

1 **Manuscript type:** Practical Tool

2

3 **Title:** An inexpensive setup for robust activity tracking in small animals: Portable Locomotion Activity
4 Monitor (pLAM)

5

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17

18 **Keywords:** activity detector, animal motion tracking, circadian rhythm, insect diel-activity, low-cost

19

20 **Abstract:**

21

22 1. Advances in computer vision and deep learning have automated animal behaviour studies that
23 previously required tedious manual input. However, tracking activity of small and fast flying animals
24 remains a hurdle, especially in a field setting with variable light conditions. Commercial locomotor
25 activity monitors (LAMs) can be expensive, closed source, and generally limited to laboratory settings.

26

27 2. Here, we present a portable locomotion activity monitor (pLAM), a mobile activity detector to
28 quantify small animal circadian activity. Our setup uses inexpensive components, is based on open-
29 source motion tracking software, and is easy to assemble and use in the field. It runs off-grid, supports
30 low-light tracking with infrared lights, and can implement arbitrary light cycle colours and brightnesses
31 with programmable LEDs. We provide a user-friendly guide to assembling pLAM hardware and accessing
32 its pre-configured software and guidelines for using it in other systems.

33

34 3. We benchmarked pLAM for insects under various lab and field conditions, then compared results to a
35 commercial activity detector. They offer broadly similar activity measures, but our setup captures flight
36 and bouts of motion that are often missed by beam-breaking activity detection.

37

38 4. pLAM will enable high-throughput quantification of small animal location and activity in a low-cost
39 and accessible manner, crucial to studying behaviour that can help inform conservation and
40 management decisions.

41

42

43 **Introduction**

44 Improvements in computing speed, memory, and technology have helped automate animal behaviour
45 studies that were once performed manually (M. W. Mathis & Mathis, 2020). For pose estimation, multi-
46 animal recognition, and motion tracking, computers can sometimes exceed human accuracy (A. Mathis
47 et al., 2018; Nath et al., 2019; Tadres & Louis, 2020). Unfortunately, many tools are inaccessible without
48 sufficient computational power and programming proficiency (von Ziegler et al., 2021). Further, it
49 remains challenging to track small or fast animals in the field, with variable light and weather. Although
50 commercial solutions, like the Locomotor Activity Monitor (LAM, <https://trikinetics.com/>) and
51 EthoVisionX (<https://www.noldus.com/ethovision-xt>), address some of these problems, they are often
52 expensive and bulky (Table S1). Open-source solutions, when available, are specialized for model
53 organisms, such as fruit flies or mice (Matikainen-Ankney et al., 2019)(Chiu et al., 2010), (Matikainen-
54 Ankney et al., 2019), and generally limited to laboratory use. Field activity tracking, with camera traps
55 and radio tags, are often only practical for large animals (Debata & Swain, 2018; Nunes-Silva et al., 2019;
56 Pirie et al., 2016).

57 The lack of a portable and affordable tool has hindered behavioral data collection for smaller,
58 non-laboratory animals, such as nocturnal arthropods, and only a handful of studies have attempted it
59 (Table S2). They used either manual observation (Fullard & Napoleone, 2001), which is difficult to scale
60 up, or non-portable setups (Edwards, 1960; J. L. Smith et al., 2016; P. H. Smith, 1983), which are difficult
61 to replicate in the field. Light traps are easy to use and portable, but have inherent problems for
62 inferring activity of phototactic animals (Lamarre et al., 2015; Lewis & Taylor, 1965). Because trap
63 effectiveness decreases with distance, they bias towards animals present around a trap (Baker &
64 Sadovy, 1978). Further, trap light can activate otherwise inactive animals, such as those waiting for
65 dawn (Donahue, 1962), pers. obs. last author). Additionally, many animals are blinded by bright trap
66 lights, and become inactive after they settle (Frank et al., 2006), making actual activity even more
67 difficult to establish. Suction traps, that sample captured insects hourly, monitor activity without the
68 problems associated with bright lights (Wright & Morton, 1995), but these are strongly affected by the
69 spatial distribution of insect populations (Taylor & Carter, 1961), and require cumbersome manual
70 sorting.

71 Many insects face growing extinction risks (Boyes et al., 2021; Janzen & Hallwachs, 2021;
72 Wagner, 2020; Yang et al., 2021), yet with the exception of certain pest species (Lima et al., 2020), little
73 attention has gone toward automated monitoring. Such systems are crucial to understanding and
74 documenting baseline behaviors, especially as anthropogenic factors, such as light pollution, alter and
75 stress the environment. Here, we address several limitations of previous methods by introducing the
76 Portable Locomotion Activity Monitor (pLAM) to automate activity monitoring of small animals under

77 arbitrary light conditions. We benchmark this equipment under different conditions, provide examples
78 of field and laboratory use, and compare results with an existing commercial package. With low-cost,
79 open documentation, and portability, anyone can monitor locomotion of multiple small animals
80 simultaneously, making this a novel tool to reveal fine-scale, small animal activity patterns.
81

82 Description and Implementation

83 The monitor logs activity events for an individual or group of animals in a small enclosure, detecting
84 activity by comparing the difference in pixel values between successive images from a video stream.
85 When pixel differences cross a predefined threshold, the program saves images or videos and logs the
86 time and duration of the motion event. The pLAM can operate over a range of natural light conditions,
87 or in the lab with programmable LEDs that simulate natural 24 light-dark cycles. It can run on power
88 banks or battery packs in the field (Fig. 1,2, Table S3).

89 pLAM consists of: (1) An infrared camera and visible lights, both controlled by a RaspberryPi single-
90 board computer, and external infrared lighting for motion capture in the dark (Fig. 1,2, Table S3). (2) A
91 command line interface for setting activity capture parameters. (3) Wrappers and pre-configured
92 settings for running *motion* (<https://github.com/Motion-Project/motion>), an open-source library that
93 detects motion between successive video frames. (4) A python-based processing pipeline to automate
94 capture, logging, and analysis of activity with text files or images. (5) Scripts to control light settings in a
95 light chamber of choice. We provide a step-by-step guide to building your own pLAM and using the
96 associated software (Appendix 1). We also provide a detailed troubleshooting and optimisation guide to
97 use the pLAM in different animals and conditions (Appendix 2). All code is available on github
98 (www.github.com/yashsondhi/diel-light-pi) and the disk image of the pre-configured pLAM OS along
99 with other instructables are hosted on OSF server (<https://osf.io/8p5kw/>).

100

101 Methods

102

103 Benchmarking

104 We benchmarked the pLAM with objects of different sizes and various test insects. We compared results
105 to data from a commercial activity detector and tested our device with 15 insect species in the field.

106 **a) Object size and distance detection limit:** To test the detectable range of object size and distances, we
107 suspended plastic beads in a chamber and moved them at random intervals with a fan. We then
108 monitored diel-activity of three differently sized lab reared insects (see Supplementary Methods).

109 **b) Comparison with commercial activity detector LAM:** The LAM (Locomotor activity monitor) and DAM
110 (Drosophila activity monitor) systems are commercially available activity monitors that are commonly
111 used in the lab with small insects like fruit flies and mosquitoes. They are designed and distributed by
112 Trikinetics (<https://trikinetics.com/>) and use infrared beams and detectors to monitor insect activity.

113 Similar to a burglar detection system, an insect passing through a ring of infrared beams interrupts the
114 signal to the detector, and the LAM device records this as a motion event (Fig. 2, [LAM25 Data Sheet](#)).

