76.2% of released *Ae. aegypti* males were collected in the sound trap, compared with 2% in the control trap (Fig. 5). In the greenhouse enclosure, 49.7% of released males were recaptured using the sound trap, compared with 0.7% in the control trap. In the first laboratory cage experiment with *Ae. polynesiensis,* 50.8% of males were recaptured in the sound trap and 2.6% in the control. In the second—in which a silent speaker was placed in front of the control trap to ensure that attraction to the sound trap was not explained by visual attraction to the speaker— 46.5% were recaptured in the sound trap against 2.2% in the control. All differences between control and treatment in these four experiments were significant (exact binomial test, $P < 0.0001$).

Efficacy of Sound Traps in a Field Setting. The results of field trials performed in American Samoa to test the usefulness of traps for sampling male *Ae. polynesiensis* are presented in Fig. 6. When both the sound trap and the control were placed by a consistent swarm site for *Ae. polynesiensis* males, an average of 29 male *Ae. polynesiensis* were captured in the sound trap against 0.2 males per 1-h trapping period in the control trap (exact binomial test, $P < 0.0001$). An average of 1.6 *Ae. aegypti* males were caught per trapping event in the same sound traps, but none in the control traps (exact binomial test, $P = 0.008$). An average of 1.0 female *Ae. polynesiensis* was caught in the control traps as opposed to 0.2 in the sound traps (exact binomial
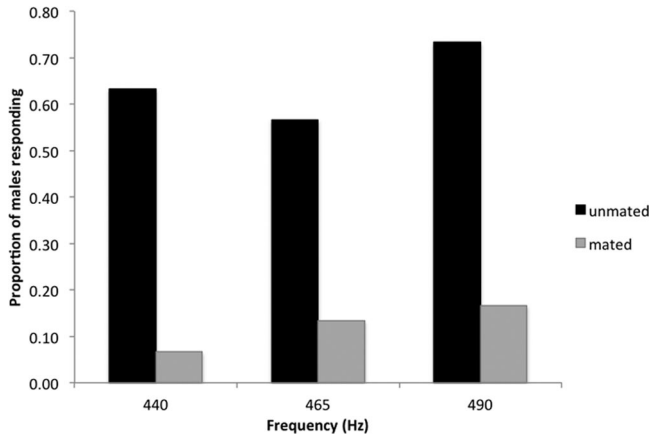
Fig. 3. Proportion ($N = 180$) of 6-d-old male *Ae. aegypti* responding to different frequencies when held individually in a small cup, according to male's mating status.

test, $P = 0.22$). When both traps were placed at a distance of 16.5 m away from a swarm site, by a breadfruit tree that did not harbor male swarms, there were no significant differences for *Ae. aegypti* or *Ae. polynesiensis* between numbers of males caught in the sound trap and the control trap. When a sound trap placed at the same mango tree used a frequency of 465 Hz, instead of 440 Hz, a mean of 20.8 *Ae. polynesiensis* males were captured with the sound trap, compared with 1.0 in the control (exact binomial test, $P < 0.0001$). Neither the number of female *Ae. polynesiensis* nor male *Ae. aegpyti* caught in the sound trap versus the control trap differed significantly (exact binomial tests, $P = 0.5$ and 0.63, respectively). No female *Ae. aegypti* were caught in these experiments. A BG-Sentinel trap with a speaker producing a 440 Hz tone caught an average of 26 male *Ae. polynesiensis,* compared with 1.0 in the control (exact binomial test, $P < 0.0001$). Differences between female *Ae. polynesiensis*—an average of

1.0 in the treatment and 0.8 in the control—and male *Ae. aegpyti*—an average of 0.2 and 0 in treatment and control, respectively—were not significant.

