Journal of the American Mosquito Control Association, 35(3):169–177, 2019

A NOVEL METHODOLOGY FOR RECORDING WING BEAT FREQUENCIES OF UNTETHERED MALE AND FEMALE *AEDES AEGYPTI*

KYRAN M. STAUNTON,[1,2] LILI USHER,[1,2] TIM PRACHAR,[3] SCOTT A. RITCHIE,[1,2] NIGEL SNOAD[3] AND BRIAN J. JOHNSON[1,2]

ABSTRACT. *Aedes aegypti* is a vector of many significant arboviruses worldwide, including dengue, Zika, chikungunya, and yellow fever viruses. With vector control methodology pivoting toward rearing and releasing large numbers of insects for either population suppression or virus-blocking, economical remote (sentinel) surveillance methods for release tracking become increasingly necessary. Recent steps in this direction include advances in optical sensors that identify and classify insects based on their wing beat frequency (WBF). As these traps are being developed, there is a strong need to better understand the environmental and biological factors influencing mosquito WBFs. Here, we developed new untethered-subject methodology to detect changes in WBFs of male and female *Ae. aegypti*. This new methodology involves directing an ultrasonic transducer at a free-flying subject and measuring the Doppler shift of the reflected ultrasonic continuous wave signal. This system's utility was assessed by determining its ability to confirm previous reports on the effect of temperature, body size, and age on the WBFs generated from acoustic or optical-based experiments. The presented ultrasonic method successfully detected expected trends for each factor for both male and female *Ae. aegypti* without the need for subject manipulation and potential impediment of natural flight dynamics due to tethering. As a result, this ultrasonic methodology provides a new method for understanding the environmental and physiological determinants of male and female WBFs that can inform the design of remote mosquito surveillance systems.

KEY WORDS *Aedes aegypti*, dengue, wing beat frequency, *Wolbachia*, Zika

INTRODUCTION

Aedes aegypti (L.) is the primary vector responsible for transmitting dengue, Zika, yellow fever, and chikungunya viruses. Controlling *Ae. aegypti* has been extremely challenging because of complications such as the mosquitoes' endophilic behavior, their propensity to oviposit within containers transported long distances, and increasing insecticide resistance (Ritchie 2014). Subsequently, tropical communities are experiencing more frequent and intense dengue outbreaks than ever before (Bowman et al. 2016, Regan et al. 2016, Ritchie et al. 2018). In response, new methods of controlling *Ae. aegypti*, and potentially the diseases they transmit, are being developed. These methods include releasing males that have been sterilized (sterile insect technique) and/or males that have been altered (such as by being infected with *Wolbachia*), so that their offspring are not viable (incompatible insect technique; Alphey 2014, Carvalho et al. 2015, Zhang et al. 2016). To succeed, these "rear and release" strategies require wide-scale population monitoring before, during, and after releases to assess operational efficacy. However, such monitoring is a daunting task that represents an enormous logistical and monetary investment. Therefore, there is a clear need for the development of methods to classify mosquitoes in the field (Mukundarajan et al. 2017) as well as economical and automated remote (sentinel) surveillance methods for release tracking.

Recent advances in sentinel surveillance of mosquito populations include the development of optical sensors that identify and classify insects based on their wing beat frequencies (WBFs) (Batista et al. 2011, Chen et al. 2014, Potamitis and Rigakis 2015), as well as male-focused traps that capture males by exploiting their attraction to female WBFs, the primary means of female identification and tracking for urban male *Aedes* spp. (Stone et al. 2013, Johnson and Ritchie 2015, Balestrino et al. 2016). Although several trap concepts have shown great promise, much work is still needed to understand the factors influencing mosquito WBFs so that efficacy is maintained during different trapping conditions and against populations of variable age and size (Scott et al. 2000, Harrington et al. 2001).

Commonly, methodological approaches have used tethered mosquitoes (subjects are fixed to fine steel wire) and particle velocity microphone-based recording systems when investigating environmental or physiological influences on mosquito WBFs (Cator et al. 2009, 2010; Arthur et al. 2014). Unfortunately, tethering may cause lower WBFs and, by excluding free-flight, restrict detection of differences in frequencies between hovering or forward flight and even increase the variance of WBF distributions detected (Aldersley et al. 2016, Mukundarajan et al. 2017). To reduce complications from tethering mosquitoes, "semitethers," in conjunction with particle velocity microphones, have also been used where the steel

---

[1] College of Public Health, Medical and Veterinary Sciences, James Cook University, Smithfield QLD 4878, Australia.

[2] Australian Institute of Tropical Health and Medicine, James Cook University, Smithfield QLD 4878, Australia.

[3] Verily Life Sciences, 259 East Grand Avenue, South San Francisco, CA 94080.

tether is replaced with a human hair (Cator and Harrington 2011, Villarreal et al. 2017). Also, observations of free-flying mosquitoes have been performed both in the laboratory using microphones to record the WBF of mosquitoes restricted to a container (Brogdon 1994, Villarreal et al. 2017) or in the field using an array of particle velocity and pressure sensitive microphones (Cator et al. 2011).