115 Unlike the pLAM which monitors the collective activity of all individuals present in the field of view,
116 LAMs monitor individual activity. They work well for small insects in the lab, but are expensive, with

117 setup costs between 1000-4000 USD, and cannot house larger animals or function in natural settings.
118 We generated comparative reference diel activity data with the LAMs for the species used in the pLAM
119 trials (Fig. 3, Supplementary Methods).

120 **c) Field tests:** To benchmark the pLAM in the field, we conducted trials in Monteverde, Costa Rica. We
121 set up 6 pLAMs outdoors at the edge of a cloud forest reserve. We collected different insect species
122 from light traps, then monitored activity with the pLAMs. We used 4-10 individuals of each species for
123 up to 48 hours under natural illumination and environmental conditions (Fig. S4, Supplementary
124 Methods).

125

126 **Data analysis**

127 **pLAM:** Our software logged motion events in a text file and saved corresponding images of frames with
128 maximal pixel differences. For bead benchmark trials, we examined motion event frequency as density
129 functions. For insect activity trials, we measured the scaled pixel difference (normalised to the maximum
130 pixel difference detected) of each image of every motion event, across all the days of the trial (Fig. 3A,C),
131 and summary data, representing hourly frequency counts of motion events (Fig. 3A,C). Field-trials (Fig.
132 S2) generated noisier data, and we therefore only included trials where activity patterns were consistent
133 across both days without manual filtering of background motion. **LAM:** LAMs count beam breaking
134 events in each channel per minute. We combined channel data and compared scaled motion event
135 counts (Fig. 3 B,D). **Light:** We measured illuminance (lux) over the trial period (Fig. S3), also represented
136 as a light bar above the plots.

137 **Results**

138 **Object size and distance detection limit**

139 To test the range of pLAM object detection, we suspended beads of different sizes at selected distances
140 from the camera, and generated erratic motion with wind from a fan. Flat lines would represent no
141 motion, but the monitor detected events for all conditions (Fig. S1). Large beads generated longer
142 motion bouts, and small beads generated high frequency, short duration events. The smallest
143 detectable beads were 4mm at over 60 cm away. We then proceeded to test various insects (wax moths
144 ~10mm, mosquitos ~3mm and fruit flies ~2mm) with an artificial, graded light-dark cycle in lab
145 conditions. The device worked for all three, monitoring 4-5 days of activity (Fig. 3 A,C, Fig. S2).

146 **Comparison with commercial activity detector LAM**

147 To test the accuracy of the pLAM activity data, we compared the same three species in standard
148 commercial LAM activity detectors. Wax moths showed nocturnal activity with a peak in the first few
149 hours of the night, and an overall pattern consistent across the LAM and the pLAM (Fig. 3 A,B) setups.
150 Fruit flies were mostly diurnal and displayed peaks of activity at dawn and dusk. The pLAMs recorded
151 mosquito activity exclusively during the day, peaking near dusk, but the LAM showed a symmetrical
152 activity at dawn and dusk. (Fig. S5). The pLAMs recorded less baseline fruit fly activity than the LAMs
153 (Fig. 3 C,D, Fig. S5). Images revealed pLAMs exclusively counted bouts of flight, rather than walking,
154 which could be changed by altering the camera angle.

155 **Field tests with multiple species**

156 To ensure devices were robust for field-use, we tested them at Estación Biológica Monteverde (EBM) in
157 Costa Rica (see Supplementary Methods, Fig S4). We monitored 15 species (Fig. S4. see Table S4) in
158 natural light and weather conditions, demonstrating pLAMs functioned well in the field, but are prone to
159 signal noise, and require software and hardware modifications not necessary in the lab (Appendix 1). We
160 found large variations in diel activity of species collected at light screens, contradicting suggestions that
161 nocturnal collection indicates natural nocturnal activity.

162 **Discussion**

163 The portable locomotion activity Monitor (pLAM) can track collective and individual small animal
164 activity using frame-based difference methods for detecting motion under various light conditions (Fig.
165 1,2). We have evaluated its performance under lab and field conditions, and provide a detailed guide to
166 hardware and software, and offer logistical advice for using it outdoors. (Appendix 1) We discuss our
167 rationale for choosing pLAM components, compare it with other options, and discuss its limitations
168 (Table S4). We detail the issues we had with lab and field trials and provide some general
169 recommendations for using the pLAM in both conditions (Appendix 2).

170

171 **Rig design**

172 We tested several camera and light combinations (Appendix 1-2) but eventually used (1) the raspberry
173 NOIR cameras modified by Arducam with IR cut filters, for their wide field of view and function under
174 both bright and dim, infrared lit, scenes. (2) IR LED illuminators, which are inexpensive and sufficiently
175 light for moderately sized cages. (3) Raspberry Pi 3's. Newer models increase cost but add little to data
176 collection performance (although they do offer faster transfer speeds and USB3 ports). All components
177 together typically cost \$130-\$180 USD (Table S3), roughly 10-fold cheaper than commercial options.

178 **Comparison of pLAM with other methods and commercial tools**

179 Aside from their price, several commercial tools offer capabilities comparable to pLAM (Table S1).
180 Options vary in interface, coding environment, and methodology but they come into two main
181 categories useful for research. First, commercial software and hardware marketed towards tracking
182 animal activity using either IR beams or video and can be used for circadian rhythm monitoring. These
183 are often expensive and can require a subscription. Second, open-source programs which, although free,
184 rarely work in field conditions without modifications to the code and equipment and can rely on
185 contrasting backgrounds or ample lighting. The pLAM offers an open-source solution that functions in
186 the field with inexpensive hardware. It does have limitations, the false positives are high if the camera
187 detects background motion from trees, wind or humans, and it may fail to capture the entire field of
188 view.

189 **Guidelines for recording diel-activity with pLAM:**

190 pLAM works consistently in the lab with controllable lighting. Isolating the setup in temperature-
191 controlled incubators is ideal, but at minimum, shield the pLAM from external light and motion. The Pi
192 supports ambient light and temperature with extra sensors, but commercial wireless loggers are more
193 convenient. Troubleshooting the pLAM and lights is easier without live animals, although further

194 optimisation with test animals is usually required to getting robust data. For mosquitos, flies and moths,
195 we found when keeping humidity levels high and providing a food source, we got data for 5-7 days post
196 eclosion. With small cages, 20-25 flies or mosquitoes were needed to get an accurate representation of
197 the activity, but with medium cages for the moths, 4-8 individuals were enough. It is possible to record
198 even a single individual, but since the animal occasionally leaves the field of view, the activity data could
199 be incomplete. We limited the insect motion to smaller chambers entirely within the camera's field of
200 view, but this reduces the animal's tendency to fly. We noticed for flies and mosquitos, the pLAM was
201 more likely to pick up flying behaviour than walking behaviour, but this was not an issue for the larger
202 moths. While setting up pLAM experiments in different systems, we recommend altering animal density,
203 cage size, using higher resolution cameras, lowering the motion threshold and using stronger IR
204 illumination to get more robust data.

205 Conducting pLAM trials in the field is slightly more challenging. Access to electrical mains,
206 internet and shelter from wind and rain and light pollution are ideal, but often hard to obtain. Portable
207 routers, UPSes and lightweight tents offer relatively inexpensive solutions, however even they fail to
208 withstand prolonged harsh weather. Severe weather causes power fluctuations, random background
209 motion of the forest and water leakage on the electrical equipment and should be avoided. We had two
210 setups get blown away during the field trials and eventually used a large indoor library with windows to
211 provide access to light while shielding the setups from wind.