Discussion

The main objective was to test whether a sound-baited trap could be successfully used to collect free-living male *Ae. polynesiensis*, to help enable further field studies on male ecology. With certain caveats, the sound trap design we used succeeded in this regard. The main drawback to this approach is the apparent short distance at which attraction occurs; likely a result of the Johnston's organ being sensitive to the displacement of air particles in the acoustic near field, rather than to sound pressure (Göpfert et al. 1999). Thus, spatial knowledge of swarm locations of this species will therefore facilitate the use of sound traps.
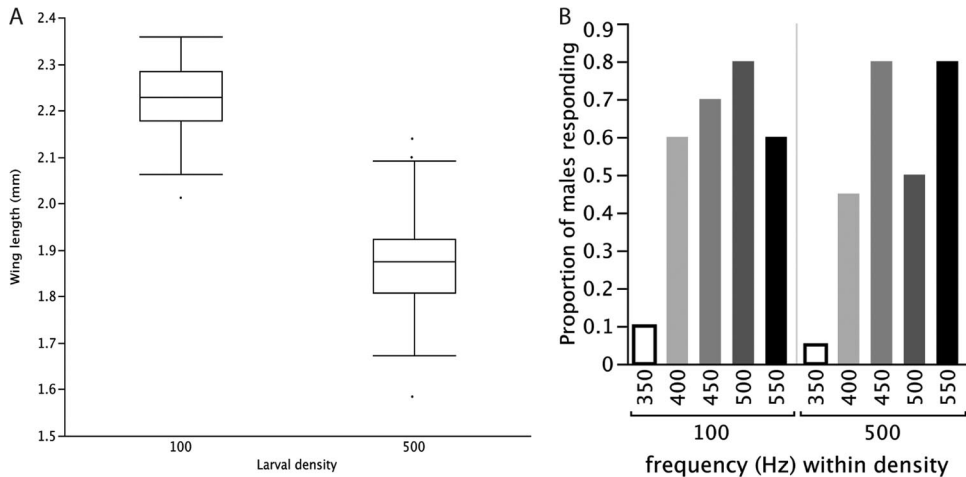

