Applied Acoustics 109 (2016) 54–60

Contents lists available at ScienceDirect

Applied Acoustics

journal homepage: www.elsevier.com/locate/apacoust

Technical note

Measuring the fundamental frequency and the harmonic properties of the wingbeat of a large number of mosquitoes in flight using 2D optoacoustic sensors

Ilyas Potamitis [a,*], Iraklis Rigakis [b]

[a] Dept. of Music Technology & Acoustics, Technological Educational Institute of Crete, E. Daskalaki Perivolia, 74100 Rethymno Crete, Greece
[b] Dept. of Electronics, Technological Educational Institute of Crete, Romanou 3 – Chalepa, Chania 73133, Greece

ARTICLE INFO

Article history:
Received 6 November 2015
Received in revised form 4 March 2016
Accepted 10 March 2016

Keywords:
Culex pipiens
Anopheles gambiae
Aedes albopictus
Insects' wingbeat

ABSTRACT

Mosquito flight tones occur during locomotion and courtship and are mostly analyzed using microphones. The use of microphones is impractical for analyzing the wingbeat of non-tethered insects especially if one is interested in studying the frequency content of wingbeats of a large number of insects. In this study we present a practical setting based on a novel 2D optical sensor that we embed inside insectary cages to record the wingbeats of three mosquito species belonging to three different genera, namely *Culex pipiens molestus*, *Anopheles gambiae* and *Aedes albopictus*. We show that this setting allows to automatically create distributions of parameters related to wingbeat frequency and harmonic properties derived from many non-tethered wingbeats and therefore characterize the wingbeat properties of a whole species with increased confidence. Implications for potential applications are discussed.

1. Introduction

From an engineering point of view, flying insects can be seen as biological micro-vehicles equipped with multi-sensors able to demonstrate admirable maneuvering capabilities [1]. From another point of view, many species such as most mosquitoes are nuisances and potential carriers of serious vectors for humans and livestock and, therefore, there is a compelling need to develop novel devices and procedures to counter-measure their presence [2]. An informative cue of the presence of these elusive insects is their wingbeat that produces an audible tone. The sound of insects' wingbeat in general [3] and of mosquitoes in particular [4–10] has been studied extensively. The most common recording device for the wingbeat is the microphone [3–6,8–10]. A different recording modality also applied to wingbeat measurements is the optical sensor [7,11–14]. The basic principle of the optical sensor as applied to this particular task is the gradual interruption of the path of light between an emitter and a receiver of light at the rhythm of the wing movement. In [12] we show that though the generative process of audio and optical recordings are completely different, they sound and their associated spectrum looks quite similar. Quality microphone recordings are hard to obtain for insects performing a free flight. Indeed, most reported literature is based on measuring the wingbeat of one or few tethered specimens (see e.g. [3,5,8–10]). Tethering is suspected to affect movement as it alters wing kinematics (see [8] and the references to this point therein). Microphone recordings of wingbeat audio stemming from non-tethered insects can be taken in the case of mosquitoes enclosed in a spacy insectary box. In such case, even a gun-shot small aperture microphone cannot avoid the background noise of a swarm of insects. The use of a single insect is possible but in such a case the acoustic properties one obtains refer to the specific specimen under study. Measuring wingbeat's acoustic properties of insects treated on one-by-one basis is not practical and if applied it can only be applied to few insects and therefore one cannot know if the properties recorded can characterize a whole species. There are more problems with the acoustic modality: the insectary must be placed in an anechoic chamber as noise penetrates environments more often than expected. Even a quiet laboratory needs to switch-off vital devices in order to take a good quality audio recording. If the microphone is placed in the field (e.g. inside a trap), then it is exposed to a large number of uncontrolled sound sources that will be picked up by the microphone [6]. In our study we introduce a practical way to observe true flight from a large number of mosquitoes and analyze automatically their collective flying properties. We place 50–120 mosquitoes of

* Corresponding author.
E-mail addresses: potamitis@staff.teicrete.gr (I. Potamitis), rigakis@chania.teicrete.gr (I. Rigakis).

http://dx.doi.org/10.1016/j.apacoust.2016.03.005
0003-682X/

the same species in an insectary and we insert a novel optoacoustic sensor inside the insect rearing cage. The novelty of our sensor with respect to the work presented in [12–14] is that the receiving part of the sensor is a surface and not a linear array. This modification allows the insect to spend more time in the field of view and therefore analyze in greater detail the full wingbeat motion. The optical sensor may record snippets as the mosquito flies past, but is blind to the flight tones of other nearby mosquitoes in contrast to microphones. Due to its optical nature it is not affected by voices and sound interferences commonly encountered in the laboratory and in the field (due to interferences from bird vocalizations, cicadas' songs, mechanical sounds, wind, etc.). A rather strong statement of this study is that: Optical sensors can replace microphones as a means to record insect' wingbeat. They are more practical in their use and embeddable in insectary cages and devices (e.g. traps [13–14]). They can also function as a standalone metric instruments operating in the field or in the laboratory. We enclose them in insectary cages to analyze the quasi-periodic movement of wings of a large number of mosquitoes from three important and widespread genera and to derive distributions of the fundamental frequency (f_0) – that coincides with the wing beating rhythm – and also distributions of several properties of the higher harmonics.

