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Multimodal photonic and acoustic stimuli drive sex-specific locomotory responses in Aedes aegypti mosquitos

Raiza D. Sauciri
Eye & ENT Hospital of Fudan University
Andrea Michinaux
Andean Road Countries for Science and Technology
Riya Saini
Andean Road Countries for Science and Technology
Nora Medrano
Laboratorio de Chagas e Inmunoparasitología, Departamento de Biología, Facultad de Ciencias y Tecnología
Marco A. Cabero Z.
editorial@journalasc.org

Andean Road Countries for Science and Technology

Article

Keywords: Aedes aegypti, phototaxis, acoustic response, vector control, dengue, multimodal stimuli

Posted Date: May 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9463922/v1

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Additional Declarations: No competing interests reported.

Abstract

Dengue, Zika, chikungunya, and yellow fever are mosquito-borne viral infections affecting approximately 100 million people annually (Bhatt et al., 2013). Mosquito-borne viruses pose a continuing threat to public health. The presence of the urbanized mosquito *Aedes aegypti*, carrier of the four aforementioned diseases, indicates that future outbreaks of emerging diseases transmitted by mosquitos may be on the horizon. A comprehensive understanding of mosquito behaviour is essential for the development of novel tools for vector control. Here, using behavioral assays with a multimodal approach and photogrammetry, we report the effect of two independent stimuli on *Aedes aegypti* mosquitos. We found that negative phototaxis in pupae was modulated by photonic energy (one-way ANOVA: $F(12, 143) = 1353$, $p < 0.001$). For adult mosquitos, photonic stimuli from 2.1 to 2.2 eV produced a remarkable negative phototaxis differentiated by dietary intake. Unfed males showed 75.7% negative phototaxis compared to 42.3% in fed males ($\chi^2 = 38.47$, $df = 1$, $p < 0.001$, OR = 4.24, 95% CI: 2.76–6.52). Unfed females showed 65.0% negative phototaxis compared to 47.8% in fed females ($\chi^2 = 8.19$, $df = 1$, $p = 0.004$, OR = 2.03, 95% CI: 1.27–3.23). These results indicate that a device emitting 2.1–2.2 eV light combined with 320 Hz tones could selectively target male mosquitos, suggesting potential for male-targeted control strategies. Acoustic stimuli produced locomotory reactions of the hind legs only in adult male mosquitos at 320 ± 10 Hz (68.75% response rate; Fisher's exact test, $p < 0.001$). These findings have important implications for the development of new, non-chemical mosquito control devices exerting phototaxis and acoustic cues, prompting next steps to evaluate multimodal prototypes in field settings.

Introduction

Dengue, Zika, chikungunya, and yellow fever are acute systemic viral infections transmitted by the mosquito *Aedes aegypti* (Simmons et al., 2012). In 2010, there were approximately 100 million mosquito-borne viral infections worldwide (Bhatt et al., 2013). Bolivia in particular experienced a 370% increase in reported cases of dengue in 2020 compared to 2019, affecting more than 7,000 people (Escalera-Antezana et al., 2018). On the other hand, chikungunya showed high incidence rates estimated at 30.47 cases per 100,000 inhabitants (Escalera-Antezana et al., 2018). Existing disease prevention strategies are based on reducing the mosquito vector population utilizing insecticides; however, there is an alarming increase in the strength and distribution of behavioural resistance to insecticides within vector populations, compromising the long-term usefulness of these control measures in halting mosquito transmission in endemic countries (Garcia et al., 2020; Escalera-Antezana et al., 2018). Recent data reveals that Ae. aegypti populations have developed high-intensity resistance to pyrethroids, with field populations exhibiting deltamethrin resistance ratios as high as 249-fold and permethrin resistance exceeding 500-fold (Hernandez & Pietrantonio, 2025). Furthermore, the frequency of knockdown resistance (kdr) mutations, such as V1016G and F1534C, now exceeds 90% in highly resistant populations, while resistance to organophosphates like malathion and temefos has become extensive across regions such as Southeast Asia. This alarming escalation in physiological resistance strengthens the urgent need for alternative, behavior-based control technologies that do not rely on chemical toxicity.

The increasing trend of dengue cases reported in the Americas underscores the urgent need for preparedness (PAHO, 2014), particularly as *Aedes aegypti* has become urbanized (Kraemer et al., 2019). A recent study suggests that climate change will expose half of the world's population to disease-spreading mosquitos by 2050 (Kraemer et al., 2019); therefore, further mechanisms of vector control must be developed.

Mosquito-borne diseases have been associated with neurological disorders such as Guillain-Barré syndrome in adults (Oehler et al., 2014) and microcephaly in infants (Cordeiro et al., 2016). Mosquito-borne viral infections have been circulating intensively in the tropical regions of Bolivia, where seroprevalence remains limited, and the density of *Aedes aegypti* populations allows greater spread of diseases (Saba Villarroel et al., 2018). The acceleration of the spread of arboviruses, such as Zika virus (ZIKV) and others, underlines the need for more proactive mosquito surveillance to contain arbovirus outbreaks (Fernandes et al., 2018). There is intense debate on the effectiveness of mosquito population control through fumigation. The combined pathogen monitoring and detection programmes currently in place in low- and middle-income countries are essential, as well as the development of new strategies and mechanisms to halt future outbreaks (Fernandes et al., 2018).

Mosquitos are very sensitive to external stimuli (Carnaghi et al., 2021). Cator et al. (2009) reported the mating behavior of mosquitos and the acoustic interactivity between genders at frequencies considered to be higher than their auditory range, and suggested reviewing the widely accepted limits of mosquito acoustic behaviour. The study demonstrated that mosquitos have an auditory range of 125–2000 Hz, as revealed in the neurophysiological study conducted on the Johnston's organ (JO; receiving antennae) through laser vibrometry and tungsten electrodes. These results refuted the myth that female mosquitos are deaf and that males have only an auditory range of 300–800 Hz (Cator et al., 2009).