Fig. 4. (A) Distribution of wing lengths of *Ae. aegypti* males reared at high (500 first instar larvae per pan) or low (100 first instar larvae per pan) density; (B) the proportion of male *Ae. aegypti* responding to tones of different frequencies (350, 400, 450, 500, and 550 Hz) when reared at high or low density. Assays were performed with individual males in small cups ($N = 200$).
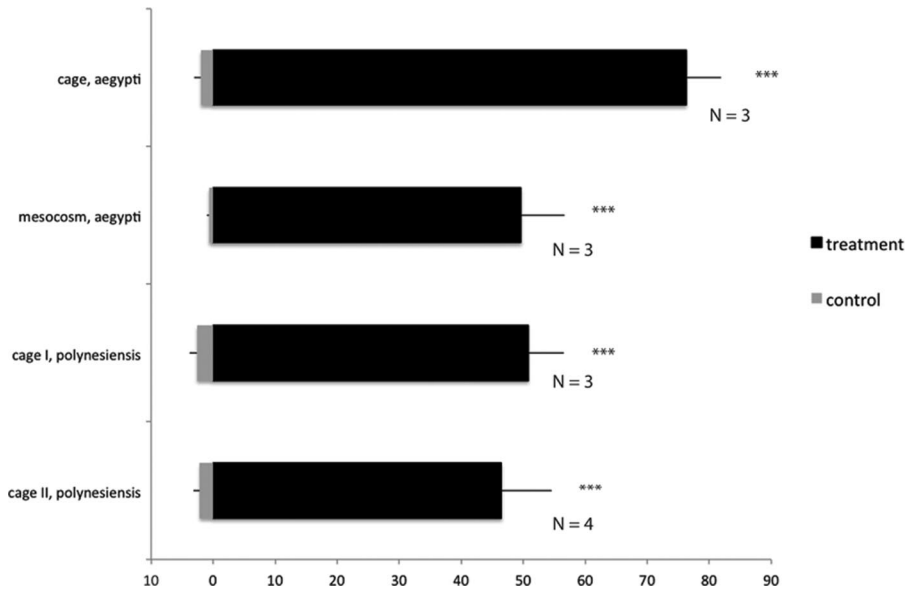
Fig. 5. Percentage of male mosquitoes retrieved in treatment and control traps over all replicates. Numbers of males released were 38–60 in laboratory cages and 100 in mesocosms per replicate. Asterisks (***) indicate a significant difference at a level of $P < 0.0001$ using the exact binomial test.
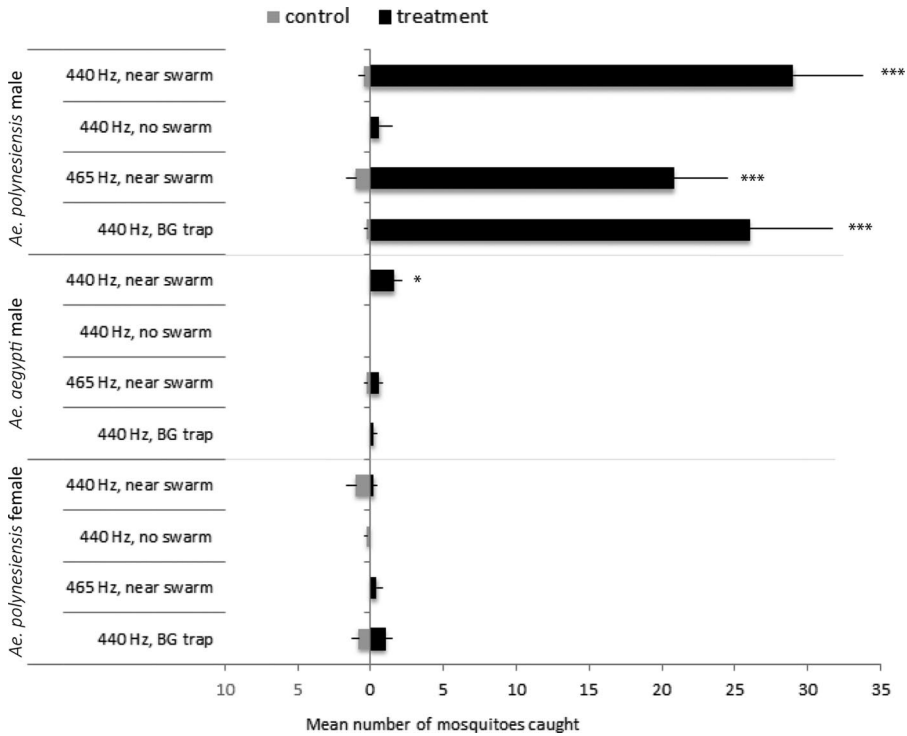

Fig. 6. The mean number $\pm$ SE of *Ae. polynesiensis* males and females and *Ae. aegypti* males caught over five replicate trapping events in control and treatment sound traps in American Samoa. Traps set at the mango tree were at 440 Hz, and a swarm of *Ae. polynesiensis* males was consistently present. The breadfruit tree was 16.5 m away from the nearest tree with a swarm present. The mango tree was likewise used for traps run at 465 Hz and for BG traps (440 Hz). Asterisks (*** and *) indicate a significant difference between treatment and control at a level of $P < 0.0001$ and $P < 0.01$, respectively, using the exact binomial test.

Advantages of using a trap at such sites, instead of sweep nets or backpack aspirators, are ease of standardization and passive trapping, potentially greater capture rates, and possible integration with female population monitoring (e.g., the use of BG-Sentinel traps with a chemical lure).

The low numbers of male *Ae. aegypti* that were caught at 440 Hz when traps were placed by an *Ae. polynesiensis* swarm site suggests that these males were attracted from nearby resting vegetation. Direct collections from swarms by backpack aspiration (Tuten et al. 2013) suggest that *Ae. aegypti* males do not co-occur in these swarms. The numbers of trapped *Ae. aegypti* males did not increase, and may have decreased, when a frequency of 465 Hz was used instead of 440 Hz, which contradicts previous lab-based studies that indicated an optimal response of *Ae. aegypti* to tones close to 465 Hz (Ikeshoji 1981, Duhrkopf and Hartberg 1992). A subject of further investigation would be whether the higher numbers we caught at 440 Hz reflect a difference in behavior of the *Ae. aegypti* population in the field, in American Samoa, or whether this is merely a result of fluctuations in temporal abundance of the species. The low numbers caught when using a BG-Sentinel trap with a tone of 440 Hz supports the latter notion. Given the short-range attraction of these traps and the lack of stationary swarms in *Ae. aegypti*, it remains to be tested in areas of higher *Ae. aegypti* abundance whether a sound trap approach would be useful for male collections in the field. It may require the incorporation of a long-range attractant such as light, or olfactory cues, as used by Kanda et al. (1987).