Recently, Villarreal et al. (2017) directly compared microphone recordings of *Ae. aegypti* that were either semitethered or free-flying. They found that these treatments did not affect the WBFs they recorded but suggested that studies concerning male-specific sound traps use data gathered from free-flying females. To record free-flying mosquito WBFs, researchers have also used a photosensor to monitor transient waveforms resulting from free-flying mosquitoes passing through a light beam (Moore et al. 1986, Tripet et al. 2004). Although optical systems, such as the one used by Tripet et al. (2004) and Moore et al. (1986), have the benefit of sampling WBFs from free-flying mosquitoes, their focal areas are generally very localized, covering only small flight samples; these methodologies also require additional external stimuli such as a beam of light being shone on specimens that are negatively phototactic, such as *Ae. aegypti* (Christophers 1960). Subsequently, there remains a need to develop a less invasive methodology for recording WBFs from free-flying mosquitoes in the laboratory setting as well as future field applications during which high levels of background noise limit the usefulness of microphone-based systems.

Here, we report the development of a novel ultrasonic-based method to detect and record male and female WBFs by measuring the Doppler shifts of reflected ultrasonic continuous wave signals from free-flying subjects. Such a system has an inherently larger focal area than optical-based systems and overcomes contamination issues due to the presence of background noise commonly encountered in acoustic-based systems, particularly in field applications. To assess the utility of the ultrasonic recording methodology, we tested its ability to detect known effects of temperature, body size, and age on the WBFs of male and female *Ae. aegypti* generated from experiments using acoustic and optical methodologies. The presented ultrasonic system confirmed expected patterns supporting its adoption in laboratory experiments and potential field applications in the design of male-specific sound lures (Johnson et al. 2018) and "smart" traps aimed at detecting and separating captured mosquitoes by sex and species based on their unique WBF profiles (Raman et al. 2007, Batista et al. 2011).

MATERIALS AND METHODS

Mosquito rearing and maintenance

Mosquitoes used in this study were wild-type *Ae. aegypti* sourced from routine ovitraps throughout Innisfail, Australia, in 2016 and maintained in colonies (ca. F2–F4 generations) using standard laboratory rearing protocols (Hoffmann et al. 2011, Ritchie et al. 2015). In short, larvae were reared in 3.4-liter white buckets with approximately 2 liter of water (ca. 500 larvae/bucket) from eggs hatched in a yeast solution (0.20 g baker's yeast per liter of water). After 24 h, the yeast solution was filtered out and fresh water added. The larvae were then maintained on a diet of fish food (Tetramin; 0.45 g on day 2 and 0.80 g on day 5), resulting in a mean pupation time of 6 days. Temperature was maintained at 28°C with a 12:12 h photoperiod. All individuals per cohort were collected as pupae on the same day, producing cohorts of identical age. Only cohorts of similar body size, determined by measuring wing length postrecording, were used in the age and temperature experiments. If a cohort was found to differ significantly in body size, a new cohort of similarly size individuals was recorded. Adults were maintained at 28°C and 70% relative humidity (RH) and allowed to feed from a 50% honey solution ad libitum. All adults used in the body size and temperature experiments were 5–7 days old at the time of recording. Laboratory experiments were conducted at the James Cook University (JCU) Tropical Medicine Mosquito Research Facility in Cairns, Queensland, Australia. Mosquitoes were blood-fed on human volunteers under JCU ethics approval H6286.

Free-flight WBF recording

The recording chamber was a 60-ml aspirator vial (Bioquip, 2809V, Compton, CA) inserted onto a test fixture containing 2 ultrasonic transducer sensors (Murata, MA40S4S, Nagaokakyo, Japan). A single male or female mosquito was aspirated from the holding cage into the test vial to record >10 sec of uninterrupted flight (i.e., no landing and resting on the sides of the container). Flight sound was recorded using a TASCAM portable handheld recorder (DR-22WL; Montebello, California) connected to a Keysight Waveform Generator (33500B; Santa Rosa, California; configuration, sine wave output, 5Vpp, 46.2 kHz) via a SRS Small Instrumentation Modules (SIM900) mainframe (Fig. 1).

Wing beat frequency data were collected by measuring the Doppler shift of a reflected ultrasonic continuous wave signal. In this setup, the function generator directly drove an ultrasonic transducer, which was aimed at the specimen. A 2nd ultrasonic transducer was used as a microphone to detect reflections of the incident signal, which was modulated by the motion of the mosquito's body and wings. The signal that was received was mixed with the transmitted signal to remove the modulation frequency (nominally 46.2 kHz), leaving the Doppler shift signal.

Recording insects in this way produces a signal mainly composed of 2 components, one a low