212 **Variation in diel-activity of insects in the field**

213 Although this study was meant to be a test of pLAM in the field, we were surprised at the diel-
214 activity patterns we found across various species. Despite using only species that were attracted to light
215 traps, which have often been used as an indicator of being nocturnal (Akite et al., 2015), we found
216 tremendous variation in their diel activity. Some were completely nocturnal, but some were active
217 throughout the day and night and others showed peaks of activity at dawn and dusk. This data suggests
218 that a much more detailed and systematic survey of diel-activity is required across small animals
219 especially nocturnal species.

220 In conclusion, we plan to optimize the device, add more features, and improve the software. We
221 learnt from our failures and plan modifications that will improve the robustness of the setup in field-
222 conditions including implementing machine learning filters post-data collection. We hope that this tool
223 and the accompanying guide to building and using your own pLAM will help promote field-based studies
224 of diel-activity periods and eventually lead to the creation of a large database of diel-activity periods
225 across animal taxa.

226

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238 Philosophical Society.

239

240 **Author Contributions**

241 YS: Conceptualisation, writing,
242 NJJ: Data collection, analysis, writing
243 BA: Data collection, writing
244 AM: Data collection, Rig testing
245 STF: Writing+illustrations
246 CR: Rig design, writing
247 EB: Data Collection and writing
248 MD: Conceptualisation, funding.
249 JPC: Rig design, Data Collection, Data Curation, Data analysis

250 CT: Rig design

251 *AYK: Conceptualisation, supervision, writing, funding

252 *JCT: Conceptualisation, supervision, writing, funding, data analyses

253 *contributed equally to the project

254

255 **Supplementary Information**

256

257 *Appendix 1: Step by step instructable*

258 https://www.dropbox.com/s/wxldf7h1h2wvnn6/Instructable%20for%20activity%20chamber_2021_07_01_shared.docx?dl=0

260

261 *Appendix 2: Guide to troubleshooting and setting up the various components:*

262 <https://www.dropbox.com/s/b0zvndsggrne3p/Troubleshooting%20and%20optimisation%20Guide.docx?dl=0>

263

264 *Supplemental methods*

265 <https://www.dropbox.com/s/n92kl9iumn39jev/Supplemental%20Methods.docx?dl=0>

267

268 **References**

269 Akite, P., Telford, R. J., Waring, P., Akol, A. M., & Vandvik, V. (2015). Temporal patterns in Saturniidae
270 (silk moth) and Sphingidae (hawk moth) assemblages in protected forests of central Uganda.

271 *Ecology and Evolution*, 5(8), 1746–1757.

272 Baker, R. R., & Sadovy, Y. (1978). The distance and nature of the light-trap response of moths. *Nature*,
273 276(5690), 818–821.

274 Basset, Y., & Springate, N. D. (1992). Diel activity of arboreal arthropods associated with a rainforest
275 tree. *Journal of Natural History*, 26(5), 947–952.

276 Bjerge, K., Nielsen, J. B., Sepstrup, M. V., Helsing-Nielsen, F., & Høye, T. T. (2021). An Automated Light
277 Trap to Monitor Moths (Lepidoptera) Using Computer Vision-Based Tracking and Deep Learning.
278 *Sensors*, 21(2). <https://doi.org/10.3390/s21020343>

279 Boyes, D. H., Evans, D. M., Fox, R., Parsons, M. S., & Pocock, M. J. O. (2021). Is light pollution driving
280 moth population declines? A review of causal mechanisms across the life cycle. *Insect Conservation
281 and Diversity / Royal Entomological Society of London*, 14(2), 167–187.

282 Broadhead, G. T., Basu, T., von Arx, M., & Raguso, R. A. (2017). Diel rhythms and sex differences in the
283 locomotor activity of hawkmoths. *The Journal of Experimental Biology*, 220(8), 1472–1480.

284 Chiu, J. C., Low, K. H., Pike, D. H., Yildirim, E., & Edery, I. (2010). Assaying Locomotor Activity to Study
285 Circadian Rhythms and Sleep Parameters in *Drosophila*. In *Journal of Visualized Experiments* (Issue
286 43). <https://doi.org/10.3791/2157>

287 Debata, S., & Swain, K. K. (2018). Estimating Mammalian Diversity and Relative Abundance Using
288 Camera Traps in a Tropical Deciduous Forest of Kudliha Wildlife Sanctuary, Eastern India. In
289 *Mammal Study* (Vol. 43, Issue 1, pp. 1–9). <https://doi.org/10.3106/ms2017-0078>

290 Donahue, J. P. (1962). Observations and records of butterflies attracted to light in India. *Journal of the
291 Lepidopterists' Society*, 16(12), 131–135.

292 Donelson, N. C., Kim, E. Z., Slawson, J. B., Vecsey, C. G., Huber, R., & Griffith, L. C. (2012). High-resolution
293 positional tracking for long-term analysis of *Drosophila* sleep and locomotion using the “tracker”
294 program. *PLoS One*, 7(5), e37250.

295 Edwards, D. K. (1960). A method for continuous determination of displacement activity in a group of
296 flying insects. *Canadian Journal of Zoology*, 38(6), 1021–1025.

297 Feer, F., & Pincebourde, S. (2005). Diel flight activity and ecological segregation within an assemblage of
298 tropical forest dung and carrion beetles. In *Journal of Tropical Ecology* (Vol. 21, Issue 1, pp. 21–30).
299 <https://doi.org/10.1017/s0266467404002056>

300 Frank, K. D., Rich, C., & Longcore, T. (2006). Effects of artificial night lighting on moths. In T. Longcore
301 (Ed.), *Ecological Consequences of Artificial Night Lighting* (pp. 305–344). Island Press Washington
302 DC, US.

303 Fullard, J. H., & Napoleone, N. (2001). Diel flight periodicity and the evolution of auditory defences in the
304 Macrolepidoptera. *Animal Behaviour*, 62(2), 349–368.

305 Janzen, D. H., & Hallwachs, W. (2021). To us insectometers, it is clear that insect decline in our Costa
306 Rican tropics is real, so let's be kind to the survivors. *Proceedings of the National Academy of
307 Sciences of the United States of America*, 118(2). <https://doi.org/10.1073/pnas.2002546117>

308 Lamarre, G. P. A., Mendoza, I., Rougerie, R., Decaëns, T., Héault, B., & Bénélu, F. (2015). Stay Out
309 (Almost) All Night: Contrasting Responses in Flight Activity Among Tropical Moth Assemblages.
310 *Neotropical Entomology*, 44(2), 109–115.

311 Lewis, T., & Taylor, L. R. (1965). Diurnal periodicity of flight by insects. *Transactions of the Royal
312 Entomological Society of London*, 116(15), 393–435.

313 Lima, M. C. F., de Almeida Leandro, M. E. D., Valero, C., Coronel, L. C. P., & Bazzo, C. O. G. (2020).
314 Automatic detection and monitoring of insect pests—A review. *Collection FAO: Agriculture*, 10(5),
315 161.

316 Mathis, A., Mamidanna, P., Cury, K. M., Abe, T., Murthy, V. N., Mathis, M. W., & Bethge, M. (2018).
317 DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nature
318 Neuroscience*, 21(9), 1281–1289.

319 Mathis, M. W., & Mathis, A. (2020). Deep learning tools for the measurement of animal behavior in
320 neuroscience. *Current Opinion in Neurobiology*, 60, 1–11.

321 Matikainen-Ankney, B. A., Garmendia-Cedillos, M., Ali, M., Krynetsky, J., Salem, G., Miyazaki, N. L.,
322 Pohida, T., & Kravitz, A. V. (2019). Rodent Activity Detector (RAD), an Open Source Device for
323 Measuring Activity in Rodent Home Cages. In *eneuro* (Vol. 6, Issue 4, pp. ENEURO.0160–19.2019).
324 <https://doi.org/10.1523/eneuro.0160-19.2019>

325 Nath, T., Mathis, A., Chen, A. C., Patel, A., Bethge, M., & Mathis, M. W. (2019). Using DeepLabCut for 3D
326 markerless pose estimation across species and behaviors. *Nature Protocols*, 14(7), 2152–2176.

