Waterproof, low-cost, long-battery-life sound trap for surveillance of male *Aedes aegypti* for rear-and-release mosquito control programmes

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Abstract

**Background:** Sterile male rear-and-release programmes are of growing interest for controlling *Aedes aegypti*, including use an "incompatible insect technique" (IIT) to suppress transmission of dengue, Zika, and other viruses. Under IIT, males infected with *Wolbachia* are released into the suppression area to induce cytoplasmic incompatibility in uninfected populations. These and similar mosquito-release programmes require cost-effective field surveys of both sexes to optimize the locations, timing, and quantity of releases. Unfortunately, traps that sample male *Ae. aegypti* effectively are expensive and usually require mains power. Recently, an electronic lure was developed that attracts males using a 484 Hz sinusoidal tone mimicking the female wingbeat frequencies, broadcast in a 120 s on/off cycle. When deployed in commercially available gravid *Aedes* traps (GATs), the new combination, sound-GAT (SGAT), captures both males and females effectively. Given its success, there is interest in optimizing SGAT to reduce cost and power usage while maximizing catch rates.

**Methods:** Options considered in this study included use of a smaller, lower-power microcontroller (*Tiny*) with either the original or a lower-cost speaker (*lcS*). A 30 s on/off cycle was tested in addition to the original 120 s cycle to minimize the potential that the longer cycle induced habituation. The original SGAT was compared against other traps incorporating the *Tiny*-based lures for mosquito capture in a large semi-field cage. The catch rates in waterproofed versions of this trap were then compared with catch rates in standard [BG-Sentinel 2 (*BGS 2*); Biogents AG, Regensburg, Germany] traps during an IIT field study in the Innisfail region of Queensland, Australia in 2017.

**Results:** The system with a low-power microcontroller and low-cost speaker playing a 30 s tone (*Tiny-lcS-30s*) caught the highest proportion of males. The mean proportions of males caught in a semi-field cage were not significantly different among the original design and the four low-power, low-cost versions of the SGAT. During the IIT field study, the waterproofed version of the highest-rated, *Tiny-lcS-30s* SGAT captured male *Ae. aegypti* at similar rates as co-located *BGS-2* traps.
Conclusions: Power- and cost-optimized, waterproofed versions of male Ae. aegypti acoustic lures in GATs are now available for field use in areas with sterile male mosquito rear-and-release programmes.

Keywords: Vector control, Gravid Aedes Trap, Female wingbeat

Background

*Aedes aegypti* is the primary vector of dengue, chikungunya, Zika and yellow fever viruses among humans. There are an estimated 200,000 yellow fever cases annually, with 30,000 deaths [1] despite the existence of an effective vaccine. There are approximately 390 million dengue infections per year, of which 96 million persons exhibit symptoms, one million contract dengue fever and 5000–10,000 die each year due to dengue [2, 3]. Chikungunya re-emerged in Kenya in 2004, and has since spread to unprecedented levels in Thailand, Malaysia, India and Italy in the past ten years [4]. Zika virus, thought to cause fetal microcephaly and Guillain-Barre syndrome [5], has quickly risen to prominence in the last few years [6, 7]. For a multitude of reasons, including cost barriers in developing countries, traditional methods of vector control have failed to eliminate these diseases [8, 9].

Rear-and-release programmes involving sterile or genetically modified mosquitoes, particularly male mosquitoes, are expanding [10]. These control efforts have created a demand for inexpensive, effective tools to survey populations of released sterile males. However, the current gold-standard, the BG-Sentinel 2 trap (BGS-2; Biogents AG, Regensburg, Germany; [11]) is too expensive to support long-term surveillance over large geographical areas and requires reliable access to power, which many areas do not have. For this reason, renewed attention has been focused on the well-known phenomenon of male mosquito attraction to sound [12–18]. Current applications of sound in mosquito control programmes are benefiting from reductions in the costs of sound production technology [19–21].