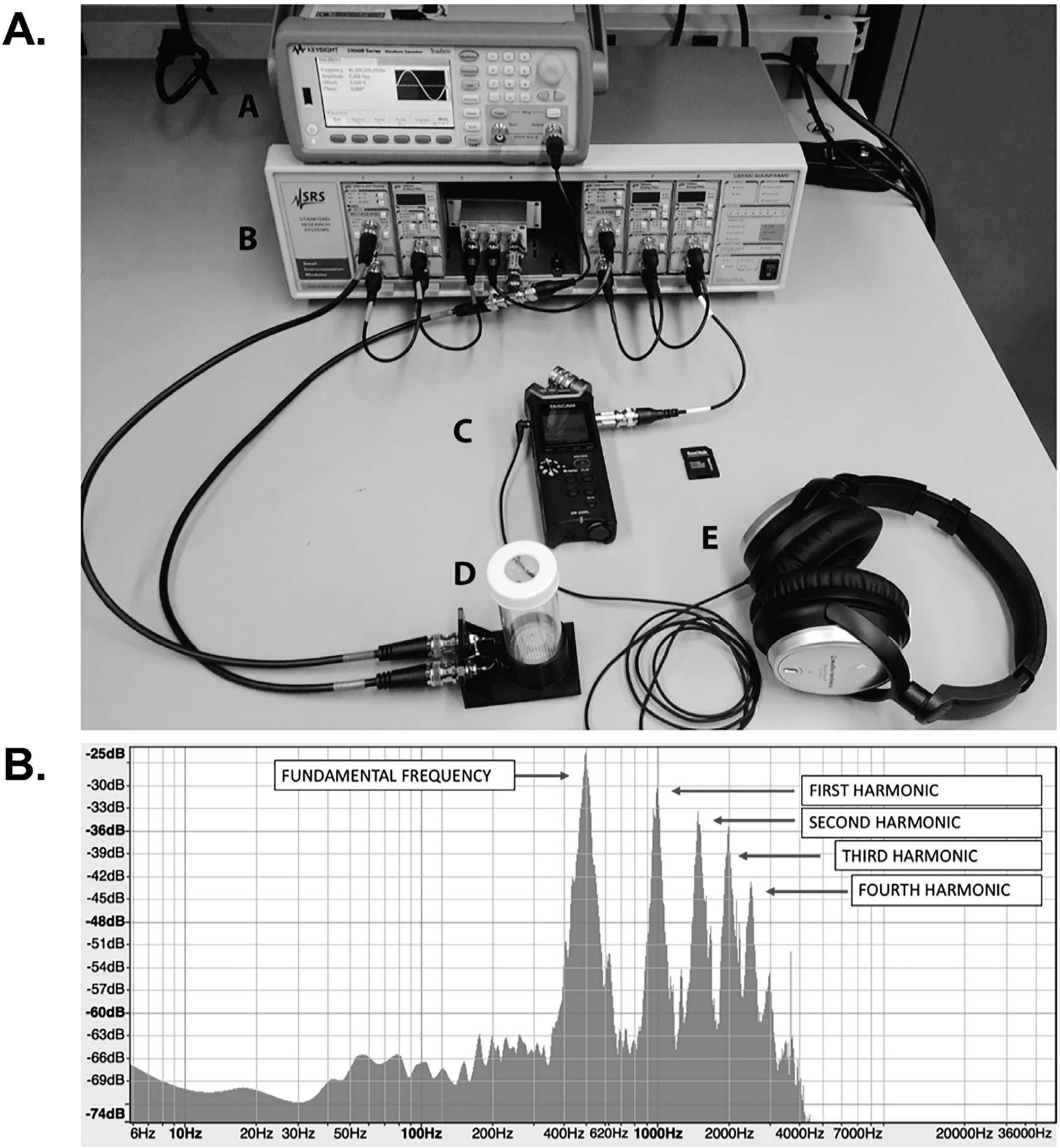

Fig. 1. (A) Recording equipment: (A) Keysight 33500B Waveform Generator, (B) SRS SIM900 mainframe, (C) TASCAM DR-22WL recorder to store wing beat recordings, (D) test fixture with vial containing free-flying mosquito, and (E) headphones to ensure viable recordings are collected. (B) Example frequency plot (Hann Window) from a female *Ae. aegypti* showing the peaks of the fundamental and harmonic frequencies.

frequency component induced by the body movement and the other a high frequency component induced by wing beating (Wang et al. 2017). The signal variation induced by the insect's turning and translational movement is slower than that induced by wing beating. This allows the wingbeat-modulated signal to be separated using high-pass filtering of low frequencies (<25 Hz) (Full and Tu 1990), well below the lower limits of recorded WBFs. We removed these lower, body-associated frequencies by high-pass filtering and amplified the resulting output. The output was further filtered to remove frequencies above the 3rd harmonic limits of the target fundamental WBFs. When there was not any motion, there were no frequency components to the received and mixed signals. The final analog signal was then recorded using the portable recording device into a high resolution .wav file (32 bit), which was analyzed using Audacity® software. The frequency spectrum of each recording was visualized (Fourier Transform; function = Hann window; size = 32,769) and the mean WBF determined as the peak of the fundamental frequency. For each individual recording, additional data (i.e., date, time, dew point [°C], barometric pressure, humidity, and origin of the mosquitoes) were also recorded.

The range of target WBFs (WBFs of interest) was determined from previous reports on *Ae. aegypti* (Stone et al. 2013, Arthur et al. 2014, Villarreal et al. 2017). These studies report that the fundamental frequencies of female and male *Ae. aegypti* range from 350–664 Hz to 571–832 Hz, respectively. Following these reports, a fundamental frequency window of 200–1,200 Hz made up our range of target WBFs, enabling us to detect the full range of reported frequencies as well as any significant variations in these frequencies. The full selection window was extended so that higher order harmonics (1st–3rd) as well as the fundamental frequencies were present.

Temperature assays

Multiple environmental and physiological factors are thought to influence mosquito WBFs. Of these, ambient temperature, mosquito age, and body size are among the most important (Tripet et al. 2004). Previous work on dipterans (genus *Musca*) has revealed a general relationship in which fundamental flight frequencies increase as ambient air temperatures rise (Sotavalta 1952, Unwin and Corbet 1984), an observation confirmed for female *Ae. aegypti* in tethered, semitethered, and free-flight experiments (Costello 1974, Villarreal et al. 2017). To determine the ability of the ultrasonic system to detect changes in WBF related to increased ambient air temperature, we recorded the WBFs of males and females exposed to 24°C, 28°C, or 32°C for 1-h periods. The changing temperature treatments were designed to assess the impact of temperature change of WBF during transitions from warmer (28°C) to cooler (24°C) temperatures and cooler (28°C) to warmer (32°C) temperatures. Wing beat frequencies were recorded from different subjects for each trial replicate ($n = 30$). After recording, each mosquito was anesthetized using CO_2 gas. Subjects were selected from cohorts reared simultaneously, so were assumed to be equal in size.

