Peer Review

Potamitis et al. **2D optoacoustic sensors embedded in mosquito insectary cages report species identity through wingbeats.** Submitted to the Journal of Medical Entomology

General Comments

- This article needs to be thoroughly edited to improve structure and grammar. In addition, a possible major flaw in the data needs to be investigated (see comments on Figure 3).
- This submission does not comply with the manuscript preparation instructions for JME:
    - Order of elements should be as follows: title page; Abstract and key words; introduction (no heading); Materials and Methods; Results; Discussion (or Results and Discussion); Acknowledgments; References Cited; footnotes; tables; figure legends; and figures. This article, as submitted, does not have a Results section and it has a Conclusions section.
    - Citations and references are not formatted correctly.
    - Figures should be on separate pages near the end of the document.
    - Figure captions should be on a single page near the end of the document.
    - Tables should be on separate pages near the end of the document.
- Acronyms are used liberally without definition on first use: LED, SNR, FOV, SD, 2D, 1D, GPRS
- A published report on similar research may be of interest to the authors:

Moore, A. 1991. Artificial neural network trained to identify mosquitoes in flight. J. Insect Behavior 4(3) 391-396

Specific Comments

1-2: The title is not very informative:

- Something like "Large aperture optical sensors embedded in mosquito insectary cages report species identity through wingbeats." would be more meaningful.
- Strictly speaking, optoacoustic sensors were not used. The sensor consists of an infrared LED array which projects light onto a rectangular light guide which routes the light to a one dimensional array of photodiodes. This article does not fully describe the sensor system: "We direct the interested reader to [15] for the full electronic board of our sensor and details of construction." Unfortunately, this reviewer was unable to download the full text of this reference, which is not published in an open source journal. I highly recommend adding a schematic of the sensor system as "Supplemental Material". Granted that the signals from the photodiode array contain insect wingbeat waveforms with fundamental frequencies and harmonics within the human audio frequency band, but that does not make the sensors "optoacoustic". The sensors are not sensing sound, but fluctuations in light intensity. That makes them optical sensors.

12 Abstract

21 "mosquitos" used here, but "mosquitoes" elsewhere in the article.

38 Introduction

38 According to the preparation instructions for the JME, there should not be a heading for the Introduction section

42-43 Change "It has been reported that 700 million people get a mosquito borne illness each year resulting in greater than one million deaths [1]." to "Seven hundred million (700,000,000) people get a mosquito borne illness each year resulting in greater than one million deaths [1]."

44 Change "Prevention of the vector borne diseases ..." to "Prevention of vector borne diseases ..."

57 automatize > automate

81-82 "The qualitative characteristics of the recorded wingbeat are very high (i.e. SNR, duration, noise)." Not clear what is meant by this sentence.

82-83 "Hereinafter, we present details on Materials & Methods followed by evaluation results and their practical implications." This sentence is unnecessary.

84 Materials and Methods

General comments:

- The Materials and Methods section does not provide enough information to allow reproduction of this research. Following the modern trend towards reproducible research, I encourage the authors to provide the following as Supplemental Material:
    - a schematic for the sensor hardware
    - code listing for data acquisition software
    - code listings for signal processing and data analysis
    - the raw dataset on which results are based

Specific comments:

85 The 2-dimensional optical Sensor

86-91 "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 they are generally slower than those. 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 to allow for their efficient classification."

- These sentences do not belong in Materials and Methods. Move to Introduction.
- Suggest changing "beating frequency of more than 300 Hz" to "wingbeat frequency of greater than 300 Hz"
- Suggest changing "they beat their wings at higher rates compared to other insects commonly encountered in domestic environments (e.g. flies, bees, hawkmoths)" to "they beat their wings at higher rates than other insects commonly encountered in domestic environments (e.g. flies, bees, hawkmoths)". There is a problem including flies here because mosquitoes are flies (Diptera) and there are small flies with wingbeat frequencies higher than that of mosquitoes.

99-100 "The light fluctuation in the output of the receiver is monitored by a microprocessor and the device is auto-triggered when it senses a wingbeat." Please provide more

information about the trigger. Please provide the make and model of the microprocessor. Is this a simple amplitude set point trigger or something more sophisticated? Is any pretrigger signal recorded? A code listing would be useful to the technical reader.

100-102 "Once triggered the light fluctuation is turned to an audio snippet of 5000 samples at 8 kHz (i.e. 0.625 s), time-stamped and internally stored in the SD of the device as a permanent record." Please include resolution (bits per sample).

102-104 "The device is immune to optical interferences from natural or electric light as it sends high frequency pulses instead of constant flow of light. We direct the interested reader to [15] for the full electronic board of our sensor and details of its construction." Please provide the pulse frequency for the IR-LEDs. This reviewer is unable to access full text for reference 15.

115 The optical Recordings

117 "The light intensity is normalized to line-out levels and is treated as an audio recording." Please clarify.

118-121 "In Fig. 2 we show characteristic wingbeat recordings of mosquitos in flight. In the same figure the upper row of each subfigure contains three typical inflight recordings one from each different species of mosquitoes. In Fig. 2 – bottom row of each subfigure we show the frequency content of these recordings." Figure 2 should be fully described by its caption, and this information should not be repeated in the main text. I suggest you simply refer to the figure along these lines: Spectral analysis shows that each recorded wingbeat waveform contains a wingbeat frequency and harmonics (Fig. 2).

121 Suggest changing "The fundamental frequency corresponds to the beating frequency of the insects' wings." to "The fundamental frequency corresponds to the insect's wingbeat frequency".

123-124 Suggest changing "The sensor can resolve the fundamental frequency (that coincides with the beating rhythm of the wings) and all harmonics up to 4 kHz." to "The sensor can resolve the fundamental frequency (wingbeat frequency) and all harmonics up to 4 kHz."