Male mosquitoes are attracted to sound only over short distances because they detect particle motion rather than sound pressure, and the amplitude of particle motion attenuates more rapidly with distance than sound pressure [22]. However, *Ae. aegypti* traps make use of long-distance visual and chemical cues that bring males within range of sound cues. Both male and female *Ae. aegypti* orient over long distances toward dark-colored, “swarm marker” visual features during peak times of the day [20]. Males then gather in swarms above these features to intercept and mate with visiting females [23]. Consequently, dark-colored traps with olfactory cues attractive to females and sound lures attractive to males can be effective *Ae. aegypti* surveillance tools [17].

Rapid developments in open-source hardware platforms (e.g. Arduino and Raspberry Pi), have enabled prototyping of several microcontroller devices for insect vector trapping and infectious disease diagnostics, e.g. [20, 21, 24, 25]. Recently, an inexpensive battery-powered, (Arduino-based) microcontroller device was developed that, when placed in a passive gravid *Aedes* trap (GAT) [26], created a dual-purpose Sound-GAT (SGAT) trap which attracted male *Ae. aegypti* mosquitoes at similar rates as the frequently used BGS trap [11].

While these general development platforms are excellent prototyping tools, further savings in cost and power usage can be achieved by focusing on the specific functions needed for mosquito trapping applications. In this study, we further modified the sound lure device to reduce costs and power demands. Also, we tested the efficacy of waterproofing the new lure, as the GAT entrance funnel is on the top of the trap and therefore potentially exposed to rainfall. We then tested the new traps with waterproof lures as part of a rear-and-release programme to determine if such devices enable effective surveillance of sterile male releases into communities, and to consider how their trap catch rates compare to co-located BGS-2 traps.

Methods

Device engineering

The acoustic signal in [21] was broadcast from a platform (Arduino Pro Mini) which includes a microcontroller (Atmel Mega (Mega), Microchip Inc, Chandler, AZ) that requires a 3.3 V electrical supply, conditioned by a voltage regulator with other external circuitry to maintain operation of particular system applications, e.g. operation of a SD (Secure Digital) card [27]. If such applications are not needed for system operation; however, the Atmel series of microcontrollers can operate on as little as 1.8 V. Therefore, a pair of new, 1.5 V AA batteries can degrade to ~0.9 V while keeping the device operational, thereby extending the battery lifetime. Using a smaller chip such as the Atmel Tiny (Tiny) which includes a microcontroller (Atmel Pro Mini) which includes a microcontroller ([20, 21, 24, 25]). Recently, an inexpensive battery-powered, (Arduino-based) microcontroller device was developed that, when placed in a passive gravid *Aedes* trap (GAT) [26], created a dual-purpose Sound-GAT (SGAT) trap which attracted male *Ae. aegypti* mosquitoes at similar rates as the frequently used BGS trap [11].

In addition, the speaker used by Johnson et al. [21], (indicated as ‘orig’) (ASE04508MR-L150-R, PUI Audio Inc, Dayton, OH), has significantly greater range and fidelity than is needed to attract male *Ae. aegypti* into a trap. Consequently, we tested a lower-cost, generic 8-ohm speaker (indicated as ‘lcS’) with a 400 Hz self-resonant
frequency in one version of the Tiny platform system to assess the effects of reduced speaker range and fidelity on male trap catches. Two devices that incorporated a Tiny microcontroller were tested, both of which used a custom printed circuit board (Itead Corp, Guangzhou, China; itead.cc), an amplifier with filter, and a two-AA-battery pack. One device broadcast sound from an orig speaker and the second from a lcS speaker. Finally, we compared male responses to tones broadcast in a 120 s on/off cycle, designated as 120s, with those broadcast in a 30 on/off cycle, designated as 30s, to determine if trap effectiveness was influenced by habituation [28, 29].

Tables 1 and 2 compare the cost and power consumption of the Mega, orig system [21] with the new Tiny systems, fitted with either the previous speaker or the new low-cost speaker. All of the lure systems were tested in a commercially available BG-GAT (Biogents, Regensburg, Germany).