Size assays

Adult size, which can be influenced by larval food availability and temperature exposure, has been found to influence mosquito WBFs, with larger individuals displaying higher WBFs in some studies (Costello 1974, Wekesa et al. 1998, Cator et al. 2010). To determine the ability of the ultrasonic system to detect changes in WBF related to differences in body size, we reared cohorts under different feeding regimes to generate “small” and “large” sized males and females following the methods of Cator and Zanti (2016). In short, cohorts of 500 1st instars were subjected to either “high” or “low” food treatments to produce large or small adults. The high food treatment groups were provided 150 mg of food daily (approx. 0.3 mg/larva), whereas the low food treatment groups were provided with 50 mg daily (approx. 0.1 mg/larva) until pupation. Because the low food groups took longer to reach pupation, adult age (i.e., age after pupal emergence) was controlled by offsetting the timing of the high food treatments such that majority of eclosion occurred within a day between the 2 treatments. After eclosion, mosquitoes were held at 28°C, 70% RH, and a photoperiod of 12:12 h (light:dark) until experiments commenced. Mean body sizes of each cohort were subsequently determined by removing and measuring 1 wing from each individual.

Age assays

The WBFs of both male and female *Ae. aegypti* increase with age (Tischner and Schief 1955, Costello 1974, Moore et al. 1986). To determine the ability of the ultrasonic system to detect changes in WBF related to age, male and female WBFs were measured at 7 days (1 wk), 14 days (2 wk), and 21 days (3 wk) postemergence. Although males rarely live this long in the field, we used extreme cases to stress the potential effect of age. Subjects were selected from cohorts reared simultaneously so were assumed to be equal in size and were maintained using standard laboratory protocols and holding conditions (28°C and 70% RH).

Statistical analyses

Groups exposed to varying temperatures were clustered by sex and exposure time, and mean comparisons were performed using 1-way analyses of variance (ANOVAs) with Tukey’s honestly significant difference (HSD) post hoc analyses. All investigations into diet effects on wing length and wing-length effects on WBF were performed using unpaired 2-tailed Welch’s *t*-tests. Wing beat frequencies of females grouped by age were compared using Kruskal Wallis rank sum test with Dunn’s test post hoc analyses, since the data were not normally distributed, even after transformation. Male data for the same comparisons were normally distributed; therefore, 1-way ANOVAs with Tukey HSD post hoc analyses were performed. Unless specified, statistical analyses were performed using Prism 6 (Graphpad Software Inc., San Diego, California) or *R* statistical software (version 3.3.3.).

RESULTS

Temperature exposure effect on WBFs

Female *Ae. aegypti* WBFs differed significantly when exposed to 24°C compared with 28°C and 32°C

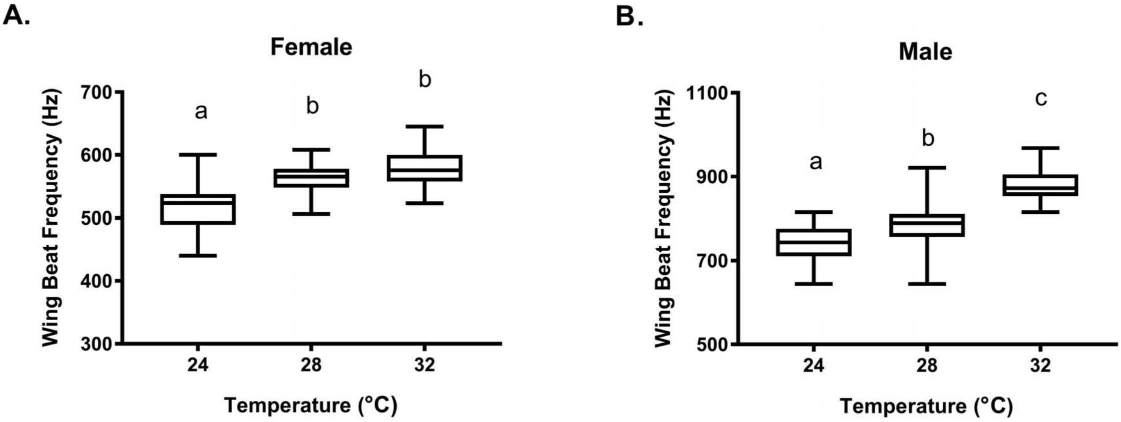

Fig. 2. Wing beat frequencies recorded for (A) female and (B) male *Ae. aegypti* exposed to different temperatures (1 h, $n = 30$). Box and whisker plots display median line with bars showing min and max values. Different letters indicate significant differences between groups (ANOVA with Tukey HSD post hoc analyses, $P < 0.05$).

($F_{2,87} = 37.98$, $P < 0.001$; Fig. 2). Mean WBFs (±standard error [SE]) for each female treatment ($n = 30$) were 515 Hz (±6.4) at 24°C, 562 Hz (±4.7) at 28°C, and 580 Hz (±5.1) at 32°C (Table 1). Male *Ae. aegypti* WBFs differed significantly when exposed to each different temperature treatment ($F_{2,87} = 75.68$, $P < 0.001$; Fig. 2). Mean WBFs for each male treatment ($n = 30$) were 740 Hz (±8.3) at 24°C, 786 Hz (±9) at 28°C, and 878 Hz (±6.7) at 32°C (Table 1).