In the case of external photonic stimuli, some reports showed the impact of photonics on the circadian timing system of mosquitos (Clements, 1963; Clements, 1999). Others have shown that photonic cues can shape 24-hour gene expression profiles (Rund et al., 2011; Rund et al., 2013) and mosquito flight activity (Rund et al., 2012; Gatton et al., 2013; Rowland, 1989). Leftwich (1954) experimented with a 90 candle-power incandescent lamp (~ 970 lumens) positioned at various distances from mosquito larvae containers. The results showed that if the lamp was moved more than one foot away, there was no response from the larvae. The research concluded that an intensity of ~ 970 lumens at one foot of distance was approximately the minimum perceptible intensity of light that affected the larvae (Leftwich, 1954). Further observations showed that larvae submerged when photonic intensity was reduced. In these experiments, the behavior of the larvae did not necessarily indicate that they could distinguish between different colors, but rather that they perceived the reduction of brightness. After approximately twelve submergent responses, the reflex stopped due to fatigue of the retinal pigment of the optic nerve, in which case a recovery time of 5 to 10 minutes was required (Leftwich, 1954). In addition, Holmes (1911) noted that there was a negative phototaxis element in the behaviour of larvae when stimulated by shadows; otherwise, they may show positive phototaxis. Finally, another experiment exposed mosquitos to white light at different intervals during dusk, dawn, and night. The study showed a dramatic reduction

in mosquito bites (Sheppard et al., 2017). Although these results provide information on the behaviour of mosquitos under photonic stimuli, further exploration is required on the locomotory reactions to different photonic energies of the electromagnetic spectrum.

Understanding mosquito locomotory response to specific external stimuli could be used to improve existing vector control tools or serve as a basis for new behaviour-oriented interventions to prevent the rapid spread of vector-borne viruses. In Latin America, where insecticide resistance is particularly widespread and resources for vector control are limited, such behavior-based approaches offer a sustainable and cost-effective alternative. Here, we begin to address this problem by combining the experimental characterization of the impact of photonic and acoustic stimuli. We generated estimates of the impact of photonic and acoustic stimuli on mosquito behaviour that can be used to inform future field trials and guide the development of electronic devices based on phototaxis and acoustic cues. This study represents an investigation of the capability of photonic stimuli to produce non-invasive phototaxis in mosquitos. Two-dimensional photogrammetry was used to analyze the locomotory response of free-moving mosquitos. The study was performed using male and female *Aedes aegypti* in the pupal and adult stages. Photonic and acoustic responses were assessed by changes in locomotory reactions and quantified over a wide range of photonic and acoustic frequencies. Our results show different reactions to photonic and acoustic cues differentiated by sex and stage of development.

Results

To evaluate the effectiveness of photonic and acoustic stimuli on mosquito behavior, a series of systematic experiments were conducted in four cohorts composed of adult males, adult females, mixed adults, and pupae. Each cohort initially contained approximately 100 mosquitos, with mosquitos reused across multiple tests. This repeated-measures design may introduce non-independence; future studies should use independent cohorts (see Methods for details on reuse protocols). Adult mosquitos were kept in cages measuring 50 × 50 × 50 cm^3. All photonic and acoustic stimulus assays were repeated six times per day for three weeks (four days per week). Acoustic stimulus assays consisted of sweeping frequencies from 100 to 1200 Hz at 10 Hz intervals. Photonic stimulus assays consisted of the application of photonic energies in the range of 1.77 to 4.9 eV. A total of 192 tests were performed, releasing an average of 80.2 ± 0.8 (SEM) mosquitos per test, using a total of 15,360 mosquitos across all replicates (each mosquito was used in multiple tests with a minimum 24-hour rest period between tests to prevent habituation).

Vector behavioral response assays

Behavioral tests using acoustic stimuli were performed on all four cohorts, one cohort at a time. The frequency was swept from 100 to 1200 Hz at an intensity of 40 dB SPL (A-weighted) measured at 0.5 m. The sampling frequency was 10 Hz, although 5 Hz steps were used in the 150–350 Hz range, as mosquitos are more sensitive to these frequencies (Menda et al., 2019). No locomotory reactions were detected in pupae or female adult mosquitos (n = 50 pupae, n = 50 females across all tests). However,

male mosquitos raised their hind legs when the frequency reached 320 ± 10 Hz (n = 160 males). This response was observed in 68.75% of male mosquitos tested (110 of 160 individuals; Fisher's exact test, $p < 0.001$). Male mosquitos adjusted the height of their legs as acoustic intensity was varied from 40 dB to 25 dB at this frequency. This response may be related to neural modulation mechanisms that produce large-amplitude oscillations selectively in male mosquitos, indicating a sexually dimorphic auditory mechanism (Su et al., 2018).

Behavioral response to photonic stimuli

Behavioral tests using photonic stimuli were performed on four cohorts, one cohort at a time. Figure 1 shows the results of the application of different photonic energies to pupae. We found that pupal speed was significantly modulated by photonic energy (one-way ANOVA: $F(12, 143) = 1353$, $p < 0.001$). Pupal speed decreased from a baseline of 1.42 ± 0.11 cm/s under dark conditions to a minimum of 0.31 ± 0.04 cm/s at 3.2 eV (Tukey HSD post-hoc: $p < 0.001$ compared to baseline), showing a U-shaped response curve. Pupae exhibited negative phototaxis across all photonic energies tested, with no evidence of positive phototaxis at any energy level.

For adult mosquito cohorts (Fig. 2), photonic stimuli from 2.1 to 2.2 eV produced negative phototaxis with a pronounced effect on unfed mosquitos. Unfed males showed negative phototaxis in 75.7% of trials (174 of 230 individuals), compared to 42.3% in fed males (55 of 130 individuals; $\chi^2 = 38.47$, $df = 1$, $p < 0.001$, odds ratio = 4.24, 95% CI: 2.76–6.52). Unfed females showed negative phototaxis in 65.0% of trials (130 of 200 individuals), compared to 47.8% in fed females (55 of 115 individuals; $\chi^2 = 8.19$, $df = 1$, $p = 0.004$, odds ratio = 2.03, 95% CI: 1.27–3.23).