### Semi-field bioassays

Comparisons of catch rates between the [21] Mega-orig and the four new prototypes were performed in tents placed within large (7 × 3.5 × 4 m), semi-field flight cages [30] at the Tropical Medical Mosquito Research Facility, James Cook University, Cairns, Australia. The bioassayed mosquitoes were wild type Ae. aegypti, collected from Innisfail, Queensland, Australia and were reared using standard laboratory protocols (temperature 28 °C, photoperiod 12:12 L:D).

Five variations of sound lures were tested in initial trials to consider whether cost per unit could be reduced without decreasing trapping effectiveness: the previously developed Mega-orig-120s, a Tiny-orig-120s, a Tiny-orig-30s, a Tiny-lcS-120s and a Tiny-lcS-30s. Subsequently, trials were conducted to test the sound lure with the highest proportion of trap catches, the Tiny-lcS-30s, in relation to a waterproofed version, constructed by placing a Tiny-lcS-30s sound lure inside a 200 ml Sistema container (http://sistemaplastics.com/products/klip-it-rectangular/200ml-rectangle) with a ziplock bag (7.5 × 14 cm) to cover the speaker.

We tested the five sound lure variations using a 5 × 5 Latin Square, replicated once within the semi-field flight cage, (n = 10). For each trial, 30 virgin male Ae. aegypti (5–7 days post-eclosion) were released within 3.4 m³ nylon tents positioned >2 m apart. This distance was set as a precaution to minimize signal interference, given that the tones were not audible from 2-m distance and no behavioural interactions were noted by mosquitoes in competing tents. The bottom of each tent was covered in a white plastic sheet and single sound lures were placed on top of the mesh inside the GATs which were positioned in the middle of each tent. After 30 min GAT entrances were covered, captured mosquitoes were knocked down with CO2 and counted, and all mosquitoes were disposed. Lures were randomly rotated throughout the Latin Square design in different trials. Trials occurred during 23–31 August 2017 when cage temperatures were 26–28 °C.

### Table 1 Device component manufacturer and cost per unit (US$) information

<table>
<thead>
<tr>
<th>Item</th>
<th>Manufacturer/Supplier</th>
<th>Mega-orig Cost</th>
<th>Tiny-orig Cost</th>
<th>Tiny-lcS Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controller</td>
<td>Sparkfun/eBay</td>
<td>1.85</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>Speaker</td>
<td>PUI Audio/DigiKey</td>
<td>6.20</td>
<td>6.20</td>
<td>0.83</td>
</tr>
<tr>
<td>Filtering</td>
<td>DigiKey</td>
<td>0.75</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Battery Case w/ Switch</td>
<td>eBay</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
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<tr>
<td>Photoresistor</td>
<td>DigiKey</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Sound Lure total not incl. batteries</td>
<td>Varta/Element14</td>
<td>9.95</td>
<td>8.54</td>
<td>3.17</td>
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<tr>
<td>AA batteries (50.33 each)</td>
<td>Varta/Element14</td>
<td>0.99</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Sound Lure total incl. batteries</td>
<td>Varta/Element14</td>
<td>10.94</td>
<td>9.20</td>
<td>3.83</td>
</tr>
<tr>
<td>Gravid Aedes Trap</td>
<td>Biogents AG</td>
<td>13.63</td>
<td>13.63</td>
<td>13.63</td>
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<tr>
<td>Total trap cost</td>
<td></td>
<td>24.57</td>
<td>22.83</td>
<td>17.46</td>
</tr>
</tbody>
</table>