Effect of size variation on WBFs

Mosquitoes reared for small and large body sizes exhibited significantly different wing lengths for both female (*t*-test, $n = 20$ and 30, $P < 0.0001$) and male (*t*-test, $n = 20$ and 30, $P < 0.0001$) groups. The mean wing size of females fed on low and high diets were 2.31 mm (±0.026) and 2.61 mm (±0.013), respectively. The mean wing size of males fed on low and high diets were 1.92 mm (±0.018) and 2.07 mm (±0.014), respectively.

Wing beat frequencies of differently sized females were significantly different (*t*-test, $n = 20$, 30, $P = 0.023$; Fig. 3). Mean WBFs of small females were 502 Hz (±9.78), whereas the mean WBFs of large females were 540 Hz (±12.35; Table 1). Like females, WBFs of differently sized males were significantly different (*t*-test, $n = 20$, 30, $P < 0.05$; Fig. 3). Mean WBFs of small males were 782 Hz (±6.48), whereas the mean WBFs of large males were 828 Hz (±9.13; Table 1).

Effect of age on male and female WBFs

One-week-old females displayed significantly lower WBFs than 3-wk-old females (H = 8.33, 2 df, $P = 0.015$, $n = 30$; Fig. 4). Mean WBFs were 503 Hz (±7.51), 524 Hz (±4.76), and 531 Hz (±6.39) for 1-, 2-, and 3-wk-old females, respectively (Table 1). One-week-old males displayed significantly lower WBFs than 2- and 3-wk-old males ($F_{2,87} = 4.46$, $P = 0.014$, $n = 30$; Fig. 4 and Table 1). Mean WBFs were 783 Hz (±7.21), 807 Hz (±5), and 806 Hz (±7.01) for 1-, 2-, and 3-wk-old males, respectively (Table 1).

DISCUSSION

Novel untethered methodology

This study successfully recorded WBFs from free-flying individuals using a novel ultrasonic-based

Table 1. Average (±SE) WBFs (Hz) recorded for each treatment and group.

Factor	*n* (±SE)
Temperature	
24°C	
Female	515 (±6.4)
Male	740 (±8.3)
28°C	
Female	562 (±4.7)
Male	786 (±9)
32°C	
Female	580 (±5.1)
Male	878 (±6.7)
Size	
Small	
Female	502 (±9.78)
Male	782 (±6.48)
Large	
Female	540 (±12.35)
Male	828 (±9.13)
Age	
1 wk	
Female	503 (±7.51)
Male	783 (±7.21)
2 wk	
Female	524 (±4.76)
Male	807 (±5)
3 wk	
Female	531 (±6.39)
Male	806 (±7.01)

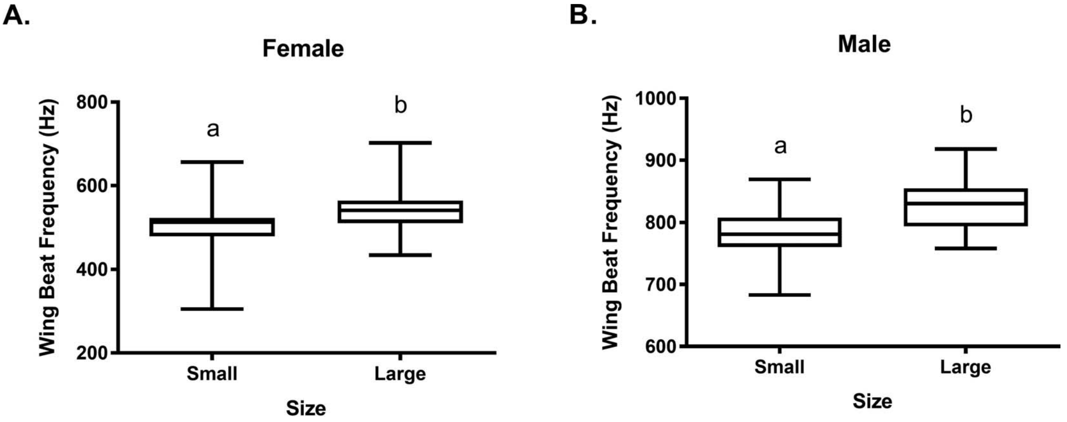

Fig. 3. Wing beat frequencies recorded for (A) female and (B) male *Ae. aegypti* exposed to different larval diets to produce small (low diet, $n = 30$) and large (high diet, $n = 20$) sized individuals. Box and whisker plots display median line with bars showing min and max values. Different letters indicate significant differences between groups (*t*-test, $P < 0.05$). All individuals were reared, maintained, and WBF recorded at 28°C.

methodology. The utility of this method was confirmed by the successful verification of previously observed effects of temperature, body size, and age on WBFs reported from acoustics and optical-based recording systems. This new methodology has the potential to improve current WBF investigations by eliminating confounding effects related to excessive subject manipulation and potential impediment of natural flight dynamics due to tethering. Additionally, this system is not impeded by background noise, which can interfere with microphone-based systems. Tethering mosquitoes has been suggested to alter specific WBFs (Cator et al. 2011, de Silva et al. 2015, Aldersley et al. 2016); change the range of tones produced from altered flight patterns, such as hovering and forward flight (Aldersley et al. 2016, Mukundarajan et al. 2017); and display varied behavioral responses (Cator and Harrington 2011). As recommended by Villarreal et al. (2017), we were able to record the WBFs of free-flying mosquitoes and therefore avoid any biases associated with tethering. Additionally, we did not employ a laser-based optical system, like those used by Tripet et al. (2004) and Moore et al. (1986). Optical systems can be very localized, whereas ultrasonic methods such as the one we used were able to cover relatively large flight areas capturing longer samples, which can encompass thousands of wing beat events. Unlike optical systems, ultrasonic methodologies can also detect vertical flight patterns and can therefore better capture the frequency of motion, as well as wing movement. Additionally, our methodology did not require external stimuli such as a beam of light being shone on this negatively phototactic species. Although determining differences in *Ae. aegypti* WBFs between different flight patterns or behavioral