327 Nunes-Silva, P., Hrncir, M., Guimarães, J. T. F., Arruda, H., Costa, L., Pessin, G., Siqueira, J. O., de Souza,
328 P., & Imperatriz-Fonseca, V. L. (2019). Applications of RFID technology on the study of bees.
329 *Insectes Sociaux*, 66(1), 15–24.

330 Pirie, T. J., Thomas, R. L., & Fellowes, M. D. E. (2016). Limitations to recording larger mammalian
331 predators in savannah using camera traps and spoor. *Wildlife Biology*.
332 [https://bioone.org/journals/Wildlife-Biology/volume-22/issue-1/wlb.00129/Limitations-to-](https://bioone.org/journals/Wildlife-Biology/volume-22/issue-1/wlb.00129/Limitations-to-recording-larger-mammalian-predators-in-savannah-using-camera/10.2981/wlb.00129.full)
333 [recording-larger-mammalian-predators-in-savannah-using-camera/10.2981/wlb.00129.full](https://bioone.org/journals/Wildlife-Biology/volume-22/issue-1/wlb.00129.full)

334 Smith, J. L., Palermo, N. A., Theobald, J. C., & Wells, J. D. (2016). The forensically important blow fly,
335 *Chrysomya megacephala* (Diptera: Calliphoridae), is more likely to walk than fly to carrion at low
336 light levels. *Forensic Science International*, 266, 245–249.

337 Smith, P. H. (1983). Circadian control of spontaneous flight activity in the blowfly, *Lucilia cuprina*.
338 *Physiological Entomology*, 8, 73–82.

339 Tadres, D., & Louis, M. (2020). PiVR: An affordable and versatile closed-loop platform to study
340 unrestrained sensorimotor behavior. *PLoS Biology*, 18(7), e3000712.

341 Taylor, L. R., & Carter, C. I. (1961). The analysis of numbers and distribution in an aerial population of
342 macrolepidoptera. *Transactions of the Royal Entomological Society of London*, 113(12), 369–386.

343 von Ziegler, L., Sturman, O., & Bohacek, J. (2021). Big behavior: challenges and opportunities in a new
344 era of deep behavior profiling. *Neuropsychopharmacology: Official Publication of the American
345 College of Neuropsychopharmacology*, 46(1), 33–44.

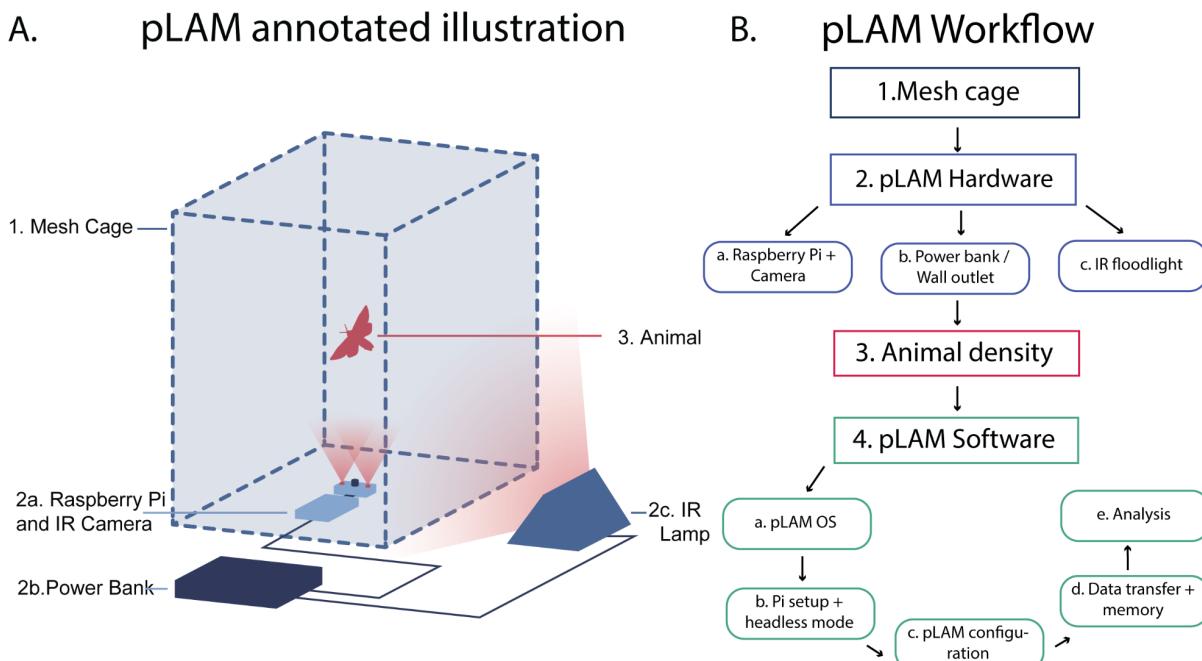
346 Wagner, D. L. (2020). Insect Declines in the Anthropocene. *Annual Review of Entomology*, 65, 457–480.

347 Wright, E. J., & Morton, R. (1995). Daily flight activity of *Trogoderma variabile* (Coleoptera:
348 Dermestidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Journal of Stored Products
349 Research*, 31(3), 177–184.

350 Yang, L. H., Postema, E. G., Hayes, T. E., Lippey, M. K., & MacArthur-Waltz, D. J. (2021). The complexity of
351 global change and its effects on insects. *Current Opinion in Insect Science*, 47, 90–102.

352

353 **Figures**



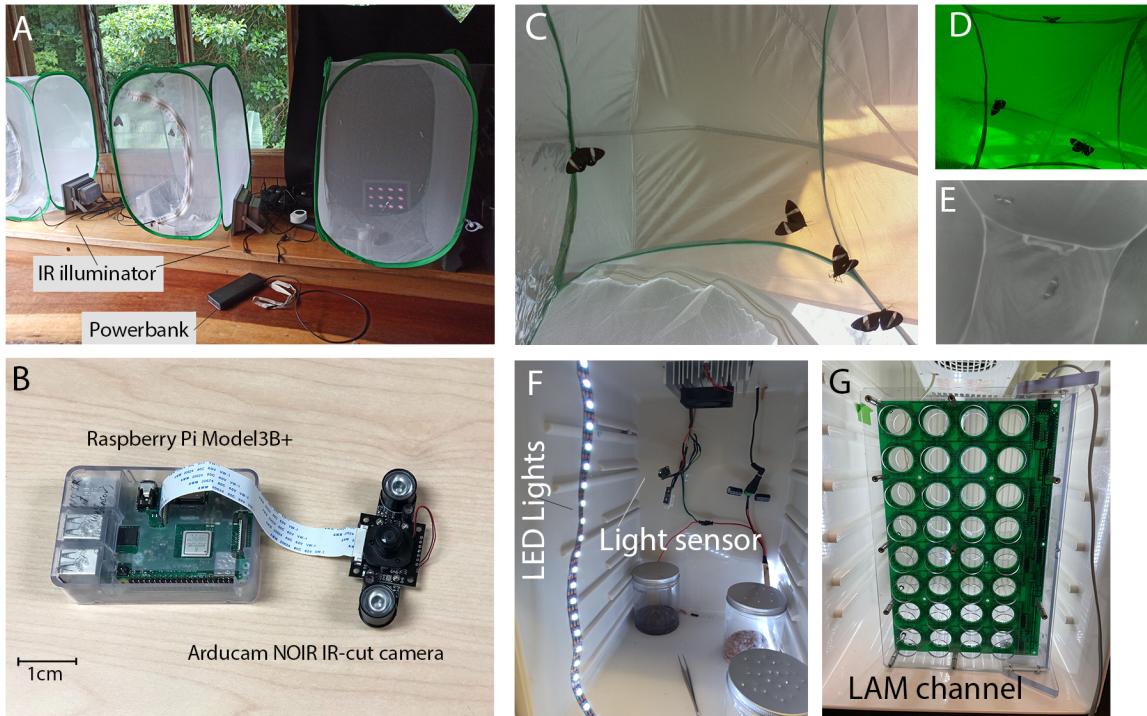
354

355

356 **Figure 1:** Annotated Illustration of pLAM A: 1. Mesh Cage, 2a. Raspberry pi and camera, 2b. Power bank,
357 2c IR floodlight 3. Small animal B: pLAM workflow (See Appendix 1-2 for more details)