Photonic energies from 1.8 to 2.0 eV and 2.3 to 2.4 eV did not activate significant phototaxis in adult mosquitos (all $p > 0.05$ compared to baseline). In the region from 3.4 to 4.9 eV, adult mosquitos showed positive phototaxis, with maximum attraction observed at 4.1 eV.

Discussion

We investigated the behavioral responses of *Aedes aegypti* to photonic and acoustic stimuli, revealing distinct, stimulus-specific patterns that vary by sex and developmental stage. Our findings highlight the potential of multimodal sensory manipulation as a basis for novel vector control strategies.

For acoustic stimuli, we observed a locomotory response—specifically hind leg elevation—exclusively in male mosquitos at approximately 320 Hz, with a response rate of 68.75%. This sexually dimorphic behavior is consistent with known differences in the structure and function of the Johnston’s organ (JO). Male mosquitos possess a more complex efferent innervation network within the JO, which enhances auditory sensitivity and enables detection of subtle frequency cues associated with mating behavior. In contrast, the reduced innervation observed in females likely limits their responsiveness to such stimuli. The frequency range identified in this study overlaps with previously reported ranges associated with

wingbeat harmonics and mating communication, suggesting that the observed response may reflect an underlying sensory bias linked to reproductive behavior. This raises the possibility that acoustic stimulation could be used to selectively interfere with male activity patterns or mating dynamics, although this hypothesis requires direct experimental validation.

Regarding photonic stimuli, we identified a narrow energy window (2.1–2.2 eV) that elicited strong negative phototaxis in adult mosquitos, particularly in unfed individuals. This finding is consistent with previous reports indicating wavelength-dependent phototactic behavior in mosquitos. The specificity of this response may be explained by the spectral sensitivity of mosquito photoreceptors, which are tuned to particular regions of the visible spectrum. The observed differences between fed and unfed mosquitos suggest that physiological state plays a role in modulating sensory responsiveness. One possible explanation is that unfed mosquitos exhibit heightened sensitivity to environmental cues as part of host-seeking behavior, leading to stronger avoidance or attraction responses depending on the stimulus.

In contrast, higher photonic energies (3.4–4.9 eV) induced positive phototaxis, indicating a bidirectional behavioral response depending on energy level. This dual response pattern suggests that mosquito phototaxis is not a simple binary mechanism but rather a graded response governed by both spectral properties and internal physiological conditions. Differences between our findings and previous studies, such as the slight shift in optimal energy for positive phototaxis, may be attributable to variations in waveform characteristics, including the presence or absence of alternating current (AC) components and duty cycles in light emission.

Importantly, our results demonstrate that both acoustic and photonic stimuli can independently modulate mosquito behavior in predictable ways. While we did not test combined stimuli in this study, the potential for synergistic or antagonistic interactions between sensory modalities represents an important avenue for future research. Multimodal approaches may enhance selectivity and efficiency in behavior-based control systems.

From an applied perspective, these findings support the feasibility of developing non-chemical vector control tools based on sensory manipulation. Devices that exploit specific photonic energies and acoustic frequencies could potentially target particular subsets of mosquito populations, such as males or host-seeking females. However, it is important to emphasize that our results are based on controlled laboratory conditions, and their translation to field settings will require careful validation. Environmental variables, including ambient light, competing stimuli, and spatial heterogeneity, may significantly influence behavioral responses in natural contexts.

Overall, this study provides experimental evidence that *Aedes aegypti* exhibits measurable and stimulus-specific locomotory responses to both photonic and acoustic cues. These findings contribute to a growing body of research exploring behavior-based vector control and lay the groundwork for future studies aimed at optimizing and integrating multimodal interventions.

Practical Applications for Latin America

Based on these findings, we recommend: (1) field trials of 2.1–2.2 eV light traps combined with 320 Hz acoustic emissions in Bolivian communities, (2) integration of these behavior-based devices with existing insecticide-based programs as part of integrated vector management strategies, and (3) technology transfer to local manufacturing partners in Latin America to ensure affordable and sustainable production. Given the widespread insecticide resistance documented in Ae. aegypti across Latin America (Hernandez & Pietrantonio, 2025), these non-chemical approaches are particularly timely and relevant for the region.

Limitations

Several limitations of this study should be acknowledged. First, laboratory conditions may not fully represent field behavior, as mosquitos in natural settings encounter additional environmental variables such as wind, humidity gradients, and competing stimuli. Second, our 2D photogrammetry underestimates true speed because the velocity vector in the third dimension was not captured; however, our analysis based on relative changes in speed before and after stimulus presentation mitigates this limitation. Third, acoustic testing used rectangular waveforms exclusively; sine waves or other waveforms may produce different behavioral responses. Fourth, we did not test combined photonic and acoustic stimuli simultaneously; synergistic or antagonistic effects between modalities remain unknown. Fifth, mosquitos were obtained from a single colony in Bolivia; geographic variation in sensory responses may exist across different populations. Sixth, the reuse of mosquitos across multiple tests (with 24-hour rest periods) may introduce potential habituation effects despite rest periods. Finally, sample size calculations were based on pilot study effect sizes, and future studies should confirm these findings with independent cohorts.

Conclusion

We determined the relative importance of photonic cues in mediating the behavior of Aedes aegypti. These findings provide a proof-of-concept for sensory-based vector control approaches under laboratory conditions. The variety of locomotory reactions produced by the application of external photonic energies could be used to design devices for attracting or repelling mosquitos. As a perspective for the future, it is desirable to assess acoustic and photonic stimuli with different waveforms and duty cycles. Given the substantial sensitivity of male mosquitos to the acoustic frequency of approximately 320 Hz, a potential acoustic circadian control, modulated by the efferent innervation of the male JO, is an intriguing possibility to control the ecologically flexible Aedes aegypti, which shows less strict circadian behavior than other species (South & Catteruccia, 2016).

The controlled combination of external stimuli alongside other interventions could enable a bespoke approach to control mosquito behavior and therefore transmission of viral infections. A multimodal approach was used to facilitate quantification of the locomotory response of mosquitos to photonic and

acoustic stimuli. Understanding mosquito responses to specific cues provides insights into behaviors that could be exploited to improve existing control tools or serve as a basis for new behavior-based interventions in a variety of epidemiological and socio-economic contexts to avoid the consequences of mosquito-borne disease outbreaks.