2. Materials and methods

2.1. Mosquitoes

Culex pipiens molestus were collected from Agios Stefanos (Greece) in 2015, *Aedes albopictus* were collected in Athens (Greece) and maintained in Athens lab for 5 years. We also used *Anopheles gambiae* Ngusso strain, a lab strain which originally came from Cameroon. Mosquitoes were reared in an environmental chamber at controlled temperature of 27–28 °C, 70–80% humidity and 12 h light/dark photoperiod. Cohorts were obtained by hatching eggs in water containers. Larvae were fed with cat food (Purina). Pupae of both sexes were transferred to insect cages with net and offered 20% sucrose solution. Counting from the time of being able to fly *C. pipiens* were 3–15 days old, whereas *Ae. albopictus* 3–20 and *A. gambiae* 1–9 respectively.

2.2. Optical recordings

The 2D optoacoustic sensor is shown in Fig. 1. It is composed of a receiver of light placed at the opposite of an infrared light emitter. The emitter is not a single LED but 2 × 4 infrared ones, operating at 940 nm and connected in row. The multiple LEDs solution forming a 2D array were chosen for two reasons: (a) to ensure uniform light distribution across the entrance of the trap, (b) to increase the field of view of the sensor as a single line of emitters will miss to illuminate insects flying on the border. The receiver is made of 20 photodiodes in a 2 × 10 arrangement and connected in parallel, thus forming a 2D light receiving surface. Details on the electronics' board used for 1D sensors can be found in [12,13]. The recordings are normalized in the range −1 and 1 in order to be treated as line-level audio signals (therefore the term optoacoustic). The sensors carry rechargeable batteries and due to their low power consumption they can record unattended for a number of days, or indefinitely by connecting the kit containing the battery to a charger.

Mosquitoes perform erratic movements during flight, they beat their wings at higher rates compared to other insects commonly encountered in domestic environments (e.g. flies, bees, hawkmoths) and are generally slower than them. Being slower and less direct in movement makes them ideal candidates for our sensors as they spend enough time inside the field of view of the sensors and since they have a beating frequency of more than 300 Hz they leave enough trace of their locomotion. In order to reduce interferences we include a high-pass filter embedded in the circuit that cuts the low frequencies below 250 Hz that are due to the main-body movements of the mosquitoes and slow-varying illumination variations. The sensor can be used for measuring tethered insects as well but was mainly designed this way in order to be inserted from the entrance window of insectary cages containing flying insects of a single species. Insects incidentally cross the field of view of the sensor on a random basis and, therefore their wingbeat is recorded. Note that the optical nature of the sensor makes it immune to sound and insects flying outside the field of view. We place several hundred adult insects strictly of a single species in cages and the recording of their wingbeat occurs the moment they pass through the rectangle of the sensors. We recorded 1707 flight cases of *Ad. albopictus*, 588 cases of *C. pipiens molestus* and 7250 flight cases of *A. gambiae* in approximately 2.5 h of recordings in total.

2.3. Analysis of measurements

The power spectral density (PSD) one-sided estimate, of each snippet sampled at 8 kHz is found using Welch's overlapped segment averaging estimator. The signal is divided into sections of length of 256 samples. The modified periodograms are computed using a Hamming window of the same length as the window. The overlapping in windowing equals to the 50% of the window length. The modified periodograms are averaged to obtain the PSD estimate. The FFT length was 256 samples. Fundamental frequency and harmonics were quantified by peak-picking the PSD (see Fig 2). One can observe that there is some spectral leakage around the fundamental frequency and the harmonics. This is more prominent in the short flights rather than in the long recording of tethered mosquitoes (Fig. 2-middle) as expected due to the larger number of available samples in the latter case. In both wingbeat modes the fundamental and frequencies are clearly resolved. In many recordings the second harmonic is found higher than the fundamental frequency. In [15] it is documented that changes in the relative strengths of the body, fundamental and second harmonic coincide with major changes in flight direction and speed. In the same work it is demonstrated that for part of the flight of a fruit fly the second harmonic can be larger than the fundamental at the time of a maneuver. We observed cases where the partials are located exactly on integer multiples of the fundamental but in most cases the partials are slightly de-tuned several Hertz (±10–20 Hz) from their corresponding harmonics.

The waveforms generated by the flight sounds of the mosquitoes, were also recorded by a low noise, small aperture gun microphone (MiniDSP, UMIK-1 omni-directional measurement microphone) inserted in the cage containing the same insects from which the optical recordings were taken. In Fig. 2-right we compare the power spectral density of the microphone recordings to the spectrum of the photodiodes sensor recordings (Fig. 2-left). The optical sensor follows closely the microphone for the first 7 harmonics. The microphone has a sharper analysis of the harmonics judging from the bandwidth of the harmonics and we are currently investigating the reason of this difference toward the use of LEDs emitting at different wave lengths. Any other difference in all three recordings is attributed to the different specimens used for the recording and small differences in the temperature. The optical sensor records an event only for the time that the light from emitter to receiver is interrupted and therefore the wingbeat events are ad-hoc shorter in time than events recorded by a gun microphone in a small cage. Special measures have been taken in order to make possible a microphone recording in the lab and these measures