128-140 "Many insects beat their wings at almost the same rhythm and, therefore, the fundamental frequency alone is an insufficient parameter to discern species [18-19]. Provided the species have morphological differences or functional differences in the wingbeating mechanism, these differences will be imprinted on the distribution of their power spectrum. Classification will be accurate if the inter-species variability of the spectrum is small and the spectrum of their wingbeats demonstrates a consistent, recognizable pattern. Advanced machine learning techniques that we will report on later in this work do not need visual resemblance in order to classify species to classes as they learn from training data the relationship among spectral coefficients (in our case among the fundamental frequency and power distribution on the harmonics). Classification accuracy is assisted if spectral variation of wingbeats is large among different species that have different wing shape, size and wingbeat frequency. As reported in [20] for the case of bumblebees, and can also be verified in Fig. 3 for mosquitoes, flying insects hold a rather constant wingbeat frequency, in a given temperature, regardless the speed and flight pattern."

- These sentences do not belong in the materials and Methods section. Move to introduction or Discussion (or Results and Discussion Section).

141-151 "In Fig. 3 we gather the spectra of 600 distinct free-flight passes, 100 for each species (Ae. aegypti, Ae. albopictus, A. arabiensis, A. gambiae, C. pipiens and C. quinquefasciatus). A close look of this aggregation of spectra allows us to visually assess the

consistency of the frequency signature in each group of specimens respectively rather than the detail of a single flight-case. The variability one observes is due to the different individuals taking part in a random experiment with different entrance angles and flight patterns once inside the field of view of the sensor. Insects of the same species will have differences in the spectrum of their in-flight wingbeat, as they are unrepeatable biological organisms. However, one can note the consistency of the spectrum within the same species and the difference among species. This entails that the spectrum (i.e. f 0 and harmonics) provided by the optoacoustic sensors are invariant to the entrance angle and behavioral mode of the flying insect."

- These sentences belong in the Results or Results and Discussion section.

157-161 "Note, that both sexes are included and since mosquitoes are dimorphic this results to a phenotypic differentiation between males and females of the same species. Incoherence in the frequency pattern within the same species group is attributed to individuals of different sex. Sex differentiation results into males being smaller in size and higher pitched than females."

- Can be expressed more clearly. Something like: "We recorded both males and females of each species. Mosquitoes are sexually dimorphic. Males are smaller and their wingbeat frequencies about 40% higher than conspecific females [Moore]"

163 Classification Experiments

General comments

- Potential flaw in the data - see comments on Figure 3.
- Information on the mosquitoes is needed. Where were they collected? How were they identified to species? How long have they been in a lab colony?
- The experimental design is not clearly described. It is not clear how recordings were made. Looks like the sensor system is a single prototype. So, in a recording session, this device was placed in a cage containing one of the six species? How long was each recording session? Was each species recorded once or multiple times? Was each species in one cage or several cages? Answers to these questions are necessary for the reader to assess the scientific veracity of the experiment.
- In the text, tables, and figures "quinx" is used as a an abbreviation of the species epithet "quinquefasciatus". Please do not do this.

Specific Comments

168-170 "This innovative setting allows us to take effortless and secure recordings of a large number of flight cases in a practical way, even for quarantine pests, as insects are generally difficult to handle in free-flight experiments."

- Move to Results or Results and Discussion section.

173-179 "In [14-15] we have shown that the generated optical fluctuations modulated by the wingbeats of insects, though short in time, offer enough information that can be used to discern species adequately. One can get a variety of features out of a recording but, as analyzed thoroughly in [22], we believe that the unprocessed spectrum containing the fundamental and its harmonics and possibly certain simple transformations of it (e.g. frequency pooling through a filter-bank, logarithmic amplitude compression) are a better choice than estimating the f 0 , the harmonics, and autoregressive features for this task."

- These sentences belong in the Introduction or Discussion (or Results and Discussion) section

181-184 Figure 3

- This figure is a very nice visualization of the spectral data. However, it seems to expose a major problem with the data. If I am interpreting the figure correctly, the background noise is not constant, but is much lower for all the *Culex pipiens* records and most of the *Anopheles arabiensis* records. This is very evident in the higher frequency bins (3000-4000 Hz) where there are distinct troughs in the three dimensional plot. I suspect that your learning algorithms are using differences in non-biological background noise signatures in classifying signals. This potential problem needs to be investigated thoroughly. It may be possible to apply a digital filter to remove background noise for all signals in the dataset.
- The amplitude of the power spectra in Figure 3 ranges from -240 to -120 dB, whereas the amplitude of the power spectra in Figure 2 ranges from -100 to -60 dB. Please explain this difference.
- Please state how the 600 samples were selected from the data set. Is this a random sample?
- *Ae. Aegypti* should be *Ae. aegypti*
- *Ae. Albopictus* should be *Ae. albopictus*

186-189 "In [21] it is documented that changes in fundamental and second harmonic ratio 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 therefore focus on the spectrum solely."

- These sentences belong in the Introduction or Discussion (or Results and Discussion) section

291 "*Anopheles/Pipiens* genus"

- *Pipiens* should be *Culex*

306 Discussion

"Mosquitoes are ectothermic and increase their wingbeat frequency along with the rise in temperature."

- Please cite a reference which shows that mosquito wingbeat frequencies increse with temperature.

340 Conclusions

General Comments

- There is no Conclusions section in a JME article. Please move the text to the Results (or Results and Discussion) section.

370 APPENDIX

General Comments

"This work is accompanied with an associated folder that can be found in the site of the paper as supporting information containing detailed recordings of insects and videos as well as the classification software."

This reviewer could not find supporting materials in the review package. This article would really benefit from inclusion of supporting materials (see comments for Materials and methods section.