As an immediate next step, we aim to translate these laboratory findings into practical applications by developing scalable prototypes that combine both photonic (2.1–2.2 eV) and acoustic (320 Hz) stimuli in a single device. While light-based traps and acoustic lures exist independently, no current device integrates both modalities to exploit the sex-specific and state-dependent responses identified in this study. Such a multimodal trap could selectively target host-seeking females (via light) and mating-competent males (via sound), potentially enhancing field efficacy. We plan to deploy and evaluate these prototypes in real-world settings in collaboration with the Laboratorio de la Escuela Técnica de Salud to validate their efficacy in reducing local vector populations in Bolivia and other Latin American countries.

Materials and Methods

Mosquito rearing

Aedes aegypti mosquitos were reared in the insectarium of the Laboratorio de la Escuela Técnica de Salud, Cochabamba, Bolivia, under identical conditions: 30.5°C ± 1°C, 60% relative humidity, and exposed to light from 9:00 AM to 5:00 PM daily. The larvae were fed with TetraMin tropical flakes (Tetra Melle). They were then transferred to a container of 30 × 20 × 5 cm^3 during the pupal stage. Pupae were transferred into cages (50 × 50 × 50 cm^3) for emergence. Adults were allowed to mate for 3 to 4 days and received 10% sugar ad libitum solution. In our experiments, separate cohorts of mosquitos were reared at the same time. The first cohort consisted of mixed unfed female and male adults (Cohort 1), the second consisted only of male adults (Cohort 2), the third consisted only of female adults (Cohort 3), and the fourth consisted of pupae (Cohort 4). Acoustic stimuli were applied from 5:00 PM to 10:00 PM. Photonic stimuli were applied from 5:00 PM to 9:00 PM, as previous studies have shown that mosquitos are very active during dusk (Dao et al., 2008; Panicker & Rajagopalan, 1984; Yuval, 2006; Diabate et al., 2009). The tests were performed on days 5–8 after adult mosquito emergence.

Study design

This was a prospective observational study that used photonic and acoustic stimuli to assess mosquito behavior. Sample size was determined by power analysis ($\beta = 0.80$, $\alpha = 0.05$) based on pilot study effect sizes (Cohen's $d = 0.6$ for phototaxis response, $d = 0.8$ for acoustic response), yielding a minimum required sample size of 45 mosquitos per group. We oversampled to approximately 100 mosquitos per cohort to account for potential mortality and non-response. The study covered one dengue season (four weeks from December to January). The pre-determined hypothesis was that photonic and acoustic stimuli influence the locomotory reaction of mosquitos. All laboratory assays were carried out with the assistance of the technicians of the insectarium of the Laboratorio de la Escuela Técnica de Salud, Cochabamba. Studies were carried out using pupae from outcrossed colonies maintained at the

Laboratorio de la Escuela Técnica de Salud. The colonies were maintained at population sizes of 100 with a 50:50 sex ratio. Each mosquito was used in multiple tests across the study period, with a minimum of 24 hours between tests to prevent habituation. A total of 15,360 mosquito observations were recorded across 192 tests, with each test using approximately 80 mosquitos (mean 80.2 ± 0.8 SEM).

Experimental setup

Acoustic stimuli. Rectangular acoustic waves were generated by combining a function generator (HP/Agilent 33120A-001-RFRB) and a 25W sound generator (DROK 3 inches Square Shape). Typically, approximately 100 flying mosquitos were present in each cage. A function generator sent a rectangular signal to the sound generator. Sound intensity was measured by a calibrated decibel meter (Keysight U8903A-200). The tests were performed in a closed room with a background noise level of 28.4 ± 2.1 dB (measured with the decibel meter under identical conditions). To avoid echoes from the mesh cage of adult mosquitos, the speakers were installed inside the cage, leaving two leads open for electrical connection to the signal generator. For pupae, the speakers were installed 5 cm from the longest edge of the container.

Photonic stimuli. The setup is illustrated in Fig. 3. Photonic energy was emitted by a multicolored Wi-Fi light bulb (LIFX Color 60W equivalent controlled lamp). Different photonic energies were calibrated using a StellarNet Inc. Greenwave spectrometer and adjusted by a Wi-Fi application on a smartphone provided by the manufacturer. The intensity of photonic energy was measured using a calibrated light meter (RS PRO IM720). The tests were conducted in darkness (~ 0 lumens). The bulb was placed vertically inside the cage and switched off and on at 1-minute intervals with a 5-minute break between each assay. The light bulb was placed vertically in four different locations 0.3 m from the corner of the pupae container.

Test conditions

Acoustic stimuli. To characterize the mosquito response to acoustic stimuli, the signal frequency was swept from 100 to 1200 Hz at a fixed intensity level of 40 dB SPL (A-weighted) measured at 0.05 m from a speaker located in the center of the cage. We selected a lower acoustic intensity for this study to prevent any damage to the mosquitos' JO (Johnston, 1855). The frequency was swept from 100 to 1200 Hz with a step of 10 Hz for 10 seconds with an interval of 5 seconds between steps to complete one cycle. This cycle was repeated three times. Then, the frequency was swept from 150 to 350 Hz with a step of 5 Hz for 10 seconds with an interval of 5 seconds between steps to complete another cycle that was repeated three times. Each cohort received sufficient time (> 5 minutes) to recover after a cycle of exogenous stimuli. Six cycles were carried out each day over four days with each cohort.

Photonic stimuli. To characterize the sensitivity of mosquitos to photonic stimuli, photonic energy was varied according to the sequence shown in Fig. 3. We chose an LED bulb to avoid infrared radiation (IR). Moreover, the low power of the LED produced no change in photonic energy due to temperature change at the junction, which was critical to ensure experimental reliability. Each photonic energy was applied for 30 seconds with 30 seconds rest. Photonic energy was swept from 1.8 to 4.9 eV to complete one

cycle. Mosquitos were given sufficient time (~ 5 minutes) to recover after application of one cycle of exogenous stimuli. Six cycles were carried out each day over four days with each cohort.