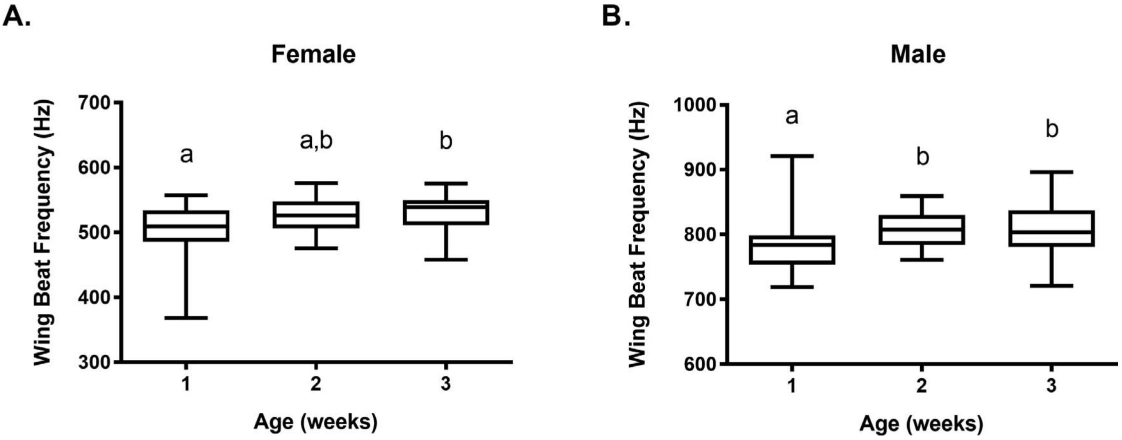

Fig. 4. Wing beat frequencies recorded for (A) female and (B) male *Ae. aegypti* of different ages ($n = 30$). Box and whisker plots display median line with bars showing min and max values. Different letters indicate significant differences between groups (Kruskal Wallis, $P < 0.05$). All individuals were reared, maintained, and WBF recorded at 28°C.

responses was outside the scope of this study, we believe that this equipment could be appropriate for such studies with only minor modifications to the flight chamber.

Effect of temperature on WBFs

Our methodology recorded increases in WBFs of both male and female *Ae. aegypti* as they were exposed to higher temperatures. We found that females changed their WBF by ~8 Hz/°C, which is consistent with both Costello (1974) and Villarreal et al. (2017), who recorded rates of change of 6.3–6.7 Hz/°C and 8–13 Hz/°C, respectively. Additionally, we found that male *Ae. aegypti* increase their WBFs at much higher rates (~17 Hz/°C between 24°C and 32°C) than females. Rowley and Graham (1968b) found that optimal flight performance for *Ae. aegypti* occurred at 21°C and subsequently decreased as temperatures increase above this threshold. Since we tested WBFs at temperatures that ranged from 24 to 32°C, it is therefore likely that increased WBFs also could be associated with decreased flight performances—a trend that may affect male *Ae. aegypti* more than females.

Effect of size variation on WBFs

Positive relationships were found between size and WBF for both female and male *Ae. aegypti*. Mosquitoes fed more as larvae were larger in size and displayed significantly higher WBFs. Similar results have been presented regarding *Anopheles gambiae* (Giles), where larger individuals of both sexes displayed higher WBFs (Wekesa et al. 1998, Cator et al. 2010). However, studies have found that size had little effect on WBF for *Aedes* mosquitoes (Mukundarajan et al. 2017, Villarreal et al. 2017). Villarreal et al. (2017) tested females within a wing-length range of 2.5–3.2 mm and stated that significant effects may be noted if smaller individuals were tested. Our study, using comparatively smaller mosquitoes, suggests that these individuals do display significant differences in WBFs.

Effect of age on male and female WBFs

Younger mosquitoes displayed significantly lower WBFs compared with older mosquitoes. These findings extend earlier works focused on males, which lived shorter life spans of 5–10 days (Costello 1974; Moore et al. 1986). Costello recorded a large increase in WBFs starting below 400 Hz and beginning to plateau from day 3 to 5 at around 500 Hz. Similarly, Moore et al. (1986) found that WBFs of both male and female *Ae. aegypti* increased until day 4 then plateaued and began to drop off by day 10. A few of the WBF data points for the youngest female cohort we sampled were low enough to cause the data set to follow a nonnormal distribution, perhaps indicating relatively slower development by this age. Costello (1974) related changes in WBF to the sexual maturation of both female and male mosquitoes, demonstrating that males are more attracted to higher female WBFs as they age. Our results extend this research to demonstrate that the increase in female *Ae. aegypti* WBFs continues to occur over the 1st 3 wk of their life from 503 to 531 Hz. Additionally, Rowley and Graham (1968a) found that female *Ae. aegypti* flew furthest during their 2nd wk after emergence (day 8–12) under laboratory conditions. Although our female WBFs increased with age between cohorts from week 1 and 3, we did not find any significant differences in WBF for the week 2 cohort, which would have suggested that WBFs could indicate a greater ability of flight in association to age.