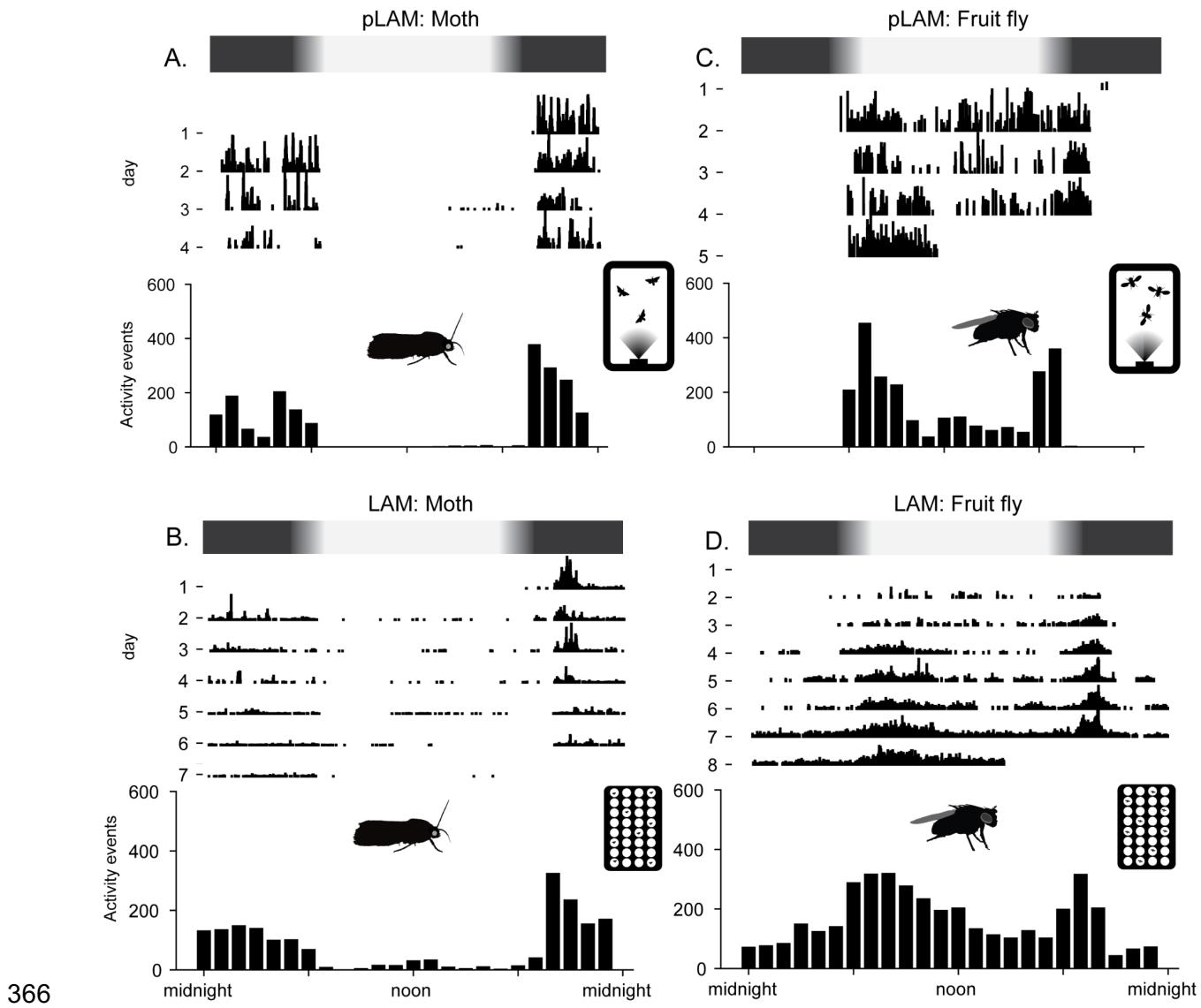
358

359



360

361 **Figure 2:** Different components of the activity chamber; A: Mesh tent containing raspberry pi and
362 power bank. B: Raspberry pi and camera C-E: Image of multiple animals (moth:*Hypocrita excellens*) as
363 seen by the pLAM under various light conditions C: Day view with visible light (IR-cut Filter) D: Day
364 without IR cut filter, E: Night without IR filter F: Rearing Incubator with controllable lights and light
365 sensor G: Commercial 32 channel LAM (TriKinetics) in modified incubator



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367
368 **Figure 3:** Comparison of pLAM data with LAM data for two different sized animals. A: pLAM data for
369 Wax moth (*Galleria mellonella*, body size ~10 mm); B: LAM data for *G. mellonella*
370 C-D: C: pLAM data for Fruit fly (*Drosophila melanogaster*, body size ~3 mm); D: LAM data for Fruit fly
371 (*D.melanogaster*). Black and white bar at the top of each graph represents the light cycle.