### Table 2 Device power and power-related cost per unit (US$) information

<table>
<thead>
<tr>
<th>Item</th>
<th>BGS-2</th>
<th>Mega-orig-GAT</th>
<th>Tiny-lcS-GAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trap Cost w/o Battery</td>
<td>274.04</td>
<td>23.58</td>
<td>16.80</td>
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<tr>
<td>Active current (mA)</td>
<td>280</td>
<td>5.8</td>
<td>6.05</td>
</tr>
<tr>
<td>Active Power (mW)</td>
<td>3360</td>
<td>26.1 (4.5V)</td>
<td>18.15 (3V)</td>
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<tr>
<td>Battery Cost per Trap</td>
<td>22.81B</td>
<td>0.99</td>
<td>0.66</td>
</tr>
<tr>
<td>Battery Capacity (mA-h)</td>
<td>10000</td>
<td>1800-2600</td>
<td>1800-2600</td>
</tr>
<tr>
<td>Active Battery Life (wks)</td>
<td>0.21</td>
<td>4.3-6.3</td>
<td>4.3-6.3</td>
</tr>
<tr>
<td>Total trap cost</td>
<td>296.85</td>
<td>24.57</td>
<td>17.46</td>
</tr>
</tbody>
</table>

a BioQuip Products, Compton, CA USA  
b 12V; Universal Power Group Inc., Coppell, TX USA
We then tested the effect of waterproofing the most successful sound lure, AT, lcS, 30s, utilizing a 2 × 2 Latin Square design, replicated four times (n = 8) in the same semi-field flight cage. Trials using the same methods as described above were conducted during 5–6 September 2017 when cage temperatures were 26–28 °C.

Field trials
Twenty SGATs were set in the Innisfail region of north Queensland, Australia, on 26 October and 14 December 2017, coinciding with the initial Debug Innisfail male releases, on 15 November-8 December 2017. The releases were of a strain resulting from a back-crossing programme that utilized an Ae. aegypti strain from the USA that was stably infected with Wolbachia (wAlbB2) from Ae. albopictus and a local Queensland strain. Male Ae. aegypti derived from this colony were released evenly throughout treatment sites. Five traps were set in Mourilyan, South Johnstone and Goondi Bend, and also in the Belvedere control site, where no males were released. Additionally, 20 unbaited BGS-2 traps were deployed in separate premises throughout each site since 2015 as part of the Debug Innisfail project.

The town of Mourilyan, comprising 256 premises, was determined to be the priority treatment site and subsequently initial releases of male Ae. aegypti were restricted to this site. During the first two weeks of releases throughout Mourilyan, approximately 177 males per week were released per trap. Males were released in low numbers in South Johnstone and Goondi Bend on 6 and 8 December. Therefore, these investigations include findings predominantly from Mourilyan.

Statistical analyses
Comparisons of mean proportions of male Ae. aegypti caught in the 5 initial semi-field treatments were performed on arcsine-transformed proportions using factorial ANOVAs. Comparisons of mean proportions caught by the standard and waterproofed versions of the Tiny lcS 30s sound lure were performed using a two-tailed independent t-test after Levene’s test for homogeneity of variance. Means comparisons of male Ae. aegypti catch rates in the field were performed using paired, two-tailed independent t-tests. Statistical analyses were performed using Prism 6 (Graphpad Software Inc., CA) and R statistical software (v. 3.3.3).

Results
Semi-field bioassays
The mean proportions of males caught were not significantly different among sound lure treatments (F(4, 45) = 1.3, P = 0.26, n = 10; Fig. 1). While not statistically significant, the Tiny-lcS-30s produced the highest proportion caught, and therefore was chosen for further testing and production.

Waterproofing modifications to the Tiny-lcS-30s sound lure did not significantly alter proportion caught (t-test, t = 0.62, df = 14, n = 8, P = 0.55; Fig. 2). On average, during the 30 min trial periods, the non-waterproofed lure captured 50 ± 5.8% standard error (SE) of released males, whereas the waterproofed version captured 45 ± 4.3%.

Field trials
SGATs weekly average male catches significantly increased in Mourilyan the week after treated male releases (t-test, t = 4.67, df = 4, n = 5, P ≤ 0.05; Fig. 3a) and remained significantly elevated when two-week averages were compared (t-test, t = 3.38, df = 4, n = 5, P ≤ 0.05; Fig. 3b). The differences among average weekly catches were not significant at the other sites, where only small numbers of treated males were released.