Data processing

Figure 1 shows the estimated speed (cm/s) of pupae in the container after application of different photonic stimuli. One hundred frames were obtained over a span of 10 seconds at 10 frames per second using a Nikon D7500 camera. We assembled the results of photogrammetry from six independent tests each day. The estimated results underestimate true speed, as the velocity vector in the third dimension was not captured. However, we based our analysis on the relative change in speed before and after introduction of photonic stimuli, and this normalized velocity was not affected by underestimation of true speed.

Algorithm for detecting speed of pupae by photogrammetry

To estimate pupal speed, we used 2D photogrammetry. The algorithm counted pixels between two frames (in the x-axis and y-axis) to obtain displacement *d* (the result of subtracting the position of a given particle in frame x ~ n~ from that of the same particle in frame x ~ n-1~). The pixel-to-distance ratio was predefined. The frames had a time interval *t* set during Nikon D7500 configuration, approximately 100 ms each. Using the speed definition v = d/t, we estimated pupal speed under different photonic stimuli. Markers in four corners of the cage and containers were used as reference points to align frames and avoid pixel position errors. To correct the effects of camera angle, the algorithm from: https://www.pyimagesearch.com/2014/08/25/4-point-opencv-get-perspective-transform-example/ was used to achieve high accuracy. Additional post-image processing was performed to minimize image noise by creating brighter and sharper images. The mask was defined, as well as threshold and binarization parameters. Background was removed and pupal positions were extracted.

Algorithm for detecting locomotory response to acoustic stimuli by photogrammetry

Using a method similar to that proposed for pupae, we detected the response to acoustic stimuli in male mosquitos at a frequency of 320 Hz. The image processing filter was set to detect narrower body parts. The center of mass (red dot) was assigned to a fixed part of the mosquito (the thorax). The hind legs were assigned a blue dot. The position of both dots was then compared. Movement of the third pair of legs was detected on stationary mosquitos and could also be appreciated by visual examination.

Statistical analysis

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2022). Normality of data was assessed using the Shapiro-Wilk test. Homogeneity of variances was confirmed using Levene's test. For pupal speed data across photonic energies, one-way analysis of variance (ANOVA) was performed followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for multiple comparisons. Effect

size (eta-squared) was calculated manually as the ratio of sum of squares between groups to total sum of squares. For adult phototaxis data, chi-square tests of independence were performed with Yates' continuity correction for 2×2 tables comparing response rates between unfed and fed mosquitos for each sex separately. Odds ratios and 95% confidence intervals were calculated using standard methods. For acoustic response data (response present/absent across sexes), Fisher's exact test was used due to small expected frequencies. All tests were two-tailed with a significance level of $\alpha = 0.05$.

Data and code availability

All data and analysis scripts supporting the findings of this study are publicly available on Zenodo at https://doi.org/10.5281/zenodo.19967980

Declarations

Acknowledgements

We thank Hugo Albarracin Veizaga and his team at the Laboratorio de Entomología Médica de la Escuela Técnica de Salud, Cochabamba, Bolivia, for their feedback and assistance with the experiments. We also thank the technicians of the insectarium for their dedicated support in mosquito rearing and maintenance.

Funding

This work was supported by the Andean Road Countries for Science and Technology (ARCST), Cochabamba, Bolivia (Grant No. 2023BIOBOL155).

Contributions:

M.A.C.Z.: Conceptualization, Methodology, Software, Writing, original draft; N.M.M.: Conceptualization, Investigation, Resources; R.D.S.: Visualization, Writing, review & editing; A.M.: Formal analysis, Validation; R.S.: Writing, review & editing, Project administration. All authors approved the final manuscript.

Competing interests:

The authors declare no competing interests.

Ethics declaration:

This study involved only invertebrate animals (mosquitos), which do not require ethical approval under Bolivian and international guidelines. All procedures followed institutional biosafety protocols.

References

1. Bhatt, S. et al. The global distribution and burden of dengue. *Nature* **496** (7446), 504–507 (2013).

2. Bidlingmayer, W. L. How mosquitos see traps: Role of visual responses. *J. Am. Mosq. Control Assoc.* **10** (2), 272–279 (1994).
3. Boo, K. S. & Richards, A. G. Fine structure of the scolopidia in the Johnston's organ of male *Aedes aegypti* (L.) (Diptera: Culicidae). *Int. J. Insect Morphol. Embryol.* **4** (6), 549–566 (1975a).
4. Boo, K. S. & Richards, A. G. Fine structure of scolopidia in Johnston's organ of female *Aedes aegypti* compared with that of the male. *J. Insect. Physiol.* **21** (5), 1129–1139 (1975b).
5. Browne, S. M. & Bennett, G. F. Response of mosquitos (Diptera: Culicidae) to visual stimuli. *J. Med. Entomol.* **18** (6), 505–521 (1981).
6. Carnaghi, M., Belmain, S. R., Hopkins, R. J. & Hawkes, F. M. Multimodal synergisms in host stimuli drive landing response in malaria mosquitos. *Sci. Rep.* **11** (1), 7379 (2021).
7. Cator, L. J., Arthur, B. J., Harrington, L. C. & Hoy, R. R. Harmonic convergence in the love songs of the dengue vector mosquito. *Science* **323** (5917), 1077–1079 (2009).
8. Clements, A. N. *The physiology of mosquitos* (Pergamon, 1963).
9. Clements, A. N. *The biology of mosquitos: Vol. II. Sensory reception and behaviour* (CABI Publishing, 1999).
10. Cordeiro, M. T., Pena, L. J., Brito, C. A., Gil, L. H. & Marques, E. T. Positive IgM for Zika virus in the cerebrospinal fluid of 30 neonates with microcephaly in Brazil. *Lancet* **387** (10030), 1811–1812 (2016).
11. Dao, A. et al. Assessment of alternative mating strategies in Anopheles gambiae: Does mating occur indoors? *J. Med. Entomol.* **45** (4), 643–652 (2008).
12. Diabate, A. et al. Spatial swarm segregation and reproductive isolation between the molecular forms of Anopheles gambiae. *Proceedings of the Royal Society B: Biological Sciences, 276*(1676), 4215–4222. (2009).
13. Escalera-Antezana, J. P., Murillo-García, D. R. & Rodriguez-Morales, A. J. Chikungunya in Bolivia: Still a neglected disease? *Arch. Med. Res.* **49** (4), 288 (2018).
14. Fernandes, J. N. et al. Rapid, noninvasive detection of Zika virus in *Aedes aegypti* mosquitos by near-infrared spectroscopy. *Sci. Adv.* **4** (5), eaat0496 (2018).
15. Garcia, G. A., Hoffmann, A. A., Maciel-de-Freitas, R. & Villela, D. A. M. *Aedes aegypti* insecticide resistance underlies the success (and failure) of Wolbachia population replacement. *Sci. Rep.* **10** (1), 63 (2020).
16. Gatton, M. L. et al. The importance of mosquito behavioural adaptations to malaria control in Africa. *Evolution* **67** (4), 1218–1230 (2013).
17. Göpfert, M. C., Briegel, H. & Robert, D. Mosquito hearing: Sound-induced antennal vibrations in male and female Aedes aegypti. *J. Exp. Biol.* **202** (20), 2727–2738 (1999).
18. Hernandez, J. R. & Pietrantonio, P. V. Pyrethroid resistance in Aedes aegypti: Genetic mechanisms worldwide, and recommendations for effective vector control. *Parasites Vectors*. **18** (1), 409 (2025).