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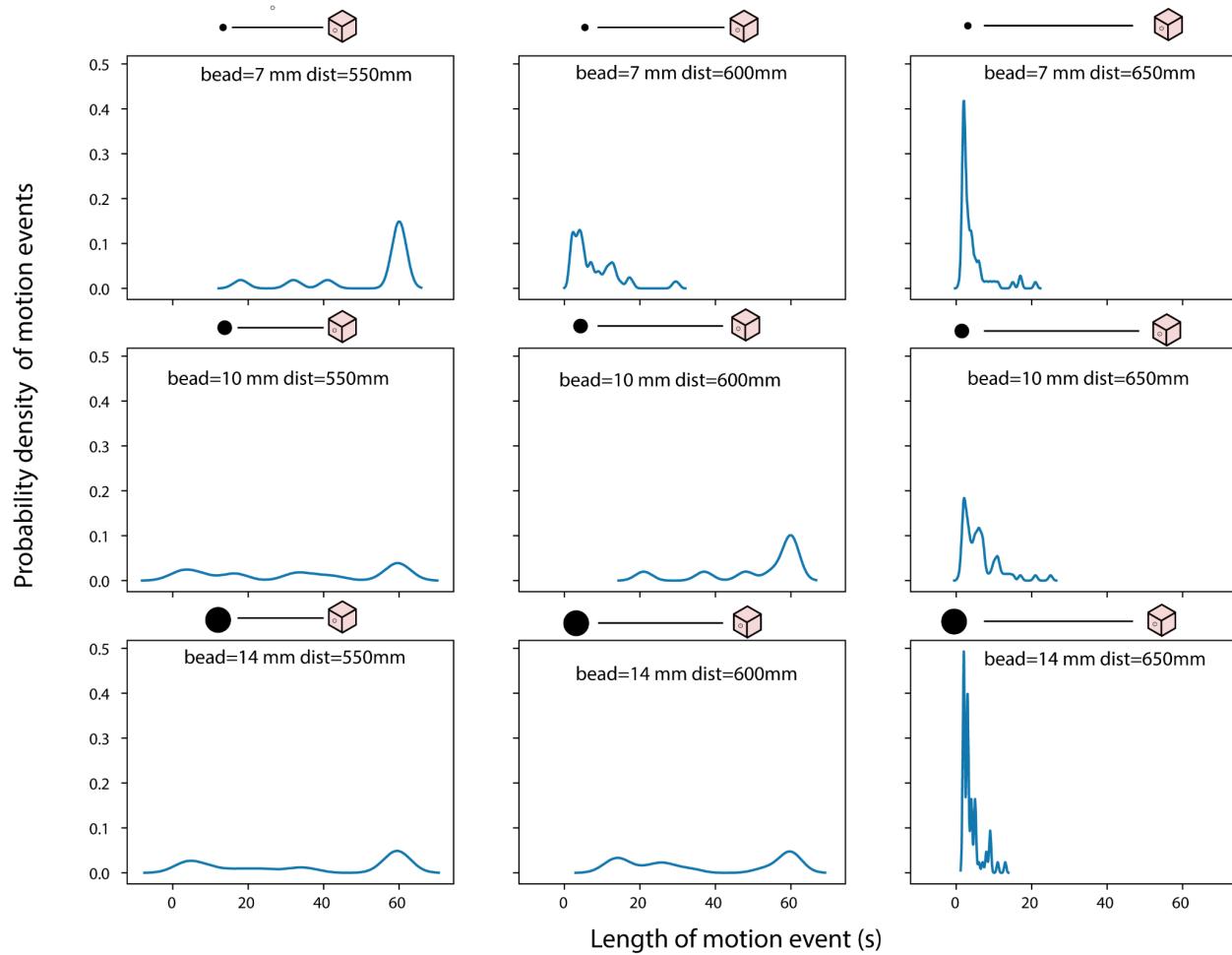
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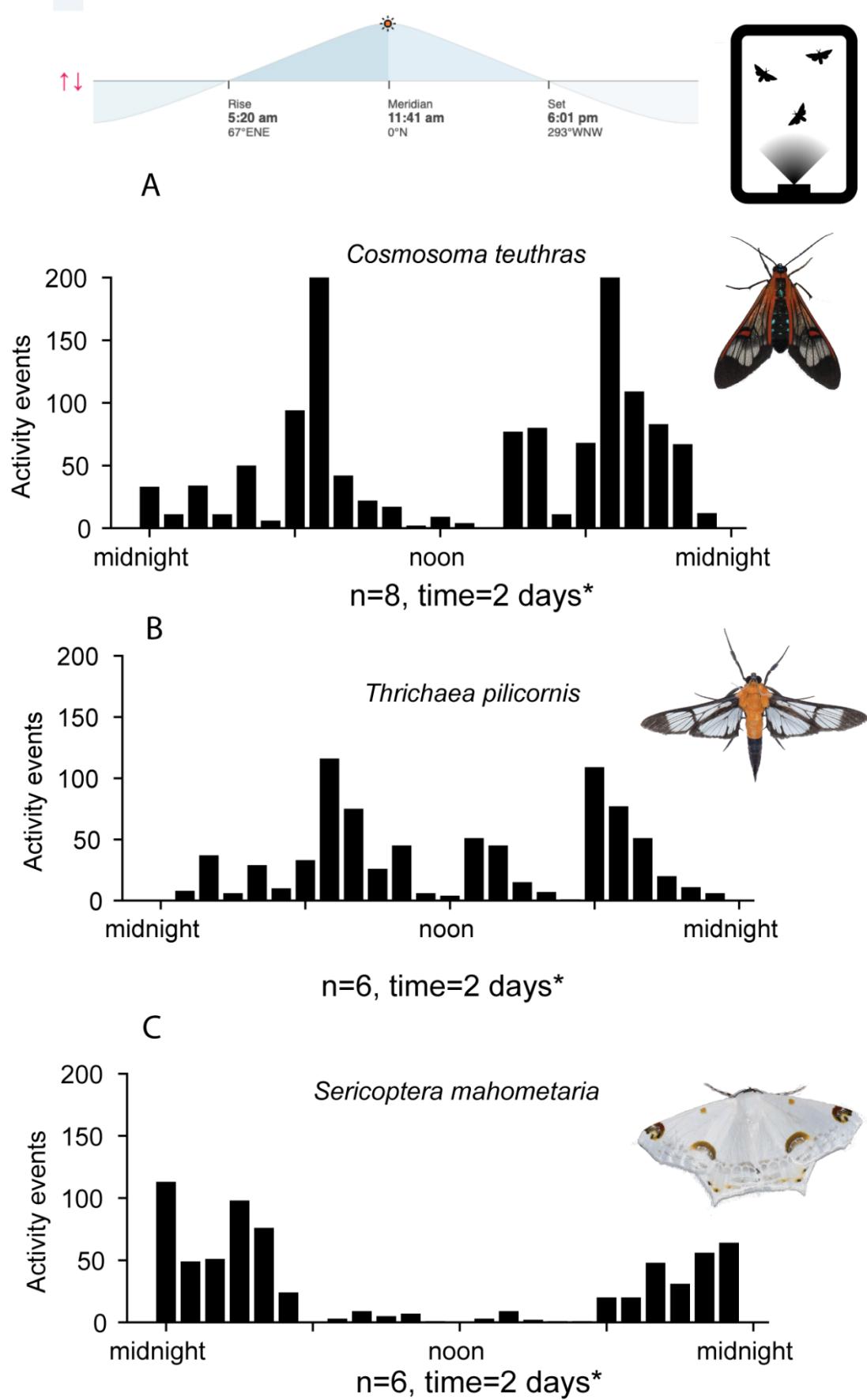
377

378 **Supplementary Figures**



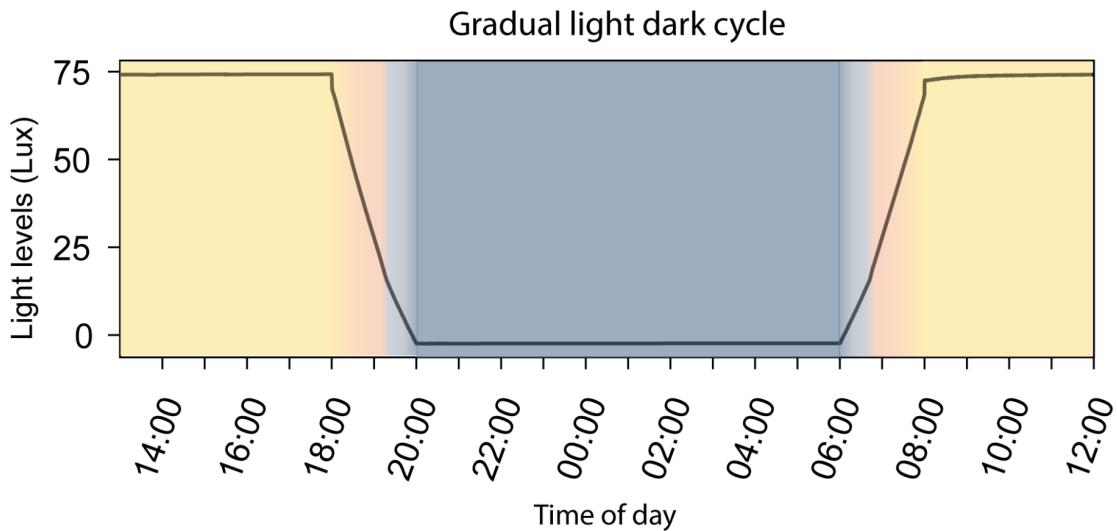
379

380 **Figure S1:** Benchmarking with beads as proxies for insects using different bead sizes and lengths from
381 the detector. The graphs depict the probability density function of the length of different motion events.



383

384 **Figure S2:** Data from field trials A-C, A: Erebidae: Arctiinae, *Cosmosoma teuthras*, n=8, t=2 days. B:
385 Crambidae: *Trichaea pilicornis*, n =6, t = 2 days. C: Geometridae: *Sericoptera mahometaria*, n = 6, t= 2
386 days. * no data for 4 hours during the afternoon on day 2 of trial due to a power failure. n = number of
387 individuals tested in one trial, time= duration of trial.



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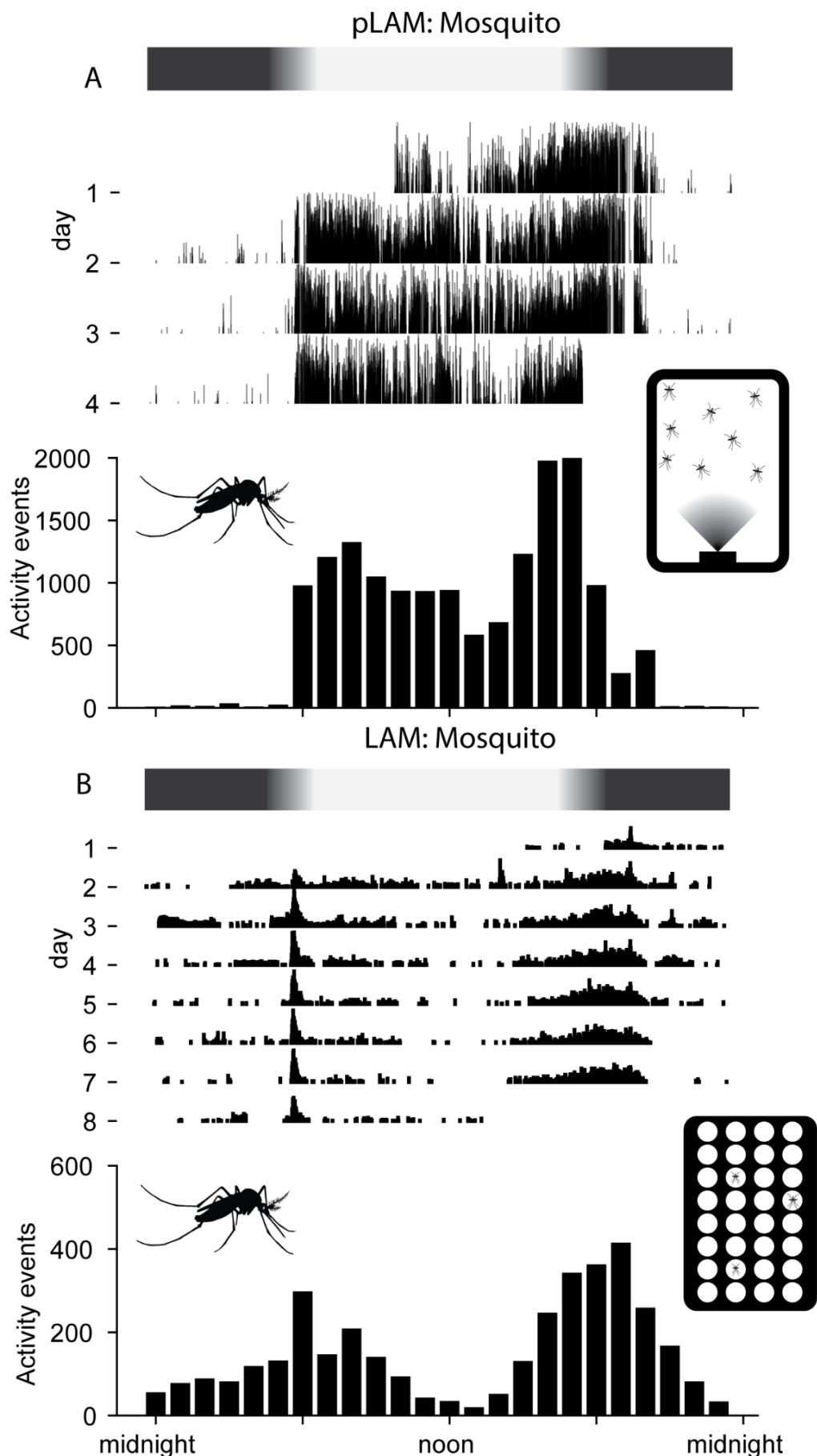
389 **Figure S3:** Gradual light dark cycle used in the lab for the pLAM and the LAMs. The absolute intensity
390 differed for different chambers, but the relative intensity was the same. The measurements were
391 obtained using a Adafruit TSL5291 sensor.

392



393

394 **Figure S4:** Field-based images of a light sheet, habitat and pLAM setup. A: Metal halide light in front of
395 at white sheet at Estación Biológica Monteverde (EBM), Costa Rica, B: Habitat around EBM C: pLAM in
396 tents at the field-station. D: View of the sky at dawn from EBM



398 **Figure S5:** Comparison of pLAM and LAM data for male mosquitoes (*Aedes aegypti*). A: pLAMs with 20-
399 25 male *A. aegypti*. B: LAMs with 32 channels with *A. aegypti*, data shown is only from males (n=16)
400 over time= 8 days.

401 **Supplementary Table S1:** Other available software that monitors circadian rhythm with information
402 about their cost, interface, user environment, tracking method and comments about their utility.

| Software Name | Open Source | Interface | Env. | Method | Comments | Link |
|-----------------|---|---------------|--------|----------------------------------|---|---|
| TriKinetics | No Hardware can be \$400 to upwards of \$3000 | GUI | App | IR beam arrays | Commonly used for recording insect locomotion in lab-reared insects. Works with LAM and DAM hardware | https://trikinetics.com/ |
| Big Brother | No \$40 a channel and paid software | GUI | App | Video camera recording movement | Uses time-based distance travelled and can be used to track circadian rhythm, not as useful for flying organisms | https://actimetrics.com/products/big-brother/ |
| “MouseActivity” | Yes | Command-based | MATLAB | Video camera | Uses a tablet to record video. Similar to pLAM, but is more stringent and seems to require better contrast with the background as it converts inverted grayscale image to binary and uses that to analyze after video is recorded. Optimised for walking behaviour with larger organisms and not applicable for outdoor field trials. | https://bmcr.esnotes.biomedcentral.com/articles/10.1186/s13104-020-4916-6 |
| “Tracker” | Yes; Not on github but on Brandeis website for free | Command-based | Java | Video camera mixed with IR beams | Similar methodology to pLAM by using text-based coordinates and images at 1 second intervals to record movement without recording a video. However, it uses a reference image to compare and requires a white background to contrast the dark flies and | https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0037250 |

| | | | | | | |
|------------------|--|----------------------------|--------|---|--|---|
| | | | | | so it cannot be applied to the outdoor field trials. Useful for recording long trials with low volume of data. | |
| ZooTracer | Yes | Command - based | OpenCV | Analysis pre-recorded video | Not directly used for circadian rhythm but it uses a pre-recorded video to analyze movement of unmarked individuals. Useful for the field but it can't detect what kind of animal is interacting and isn't useful for limiting storage usage | https://www.microsoft.com/en-us/research/project/zootracer/?from=https%3A%2F%2Fresearch.microsoft.com%2Fen-us%2Fprojects%2Fzootracer%2F |
| EthoVision XT | No. \$2000/year or \$6000 for a one-time license | GUI | N/A | Either pre-recorded video or live feed | Really expensive but has nice features including calibration features, setting field in view, and extras for specific species. Def not worth price tag | https://www.noldus.com/ethovision-xt |
| Behavior Cloud | No. \$990/year | App iOS/ mobile or mac/ PC | N/A | Either pre-recorded video or live feed | Analyzes data for you including movement tracking, coding complex behaviors, and even heart rate/EKG | https://www.behaviorcloud.com/ |
| Track 3D Insects | Price available on request (estimated cost 10-20k) | App | N/A | Records video to analyze | Tracks movement over time but it does track coordinates in 3D by using 2 separate cameras and even uses IR for night experiments. Custom solutions are designed for each lab. | https://www.noldus.com/track3d/insects |
| Graphite | Yes | GUI | App | Video recordings of human readable tags | Not directly used for diel-activity monitoring, but could be adapted for diel-activity monitoring if individual identity is | https://besjournals.onlinelibrary.wiley.com/doi/10.111/2041- |