The presented ultrasonic recording methodology successfully captured a range of environmental factors known to influence the WBFs of mosquitoes, particularly the influence of temperature and body size variation, a result of variable diet and age. Since the subjects were untethered, they were more likely to have displayed behaviors more similar to those portrayed in natural conditions, a clear goal of such analyses and an improvement over the tethered system. The results support the adoption of this system to investigate environmental and physiological determinants of male and female WBFs to advance the design of male-focused acoustic trap systems and "smart" traps aimed at detecting and separating captured mosquitoes by sex and species based on their unique WBF profiles.

ACKNOWLEDGMENTS

We acknowledge the help we received from personnel at JCU's Tropical Medicine Mosquito Research Facility, especially Sandy Taylor, Jason Anderson, Mick Townsend, Chris Paton, and Ana L. Ramirez.

REFERENCES CITED

Aldersley A, Champneys A, Homer M, Robert D. 2016. Quantitative analysis of harmonic convergence in mosquito auditory interactions. *J R Soc Interface* 13. doi:10.1098/rsif.2015.1007

Alphey L. 2014. Genetic control of mosquitoes. *Annu Rev Entomol* 59:205–224.

Arthur BJ, Emr KS, Wyttenbach RA, Hoy RR. 2014. Mosquito (*Aedes aegypti*) flight tones: frequency, harmonicity, spherical spreading, and phase relationships. *J Acoust Soc Am* 135:933–941. doi:10.1121/1.4861233

Balestrino F, Iyaloo DP, Elahee KB, Bheecarry A, Campedelli F, Carrieri M, Bellini R. 2016. A sound trap for *Aedes albopictus* (Skuse) male surveillance: response analysis to acoustic and visual stimuli. *Acta Tropica* 164:448–454. doi:10.1016/j.actatropica.2016.09.002

Batista GE, Hao Y, Keogh E, Mafra-Neto A. 2011. Towards automatic classification on flying insects using inexpensive sensors. Paper presented at the 2011 10th International Conference on Machine Learning and

Applications and Workshops. 2011 December 18–21; Honolulu, HI.

Bowman LR, Donegan S, McCall PJ. 2016. Is dengue vector control deficient in effectiveness or evidence?: systematic review and meta-analysis. *PLoS Negl Trop Dis* 10:e0004551.

Brogdon WG. 1994. Measurement of flight tone differences between female *Aedes aegypti* and *A. albopictus* (Diptera: Culicidae). *J Med Entomol* 31:700–703. doi:10.1093/jmedent/31.5.700

Carvalho DO, McKemey AR, Garziera L, Lacroix R, Donnelly CA, Alphey L, Malavasi A, Capurro ML. 2015. Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. *PLoS Neglect Trop Dis* 9:e0003864. doi:10.1371/journal.pntd.0003864

Cator LJ, Arthur BJ, Harrington LC, Hoy RR. 2009. Harmonic convergence in the love songs of the dengue vector mosquito. *Science* 323:1077–1079.

Cator LJ, Arthur BJ, Ponlawat A, Harrington LC. 2011. Behavioral observations and sound recordings of free-flight mating swarms of *Ae. aegypti* (Diptera: Culicidae) in Thailand. *J Med Entomol* 48:941–946. doi:10.1603/ME11019

Cator LJ, Harrington LC. 2011. The harmonic convergence of fathers predicts the mating success of sons in *Aedes aegypti*. *Animal Behavior* 82:627–633. doi:https://doi.org/10.1016/j.anbehav.2011.07.013

Cator LJ, Ng'Habi KR, Hoy RR, Harrington LC. 2010. Sizing up a mate: variation in production and response to acoustic signals in *Anopheles gambiae*. *Behavioral Ecol* 21:1033–1039. doi:10.1093/beheco/arq087

Cator LJ, Zanti Z. 2016. Size, sounds and sex: interactions between body size and harmonic convergence signals determine mating success in *Aedes aegypti*. *Parasite Vector* 9:622. doi:10.1186/s13071-016-1914-6

Chen Y, Why A, Batista G, Mafra-Neto A, Keogh E. 2014. Flying insect classification with inexpensive sensors. *Journal of Insect Behav* 27:657–677.

Christophers SR. 1960. *Aedes aegypti (L.) the yellow fever mosquito: Its life history, bionomics and structure*. Cambridge, UK: Cambridge University Press.

Costello RA. 1974. "*Effects of environmental and physiological factors on the acoustic behaviour of Aedes aegypti (L.) (Diptera: Culicidae)*." [Ph.D. dissertation]. Simon Fraser University, Burnaby, BC.

de Silva P, Nutter B, Bernal XE. 2015. Use of acoustic signals in mating in an eavesdropping frog-biting midge. *Anim Behav* 103:45–51.

Full RJ, Tu MS. 1990. Mechanics of six-legged runners. *J Exp Biol* 148:129–146.

Harrington LC, Buonaccorsi JP, Edman JD, Costero A, Kittayapong P, Clark GG, Scott TW. 2001. Analysis of survival of young and old *Aedes aegypti* (Diptera: Culicidae) from Puerto Rico and Thailand. *J Med Entomol* 38:537–547. doi:10.1603/0022-2585-38.4.537

Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, Greenfield M, Durkan M, Leong YS, Dong Y, Cook H, Axford J, Callahan AG, Kenny N, Omodei C, McGraw EA, Ryan PA, Ritchie SA, Turelli M, O'Neill SL. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476:454–457. doi:10.1038/nature10356

Johnson BJ, Ritchie SA. 2015. The siren's song: Exploitation of female flight tones to passively capture male *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 53:245–248.