19. Holmes, S. J. The reactions of mosquitos to light in different periods of their life history. *Anim. Behav.* **1** (1), 29–32 (1911).
20. Johnston, C. Original communications: Auditory apparatus of the Culex mosquito. *Journal of Cell Science*, *s1-3*(9), 97–102. (1855).
21. Kraemer, M. U. G. et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and Aedes albopictus. *Nat. Microbiol.* **4** (5), 854–863 (2019).
22. Lapshin, D. N. & Vorontsov, D. D. Frequency organization of the Johnston's organ in male mosquitos. *J. Exp. Biol.* **220** (21), 3927–3938 (2017).
23. Leftwich, A. W. Vision and blindness in mosquito larvae as indicated by the submerging reflex. *Physiol. Entomol.* **29** (3), 156–162 (1954).
24. Liu, Y. N. et al. Enhancement of mosquito trapping efficiency by using pulse width modulated light emitting diodes. *Sci. Rep.* **7** (1), 40074 (2017).
25. Menda, G. et al. The long and short of hearing in the mosquito Aedes aegypti. *Curr. Biol.* **29** (4), 709–714e4 (2019).
26. Oehler, E. et al. Zika virus infection complicated by Guillain-Barré syndrome: Case report, French Polynesia. *Eurosurveillance* **19** (9), 20720 (2014).
27. Pan American Health Organization. *Five-fold increase in dengue cases in the Americas over the past decade.* (2014)., December 3 https://www.paho.org/hq/index.php?option=com_content&view=article&id=9657:2014-los-casos-dengue-americas-
28. Panicker, K. N. & Rajagopalan, P. K. Field observations on the swarming and the mating behaviour of Anopheles subpictus Grassi 1899. *Indian J. Med. Res.* **80**, 60–62 (1984).
29. Rowland, M. Changes in the circadian flight activity of the mosquito Anopheles stephensi associated with insemination, blood-feeding, oviposition and nocturnal light intensity. *Physiol. Entomol.* **14** (1), 77–84 (1989).
30. Rund, S. S. C., Gentile, J. E. & Duffield, G. E. Extensive circadian and light regulation of the transcriptome in the malaria mosquito Anopheles gambiae. *BMC Genom.* **14** (1), 218 (2013).
31. Rund, S. S. C., Hou, T. Y., Ward, S. M., Collins, F. H. & Duffield, G. E. Genome-wide profiling of diel and circadian gene expression in the malaria vector Anopheles gambiae. *Proceedings of the National Academy of Sciences*, *108(*1), 421–430. (2011).
32. Rund, S. S. C., Lee, S. J., Bush, B. R. & Duffield, G. E. Strain- and sex-specific differences in daily flight activity and the circadian clock of Anopheles gambiae mosquitos. *J. Insect. Physiol.* **58** (12), 1609–1619 (2012).
33. Saba Villarroel, P. M. et al. Zika virus epidemiology in Bolivia: A seroprevalence study in volunteer blood donors. *PLoS Negl. Trop. Dis.* **12** (3), e0006239 (2018).
34. Sheppard, A. D. et al. Light manipulation of mosquito behaviour: Acute and sustained photic suppression of biting activity in the Anopheles gambiae malaria mosquito. *Parasites Vectors*. **10** (1), 255 (2017).

35. Shimoda, M. & Honda, K. I. Insect reactions to light and its applications to pest management. *Appl. Entomol. Zool.* **48** (4), 413–421 (2013).
36. Simmons, C. P., Farrar, J. J., Nguyen, V. V. & Wills, B. Dengue. *N. Engl. J. Med.* **366** (15), 1423–1432 (2012).
37. South, A. & Catteruccia, F. Sexual selection and the evolution of mating systems in mosquitos. In (ed Raikhel, A. S.) Progress in mosquito research (Vol. 51, 67–92). Academic. (2016).
38. Su, M. P., Andrés, M., Boyd-Gibbins, N., Somers, J. & Albert, J. T. Sex and species specific hearing mechanisms in mosquito flagellar ears. *Nat. Commun.* **9** (1), 3911 (2018).
39. Yuval, B. Mating systems of blood-feeding flies. *Ann. Rev. Entomol.* **51** (1), 413–440 (2006).