A main consideration for the use of sound traps is whether obtained samples will reflect the true population composition of males in the field, or whether samples will be biased toward males of particular physiological states or age classes. Using individual assays, we were not able to elucidate these issues for *Ae. polynesiensis*, owing to their minimal and ambiguous response to sound under the confined circumstances of the individual lab assays. A difference in the mating behavior of these species was also observed by Nijhout and Craig (1971), who introduced males into 4-liter cages with conspecific females and noted that while *Ae. aegypti* males responded quickly to females, coupling within a few seconds, *Ae. polynesiensis* were slow and reluctant. Further studies on the effects of male age, size, and mating status on responsiveness of *Ae. polynesiensis* to sound may thus have to be performed under large-cage or semifield conditions.

In addition to the overall responsiveness of *Ae. aegypti* males to sound, we tested whether male age, size, and mating status affected responses to sounds of different frequencies in a different manner. The rationale behind this is that the fundamental frequency of females increases with their age (Tischner and Schief 1955) and size (Cator et al. 2010). If male responsiveness to female sound tracks these differences according to male age and size (Belton 1994), this could have implications for male sampling in the field, and potentially for assortative mating and competitiveness of released modified males.

In our first lab-based experiment, we found that although males became more responsive to female flight sounds in general as they aged, there was no significant interaction between age and frequency, suggesting that males do not become more or less discriminatory in regard to female state. Ikeshoji (1985) found that responsiveness of male *Ae. aegypti* to a sound of 460 Hz peaked at 3–4 d after emergence, whereas our results indicate an overall increase that continues until day 6–7 (Fig. 2). Our conclusion contrasts with that of Roth (1948), who showed that as *Ae. aegypti* males age, they become responsive to a wider range of frequencies. However, where he measured the upper and lower frequencies to which males would respond, we were interested in the frequency at which an optimum response was found. A question that arises when considering the increase in responsiveness with age is whether this reflects a difference in responsiveness with age, or merely with increasing time since (the last) mating. Figure 3 shows that the presence of females influences the likelihood that a male will respond to sound, comparable with results found by Ikeshoji (1985), but mated males are no more affected by higher frequencies (indicative of larger, perhaps more fecund females) than they are to lower frequencies. We also did not detect a difference in the response to different frequencies by males reared at low and high densities, suggesting that if size-assortative mating occurs it is not owing to a preference among males. These individual assays used single cohorts of males from one laboratory colony of *Ae. aegypti*. Future studies may therefore more fully address factors (e.g., cohort or colony effects) that may influence male response.

An interesting follow-up question would be to see how long this reduced responsiveness to female flight sounds prevails, in particular to what extent it is affected by attempted copulations with, or cohabitation with, females versus actual sperm depletion after the insemination of several females (Jones 1973, Foster and Lea 1975), and whether responsiveness to sound returns in concert with replenishment of sperm and accessory gland secretions. If variation in male competitiveness, translating to a greater mating success, exists in nature, and such males are underrepresented in sound trap samples, this could skew samples. Likewise, responsiveness of wild-type males could differ from that of males having undergone laboratory cage adaptations or bottlenecks associated with colonization. Another question is whether male responsiveness to generated tones as used in this study reveals anything about their competitiveness in the field. If so, beyond using the male response as a method to sample populations, the response of male mosquitoes to sound could potentially facilitate screening for a loss of competitiveness in mass-rearing operations.

Acknowledgments

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