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Low-cost automated flight intercept trap for the temporal sub-sampling of flying insects attracted to artificial light at night

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TITLE:
Low-Cost Automated Flight Intercept Trap for the Temporal Sub-Sampling of Flying Insects Attracted to Artificial Light at Night

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KEYWORDS:
arthropod sampling, ecological methods, insect sampling, intercept trap, light trap, malaise trap, time-specific sampling, window trap

SUMMARY:
To study the impacts of artificial light at night (ALAN) on nocturnal flying insects, sampling needs to be confined to nighttime. The protocol describes a low-cost automated flight intercept trap that allows researchers to sample at user-defined periods with increased replication.

ABSTRACT:
Sampling methods are selected depending on the targeted species or the spatial and temporal requirements of the study. However, most methods for passive sampling of flying insects have a poor temporal resolution because it is time-consuming, costly and/or logistically difficult to perform. Effective sampling of flying insects attracted to artificial light at night (ALAN) requires sampling at user-defined time points (nighttime only) across well-replicated sites resulting in major time and labor-intensive survey effort or expensive automated technologies. Described here is a low-cost automated intercept trap that requires no specialist equipment or skills to construct and operate, making it a viable option for studies that require temporal sub-sampling across multiple sites. The trap can be used to address a wide range of other ecological questions that require a greater temporal and spatial scale than is feasible with previous trap technology.

INTRODUCTION:
There are many arthropod sampling techniques[1–3], but ecologists often have difficulty applying these methods in ways that are appropriate to their research questions (see[4]). When choosing an appropriate method for sampling insects, ecologists must consider the targeted

species, time, effort, and cost involved in different techniques. For example, a common limitation is that it can be logistically challenging to sub-sample during specific time periods over replicated sites to quantify temporal variables which influence species activity, such as changes in weather or circadian activity (but see[5]). Most passive-survey insect traps are set for long periods (e.g., over multiple days, weeks, or even months), lacking fine-scale temporal resolution[1]. For surveys targeting specific time periods across multiple replicate sites (such as nocturnal sampling only across distinct sites), a large team may be required to visit sites over multiple days at the same time points (e.g., within 30 min of sunrise and sunset) to collect specimens and reset traps[6]; otherwise, an automated trapping device is required[5,7,8].

There is a growing field of work on the impacts of artificial light at night (ALAN) on insect activity patterns and localized population dynamics[9,10]; and on the interactions between ALAN and rates of insect predation[4,11–13]. However, to study the impacts of ALAN on nocturnal insect taxa, sampling needs to be confined to nighttime. Several different active light traps have been described and used for automated temporal sampling of nocturnal insects[14]. Some examples include simple falling disk-type separation devices, where the catch falls into a narrow tube with a disk falling every hour to separate the catch[15], or turn-table separation devices that rotate collection bottles at timed intervals[7,16,17]. These previous automated light traps address the sampling challenges involved with temporal survey requirements but are often large and unwieldy and use outdated or unreliable technology. A new automated passive sampling device was recently developed and tested[8]. This device utilized a commercially available flight-interception trap paired with a lightweight custom-designed collection device consisting of a turn-table holding sampling cup that allows for collecting trap contents at user-defined intervals[8]. This new automated trap employs sophisticated programming that can be operated by a smartphone but is prohibitively expensive to build at around EURO 700 (AUD 1,000) per trap[8].

Flight intercept traps are one of the most efficient ways to survey flying insects[1,18,19] and work on the principle that flying insects fall to the ground when they collide with a vertical surface. Flight intercept traps come in a variety of designs. However, most are typically constructed with a transparent or mesh surface and a collecting container filled with water and/or a preservative. The new trap described here uses a cross vane/baffle type or multidirectional intercept trap[20], given that cross baffles have been shown to increase capture rates[14,21] and sample insects from all directions. The purpose of this trap is to survey nocturnal flying insects that are attracted to artificial lights. This phototaxis results in insects circling the light source[22]; hence a multidirectional trap is most suitable.

Described here is a low-cost automated intercept trap that requires no specialist equipment or skills to construct and operate. The trap uses a commercially available automated pet food dispenser and common items available from hardware stores. This design costs less than EURO 66 (AUD 105) per trap to construct (**Table 1**), making them a viable option for studies requiring temporal sub-sampling across multiple sites simultaneously.

PROTOCOL:

1. Trap construction

NOTE: All of the components required to build the traps can be found in the **Table of Materials**. Each trap was constructed as shown in **Figure 1** and **Figure 2** by one person within 2 h.

1.1. Use a jigsaw to cut the polycarbonate roofing sheets (8 mm x 610 mm x 2400 mm) into 610 mm x 230 mm sections (**Figure 1**, item 1 & 2). Then cut a 8 mm center groove halfway up the center of each (610 mm x 230 mm) pane to allow the two panes to slide together to form a cross baffle.

1.2. Slide the crossed baffles snuggly into the plastic funnel opening (**Figure 1**, item 4) and secure them to the funnel with 20 mm stainless steel angle brackets (Fig 1, item 5b).

1.3. With the angle bracket in place, pre-drill holes and then use M4 screws and nuts with washers (**Figure 1**, item 6b) to secure the cross baffles to the funnel.

1.4. Again using the jigsaw, cut a piece of the polycarbonate sheeting (230 mm x 305 mm) from remaining sheets and secure with 20 mm stainless steel angle brackets (**Figure 1**, item 5a) at a 90° angle to the top of the crossed baffles to form a protective roof (**Figure 1**, item 3).

1.5. With the angle bracket in place, pre-drill holes and then use M4 screws and nuts with washers (**Figure 1**, item 6a) to secure the roof to the baffle.

1.6. Trim the funnel spout to a length of ~30 mm with a hacksaw to ensure the sample trays of the automated pet feeder will rotate unobstructed at the programmed intervals.

1.7. Place the automated pet dispenser (**Figure 1**, item 8) within a 9 L (38 mm diameter) plastic basin (**Figure 1**, item 7) to protect the samples from weather conditions.

1.8. