**Editorial comments:**  
Changes to be made by the Author(s):  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.  
2. Figures 8 and 9: Please capitalize the first word in the labels on the x and y axis (i.e., Day, Time).

*MMB: The x and y axis titles were capitalized.*  
3. Please rephrase the Long Abstract to more clearly state the goal of the protocol.

*MMB: The long abstract was reworded to clearly state the goal of the protocol.*   
4. Please rephrase the Introduction to include a clear statement of the overall goal of this method.

*MMB: The introduction was reworded to clearly state the goal of the protocol.*  
5. Please define all abbreviations before use.

*MMB: All abbreviations were defined throughout the text.*   
6. Please include a space between all numbers and their corresponding units: 33 cm, 120 Ω, 15 mL, 37 °C, 60 s; etc.

*MMB: A space was included between numbers and units throughout manuscript.*  
7. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

*MMB: We removed any personal pronouns from the protocol text.*  
8. 1.7.2, 2.2, 2.2.1, 2.2.2: Please write the text in the imperative tense.

*MMB: Text was written in an imperative tense at specified locations.*

9. References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references.

*MMB: We corrected these mistakes in the references.*

10. Please upload supplemental data that are mentioned in the manuscript. Please note that files uploaded to the “Supplemental Files (as requested by JoVE)” section of your Editorial Manager account are only for JoVE’s internal use and will NOT be published with your article. If you would like your files to be available for download with your article, then please move them to the “Supplemental Code Files” section of your Editorial Manager account.  
*MMB: We will ensure that this issue is rectified in the final uploaded version.*

**Reviewers' comments:**  
  
**Reviewer #1:**  
Manuscript Summary:  
The authors have developed an innovative system to measure insect emergence patterns in experimental settings. I would suggest revising the manuscript to provide additional context about the system. For example, I work with emergent aquatic insects and the purpose/potential uses of this system were not clear to me until I read most of the manuscript. In addition, the authors should provide more details about the setup and methods to help users new to this type of experimental system (like students) get started. I have provided several specific suggestions below. Good work.  
Major Concerns:  
Long abstract  
1. 1st sentence, consider revising to "… they are limited in the maximum number of emerging insects they can detect."  
*MMB: This change was made to the first sentence.*   
Short Abstract  
1. "multiple data points" is vague. Please define "data points." I think this is number of emerging insects.  
*MMB: This part of the sentence was removed to improve clarity.*   
Introduction  
1. 1st sentence, explain what type of insect emergence is meant. i.e. terrestrial insects in experimental settings, this set up is not for aquatic insects or for use in field (i.e. non experimental) settings.

*MMB: This sentence was reworded to be more specific.*  
2. explain the "falling-ball" principle at first mention. This design is based on falling ball so a brief explanation will help new users.

*MMB: A brief description was added here.*   
3. define what is meant by "sample sizes" (i.e. number of emergent insects detected).

*MMB: This was more clearly defined here.*  
4. What does "In our hands" mean? Couldn't anyone achieve similar results? I'd suggesting removing this statement or clarifying.

*MMB*: The words were removed here.   
Protocol  
1. Before the protocol section please provide a brief overview of the system and how it works. Without this it's not really clear how the systems works until one reads all the way through the protocol.

*MMB: A brief overview of the system and how it works was added before the protocol section.*   
2. System use 2.3.2 and 2.3.3, "metal pellet" "metal ball" and "BB" are all used to describe the same thing. Please be consistent.

*MMB: This inconsistency was corrected by using “metal BB” throughout the manuscript.*

Representative results  
1. Prior to explaining the results please provide a description of how the experiment was set up/methods. i.e. pupae were loaded into centrifuge tubes, next a plastic BB and metal BB were added to each tube, etc. Again, I think this will be beneficial to people new to the system (like a graduate student using this article/video to set up their experiment).  
*MMB: A further description of this process was added to section 2.3.2 of the protocol.*

Figures  
1. Please add labels to figures so that components can be identified without color (e.g. figure 1).

*MMB: This change has been made to the figure.*

2. Some actual images of system components with wiring, centrifuge tubes, etc along with renderings would be helpful. (e.g. figure 3)  
*MMB: In response to this statement and that of reviewer #4, we have changed the final figure to a photograph of the setup. We found wiring to be difficult to convey in a photograph, and are therefore reluctant to replace Figure 6 with a photograph. Additionally, we think that these issues will be addressed in the final video.*

Minor Concerns:  
Long abstract  
1. 4th to last sentence. Replace "ran" with "run".

*MMB: This mistake was corrected.*   
Limitations  
1. two typos in this paragraph: "beingmissed" and "emergeat once"  
*MMB: These mistakes were corrected.*  
Figures  
3. Figure 9 legend replace "overly" with "relatively"  
*MMB: This replacement was made.*  
  
**Reviewer #2:**  
Manuscript Summary:  
Bennett et al. have created an autonomous system to collect data on insect emergence. This system includes both a design that can be 3D printed and constructed from purchases pieces and R code to assist with the analysis of data from this emergence trap. The system is specifically designed to measure the of emergence of a leaf cutter bee.

Major Concerns:  
It should be made clearer early in the manuscript both what types of insects this trap is optimized for and that it is for lab-based assays. As those that study insect emergence in the field (e.g. in aquatic environments) or in taxa that have a different life history (e.g. Drosophila) would be unlikely to use this trap.  
*MMB: This point was made clearer earlier in the manuscript, at the end of the introduction.*

Minor Concerns:

Abstract:  
Unclear what 'pretreatment' means in this context (and in the introduction).

*MMB: We changed this to treatment to avoid confusion.*   
Should be clear in introduction that this apparatus is primarily for lab-based work on insect emergence- with a focus on Megachile rotundata.

*MMB: A sentence was added to specify these points, at the end of the introduction.*

Introduction:  
Better to use 'exogenous factors' or 'environmental factors' than Zeitgebers to reduce sub-field specific jargon- or at least define with first use.  
*MMB: Zeitgeber was removed and replaced with environmental cues throughout the manuscript.*

Protocol:  
It would be useful to more thoroughly discuss how larval insects are arranged inside the apparatus so potential users working on different taxa can envision whether this design would be useful for them.  
What size range of insects are the sensors likely sensitive to (particularly on the small end of the spectrum)?

*MMB: A further description of this process was added to section 2.3.2 of the protocol.*

**Reviewer #3:**  
Manuscript Summary:  
This manuscript describes new apparatus for recording the timing of insect eclosion/emergence. By improving the existing system, the authors succeeded to improve the measurement accuracy, to expand the sample size, and to be automated further. I think the paper would be useful for those just starting the research in this field. The subject is suitable for the journal. Usability of this new device is guaranteed from the results obtained. I would recommend this manuscript to be accepted after minor revision.  
  
Minor Concerns:  
Is it possible to monitor the emergence status during the measurement? If so, the author should better explain this.  
*MMB: This point is discussed in the “limations” section.*

References 3 'drosophila' should be 'Drosophila'

*MMB: Drosophila was capitalized here.*

References 4 '…. Emergence in the Alfalfa Leafcutting Bee' should be '…. emergence in the alfalfa leafcutting bee'  
*MMB: The case was fixed here in this reference.*

**Reviewer #4:**  
Manuscript Summary:  
The paper describes a neat, efficient system for remotely measuring insect emergence. The instructions are easy to follow, and should be repeatable by anyone with basic technical knowledge of circuitry, or enthusiasm to learn the basic skills required.  
  
Major Concerns:  
1. In Section 1.1 and Figure 1 - The number of parts for each component don't match in the text and figure.

*MMB: This change has been made to the figure.*

2. I couldn't see any supplementary files, and I would have liked to look them over. For e.g., I couldn't assess whether the supplementary .stl files are well-named for easy reference, nor whether the R script is well annotated for easy use.

*MMB: We will ensure that this issue is rectified in the final uploaded version.*

3. The only mention of an incubator is in the results section under "Successful run". You may want to mention earlier in the text that the system can be placed in an incubator, and perhaps give some guidelines of the temperature range in which this setup would function.

*MMB: The mention of an incubator was added in the description of the system before the protocol.*

4. Provide the raw data files used in Figure 8 and 9 as supplementary materials. This way users building their own setups could check the downstream processing pipeline.

*MMB: Raw data files will be provided in supplementary materials.*

5. I would like to see a photo(s) of the completed setup included.

*The final figure has been replaced by a photo of the assembled setup.*

6. Adding dimension information to some of the figures would be helpful for readers to easily see how much bench/desk/incubator space it takes up. Some overall dimensions (length, breadth, height) in the text would also be worthwhile adding.  
*MMB: The dimensions of the assembled apparatus were added to the Figure 7 legend.*

Minor Concerns:  
Long abstract  
-1st sentence: Strange wording - Replace "that" with "the"

*MMB: This sentence was reworded for clarity.*  
Introduction  
-Provide a short description of the "falling-ball" or "bang-box" systems.

*MMB: A brief description was added here.*   
-Suggest you provide the common name for this species.

*MMB: The common name was added to the introduction.*  
-The last two paragraphs of the introduction are a little repetitive. Consider revising the text to streamline the information.

*MMB: The information was streamlined to avoid repetition.*

Protocol  
-Would any 3D printer be able to print the required components? If there are minimum requirements these should be specified.

*MMB: Added this note to the 1.1 section in system construction section.*   
-It may be nice to include the actual filenames for printing in the text

*MMB: This was added to the protocol section of the manuscript.*  
-Make sure to define abbreviations (where needed)

*MMB: Abbreviations were defined throughout.*

-1.7 Does this software work on all operating systems?

*MMB: Arduino can be downloaded for both windows and mac operating systems, specification was added 1.7.1.*  
-2.1 Does the system just "click" together? OR is adhesive needed?

*MMB: It was further specified in 2.1 how to assemble the rack platforms.*   
-2.2. Make sure it's clear in the manuscript what a channel is

*MMB: Channels are discussed throughout the manuscript.*  
-3 How is temperature recorded and logged? I see there's a sensor in the breakout board listed, but does it need calibration?

*MMB: In section 1.6.5 a note was added to test temperature sensor before use.*   
-3.2.4 Link to a "How to use R" type document/website for those not familiar with instructions like 'change the working destination"

*MMB: In section 3.2.3 a link for help with R was added.*   
Results  
-Figure 1 - I would find it helpful if the parts were labelled in the actual figure (not just the legend)  
*MMB: This change has been made to the figure.*

-Figure 3 legend - "for" should be "four"?

*MMB: This mistake was corrected.*  
-Figure 4 - A and B labels missing in figure

*MMB: This issue has been corrected.*  
-Figure 8 - I'm not sure why you need the "color" legend to be visible in the plot

*MMB: This mistake was corrected.*  
Discussion

-Limitations section - Numerous spacing issues in the 2nd sentence.

*MMB: This sentence was adjusted to be more streamlined.*