Average weekly catches of males in SGAT traps in Mourilyan were comparable in magnitude to those from co-located BGS-2 traps (Fig. 4). The increases of
mosquitoes caught the week after treated male releases were almost identical for BGS-2 (6.1 ± 1.9 SE mean captures per week) and SGAT traps (5.8 ± 1.2 SE). However, in the following weeks during releases, weekly SGAT catches were more variable than BGS catch numbers.

Discussion
Considerations of trapping cost vs benefit
Successful management of male mosquito rear-and-release programmes requires continuous census estimates on both area-wide and local (block) levels, which at Mourilyan encompassed ~8 ha and ~1.6 ha, respectively. Mosquito factories and rearing facilities require approximate population data to predict needed capacity in a given location and time of year [10]. Mosquito distributors require accurate block-by-block data to respond to emergent infestations and eliminate hotspots [31]. Achievement of both objectives requires frequent, widespread trapping, which currently is conducted at considerable cost.

This paper develops and tests the Tiny-lcS-30s, an ultra-low-cost sound lure for trapping male Aedes aegypti. Tested in a commercial GAT (Biogents), its effectiveness was similar to that observed in non-commercialized passive traps, such as the original GAT [26], as well as that of the original prototype sound GATs [21]. The use of a low-cost GATs with the add-on of the Tiny-lcS-30s can significantly reduce the costs of male mosquito surveillance in rear-and-release programs.

Low-cost trapping methods can improve the efficacy of vector control programmes throughout the world. There is potential to adapt these lures for Aedes albopictus surveillance as well, given that males of this species also are attracted to wingbeat sounds of female conspecifics [24]. Waterproofing the lure enhances operation in periurban or vegetated locations, the preferred habitats of Ae. albopictus, which may be exposed to rainfall. Other Culex spp. [19] and Anopheles spp. [32] of medical importance also are known to gather at visually attractive swarm markers and display attraction to female wingbeats; thus, the sound lure likely could be adapted to capture males of such species as well. Females of several Culex spp. [33] and Anopheles spp. [34] have been found to respond to oviposition attractants; consequently, it may be feasible to combine male sound lures and female oviposition attractants of such species into a single trapping device that serves as a swarm marker, as in this study.

SGAT and BGS-2 effectiveness for mosquito surveillance in rear-and-release programmes
Male Ae. aegypti mean weekly catches significantly increased in SGATs fitted with the ultra-low-cost
waterproofed lures (Tiny-Ics-30s) during the Debug Innisfail Wolbachia-treated male rear and release programme (Fig. 3). Significant changes in SGAT catch rates were not detected in other sites where releases had not occurred during the study period. Furthermore, male Ae. aegypti mean weekly catches in SGATs were comparable in magnitude and, at times, greater than those from unbaited BGS-2 traps during this time (Fig. 4). The BGS traps are known to be the gold standard for surveilling Ae. aegypti [21]. The previous, higher-cost version of this lure [21] was also found to capture similar numbers of male Ae. aegypti in unbaited BGS-2 traps in the field in northern Australia. Our data suggest that this current version is equally effective, with added benefits of lower costs per unit and lower power usage.

During the releases, the number of males in the release area increased. The SGAT and BGS-2 captured similar initial increases in weekly catch rates. However, SGAT data were more variable than the BGS-2 during continued releases of male Ae. aegypti. Perhaps this variation was due to lower statistical power, having deployed only five SGATs compared to 20 BGS-2 traps per trial.

Conclusions
Advances in disease containment by sterile mosquito release have created demand for cost-effective, low-power-usage male mosquito lures. With more specific and timely data, rear-and-release programmes can target more precisely the areas where the Wolbachia-infected males need to be released and reduce the resources needed to produce and release them in sufficient numbers to enable disease containment. The new SGATs described in this report are inexpensive tools potentially highly suitable for surveillance of male Ae. aegypti populations as well as those of other mosquito species during “rear and release” programmes.

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Authors’ contributions
BBR and NCZ designed and constructed the acoustic lure devices, BBR, KMS, NCZ, and SAR conducted the experiments, BBR, KMS, NCZ, RWM, and SAR analyzed data, organized and drafted versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare they have no competing interests.

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