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|--|--|--|--|--|----------|------------|
| | | | | | required | 210X.13200 |
|--|--|--|--|--|----------|------------|

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405 **Supplementary Table S2:** Studies that monitored small animal (arthropod) diel-activity, the species and
406 methods they used and comments about the study.

| Species/ Organism | Method | Reference | Comments |
|---|--|----------------------------|--|
| Multiple insect species | Suction traps | (Lewis & Taylor, 1965) | Emphasized how suction traps are less biased than light traps. |
| Beetles (dung, carrion) | Pitfall trapping | (Feer & Pincebourde, 2005) | Falling in the trap would kill insects using sodium chloride and detergent solution |
| Hawkmoths (<i>Manduca sexta</i> , <i>Hyles lineata</i>) | Periodic imaging with manual verification | (Broadhead et al., 2017) | Exposed moths to different light cycles in enclosed, individual spaces (separated by sex). Lab reared organisms |
| Moths | Light traps with automated movement detection | (Bjerge et al., 2021) | Does not report 24 hour diel-activity, but gives an idea of time of arrival at a light sheet |
| Arthropods | Interception traps with manual observation | (Basset & Springate, 1992) | Traps collected 3 times a day (5 AM, 12PM, 6 PM) |
| Blowflies (specified flying insects) | Disruption in electric field was used to measure flight activity | (Edwards, 1960) | There was no trapping here, lab reared blow-flies were used. |
| Fruitfly (<i>Drosophila melanogaster</i>) | Infrared beam based motion detector | (Chiu et al., 2010) | Lab reared flies in environmentally controlled incubators |
| Fruitfly (<i>Drosophila melanogaster</i>) | Motion capture camera/program | (Donelson et al., 2012) | Records exact location of fly in cage, adds to the locomotor tracking techniques that lacked this feature previously "Tracker program" |

407

408 **Supplementary Table S3:** Components, potential suppliers, and approximate cost in US dollars to build a
409 single pLAM.

| Sr. | Part name | Cost (USD) | Supplier | Comments |
|-----|-----------|------------|----------|----------|
|-----|-----------|------------|----------|----------|

| no. | | | | |
|-----|--------------------------------|-----------|--|--|
| 1 | Raspberry pi model 3+ kit | \$55 | CanaKit via amazon | Can buy the Pi separately for (\$35) and buy power supply and case separately |
| 2 | Adafruit NOIR camera V1 (5 MP) | \$30 | ArduCam via amazon | Can buy the 8 MP NOIR camera, but without the IR-cut feature images in the day are strangely coloured |
| 3 | Power bank/ Portable UPS | \$30-45\$ | Inui via amazon/Cyber power via amazon | A 20000 mAh power bank lasts approximately 12 hours. For longer use utilise wattage power banks/ portable UPS (Cyber power 750 VA). Alternatively connect to mains via low wattage UPS PiHat, CyberPower 325VA |
| 4 | Memory card | \$12-\$24 | Sandisk via amazon | 64GB or 128 GB are recommended depending on length of recordings required |
| 5 | Extra IR lights (optional) | \$30 | JC illuminator via amazon | Different brand of IR illuminators can work, use ones that turn-on automatically in low light |
| | | | | Total=\$127-\$184 |

410

411

412 **Supplementary Table S4:** Species tested in the field pLAM trials in Monteverde Costa Rica. * Unless
 413 mentioned otherwise, all species tested were moths. ** Had motion activity from the trees and requires
 414 additional filtering.

415

| No. | Family/Subfamily | Scientific name | Diel-period | Trial and number of individuals per trial | Comments |
|-----|------------------|-----------------|-------------|---|----------|
| | | | | | |

| | | | | | |
|---|------------------------|--------------------------------|--|-----------------------------|--|
| 1 | Erebidae: Arctiinae | <i>Agylla sp.</i> | Nocturnal with a peak at dusk | #1(n=6), #3 (n=6), #4 (n=5) | **Trial #3 and #4. |
| 2 | Erebidae: Arctiinae | <i>Cosmosoma teuthras</i> | Both diurnal and nocturnal, peak at dusk and a few hours after dawn | #1 (n=8) | Fig. S2 A |
| 3 | Pyralidae | <i>Trichaea pilicornis</i> | Diurnal with some limited dusk activity | #1 (n=6) | Fig S2. B |
| 4 | Geometridae: Ennominae | <i>Sericoptera mahometaria</i> | Nocturnal with a peak around midnight | #1(n=6) | Fig. S2 C |
| 5 | Erebidae: Arctiinae | <i>Dycladia correbioides</i> | Activity throughout the night and bursts of constant activity during the day. | #1 (n=12) | |
| 6 | Erebidae: Arctiinae | <i>Psoloptera sp.</i> | Diurnal | #1 (n=9) | |
| 7 | Erebidae: Arctiinae | <i>Halysidota sp.</i> | Mostly nocturnal, low levels of activity, peak around midnight | #3 (n=9) | |
| 8 | Erebidae: Arctiinae | <i>Cosmosoma pudicum</i> | Both diurnal and nocturnal, diurnal activity on 1st day, nocturnal activity on the second day | #3 (n=10), #4 (n=9) | 10 individuals were alive at the end of the trial (#3) ** |
| 9 | Erebidae: Arctiinae | <i>Hypocrita excellens</i> | Both diurnal and nocturnal, heavy diurnal activity paused for a few hours in the afternoon and continued till midnight. Peak at dusk | #3 (n=4) | ** |

| | | | | | |
|----|-------------------------|-----------------------------|--|-----------|--|
| 10 | Erebidae: Arctiinae | <i>Dycladia correbiodes</i> | Mostly nocturnal with less, but consistent activity during the day | #3 (n =1) | Repeated data with single individual |
| 11 | Pyralidae | | Mostly nocturnal with inconsistent dawn activity on a single day (**noise) | #3 (n=4) | Brown-silver Pyralidae ** |
| 12 | Geometridae : Ennominae | | Mostly nocturnal. Inconsistent diurnal dawn activity on a single day (**noise) | #4(n=8) | Silver ** |
| 13 | Erebidae:Erebinae | | Mostly nocturnal, low basal activity on day 1 (**noise) | #4(n=8) | Orange brown Erebidae ** |
| 14 | Pyralidae | | Nocturnal | #4 (n=9) | White Pyralidae. ** |
| 15 | Wasp* | | Both nocturnal and diurnal. But most activity stopped after day 2 | #4(n=4) | Only 1 individual survived for both days. ** |
| 16 | Jewel Beetle* | <i>Chrysina limbata</i> | | #1 (n=5) | |