Figures

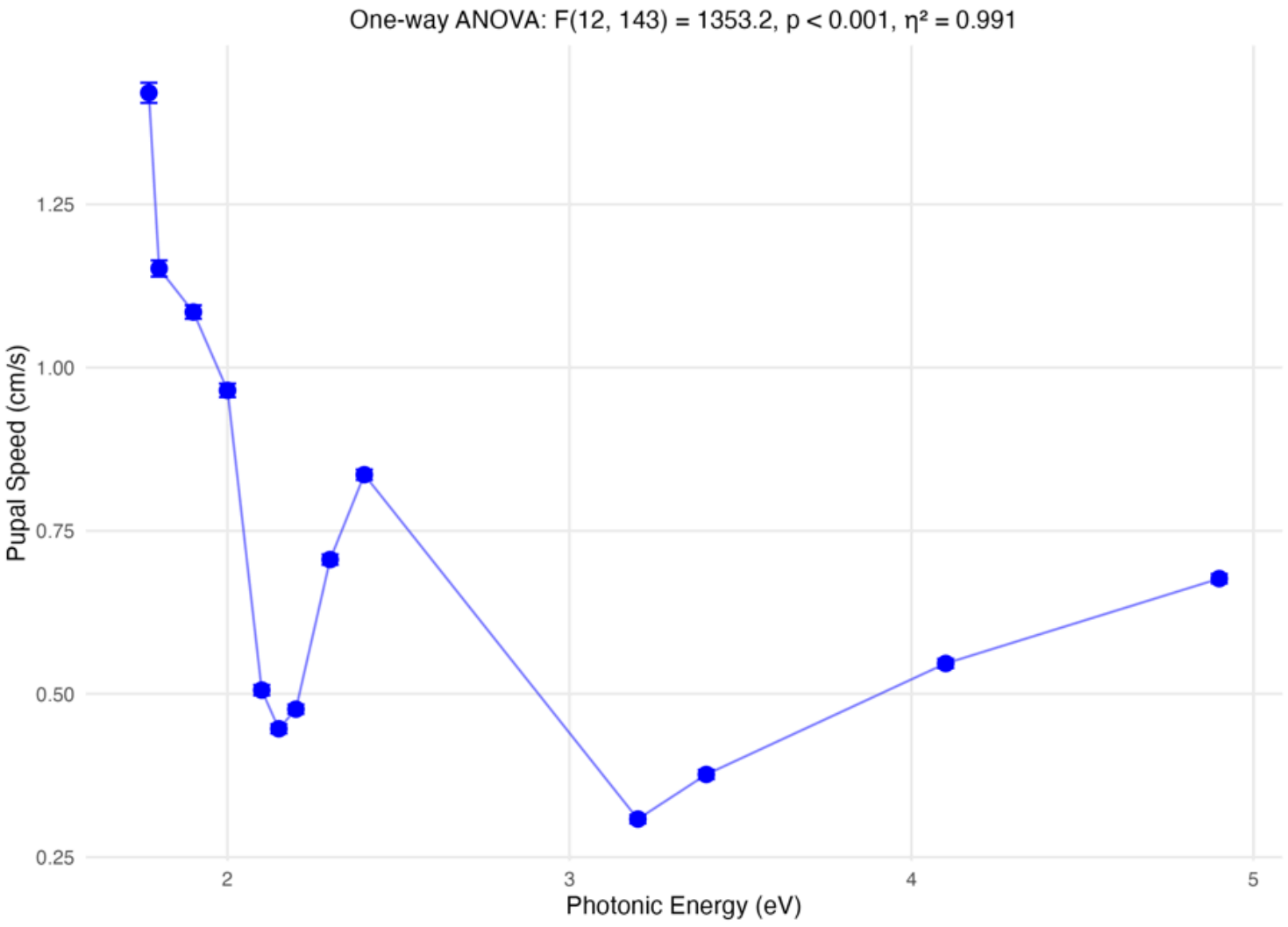


Figure 1

Pupal speed (cm/s, mean ± SEM) as a function of photonic energy (eV). Pupal mobility was significantly modulated by photonic energy, showing negative phototaxis across all energies tested with minimum

speed at 3.2 eV. n = 6 tests per energy level, with approximately 80 pupae per test. One-way ANOVA: $F(12, 143) = 1353$, $p < 0.001$. Different letters indicate significant differences at $p < 0.05$ (Tukey HSD).