Johnson BJ, Rohde BB, Zeak N, Staunton KM, Prachar T, Ritchie SA. 2018. A low-cost, battery-powered acoustic trap for surveilling male *Aedes aegypti* during rear-and-release operations. *PloS One,* 13:e0201709. doi:10.1371/journal.pone.0201709

Moore A, Miller JR, Tabashnik BE, Gage SH. 1986. Automated identification of flying insects by analysis of wingbeat frequencies. *J Eco Entomol* 79:1703–1706. doi:10.1093/jee/79.6.1703

Mukundarajan H, Hol FJH, Castillo EA, Newby C, Prakash M. 2017. Using mobile phones as acoustic sensors for high-throughput mosquito surveillance. *eLife,* 6:e27854. doi:10.7554/eLife.27854

Potamitis I, Rigakis I. 2015. Novel noise-robust optoacoustic sensors to identify insects through wingbeats. *IEEE Sensors J* 15:4621–4631.

Raman D, Gerhardt RR, Wilkerson JB. 2007. Detecting insect flight sounds in the field: implications for acoustical counting of mosquitoes. *Agr Biosyst Eng* 50:1481–1485. doi:10.13031/2013.23606

Regan D, Manrique-Saide P, Anuar Medina B, Edgar Ulises Koyoc C, Che-Mendoza A, Jones B, Liebman K, Vizcaino L, Vazquez-Prokopec G, Lenhart A. 2016. Spatial variation of insecticide resistance in the dengue vector *Aedes aegypti* presents unique vector control challenges. *Parasites & Vectors* 9. doi:http://dx.doi.org/10.1186/s13071-016-1346-3

Ritchie SA. 2014. Dengue vector bionomics: why *Aedes aegypti* is such a good vector. In Gubler DJ, Ooi EE, Vasudevan S, and Farrar J, eds., *Dengue and dengue hemorrhagic fever*, 2nd ed. Wallingford, UK: CABI. p 455–480.

Ritchie SA, Townsend M, Paton CJ, Callahan AG, Hoffmann AA. 2015. Application of *w*MelPop *Wolbachia* strain to crash local populations of *Aedes aegypti*. *PLoS Neglect Trop D,* 9:e0003930. doi:10.1371/journal.pntd.0003930

Ritchie SA, van den Hurk AF, Smout MJ, Staunton KM, Hoffmann AA. 2018. Mission accomplished? We need a guide to the 'post release' world of *Wolbachia* for *Aedes*-borne disease control. *Trends in Parasitol* 1715:1–10. doi:10.1016/j.pt.2017.11.011

Rowley WA, Graham CL. 1968a. The effect of age on the flight performance of female *Aedes aegypti* mosquitoes. *J Insect Physiol* 14:719–728. doi:https://doi.org/10.1016/0022-1910(68)90230-8

Rowley WA, Graham CL. 1968b. The effect of temperature and relative humidity on the flight performance of female Aedes aegypti. *J Insect Physiol* 14:1251–1257. doi:https://doi.org/10.1016/0022-1910(68)90018-8

Scott TW, Amerasinghe PH, Morrison AC, Lorenz LH, Clark GG, Strickman D, Kittayapong P, Edman JD. 2000. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. *J Med Entomol* 37:89–101.

Sotavalta O. 1952. Flight-tone and wing-stroke frequency of insects and the dynamics of insect flight. *Nature* 170:1057–1058.

Stone CM, Tuten HC, Dobson SL. 2013. Determinants of male *Aedes aegypti* and *Aedes polynesiensis* (Diptera: Culicidae) response to sound: efficacy and considerations for use of sound traps in the field. *J Med Entomol* 50:723–730. doi:10.1603/ME13023

Tischner H, Schief A. 1955. Fluggeräusch und schallwahrnehmung bei *Aedes aegypti* L. (Culicidae). *Zool Anz* 18:453–460.

Tripet F, Dolo G, Traoré S, Lanzaro G. 2004. The "Wingbeat Hypothesis" of reproductive isolation between members of the Anopheles gambiae complex (Diptera: Culicidae) does not fly. *J Med Entomol* 41:375–384.

Unwin DM, Corbet SA. 1984. Wingbeat frequency, temperature and body size in bees and flies. *Physiol Entomol* 9:115–121. doi:10.1111/j.1365-3032.1984.tb00687.x

Villarreal SM, Winokur O, Harrington L. 2017. The impact of temperature and body size on fundamental flight tone variation in the mosquito vector *Aedes aegypti* (Diptera: Culicidae): implications for acoustic lures. *J Med Entomol* 54:1116–1121. doi:10.1093/jme/tjx079

Wang R, Hu C, Fu X, Long T, Zeng T. 2017. Micro-Doppler measurement of insect wing-beat frequencies with W-band coherent radar. *Sci Rep* 7:1396–1396. doi:10.1038/s41598-017-01616-4

Wekesa J, Brogdon W, Hawley W, Besansky N. 1998. Flight tone of field-collected populations of *Anopheles gambiae* and *An. arabiensis* (Diptera: Culicidae). *Physiol Entomol* 23:289–294.

Zhang D, Lees RS, Xi Z, Bourtzis K, Gilles JR. 2016. Combining the sterile insect technique with the incompatible insect technique: III—robust mating competitiveness of irradiated triple *Wolbachia*-infected *Aedes albopictus* males under semi-field conditions. *PloS One,* 11:e0151864.