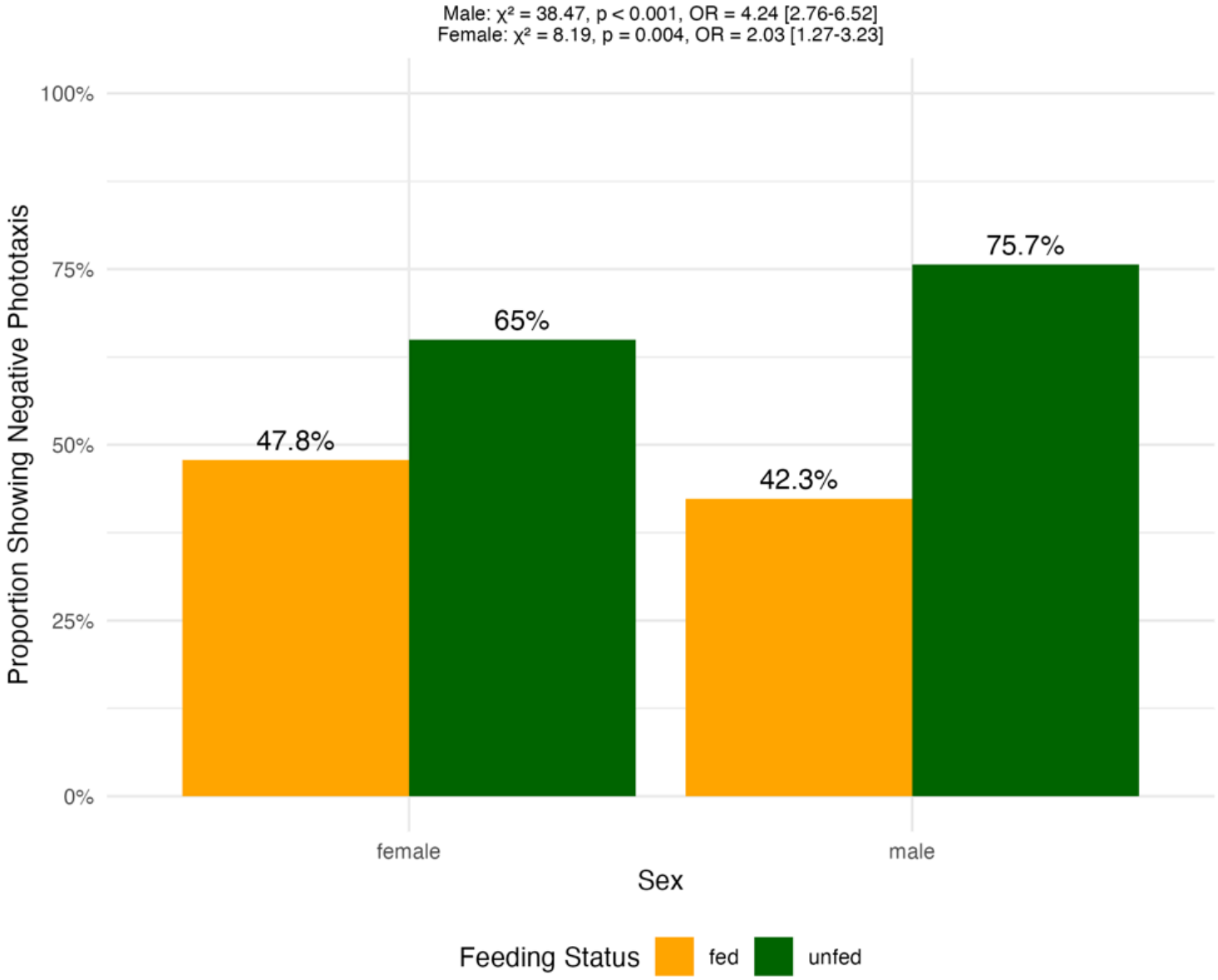


Figure 2

Percentage of adult mosquitos exhibiting negative phototaxis at 2.1-2.2 eV. Unfed males: 75.7% (174/230); fed males: 42.3% (55/130); unfed females: 65.0% (130/200); fed females: 47.8% (55/115). Male comparison: $\chi^2 = 38.47$, df = 1, $p < 0.001$, OR = 4.24 [2.76-6.52]; Female comparison: $\chi^2 = 8.19$, df = 1, $p = 0.004$, OR = 2.03 [1.27-3.23]. ***$p < 0.001$, **$p < 0.01$.

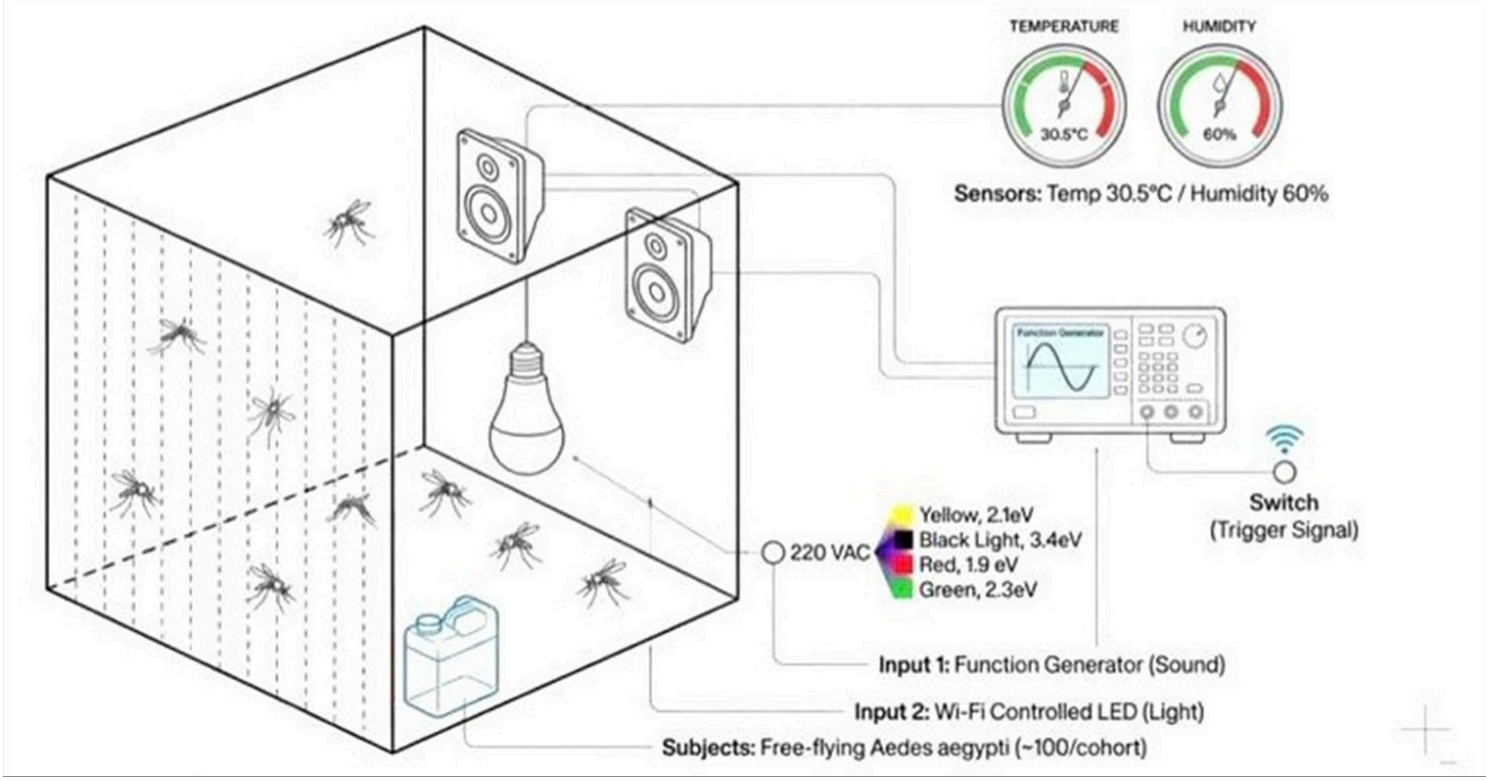


Figure 3

Experimental system for multimodal stimuli. The same setup was used to deliver both acoustic stimuli (via speakers) and photonic stimuli (via LED light). Temperature and humidity were kept constant. A digital camera recorded mosquito locomotory responses for 10 seconds during stimulus presentation.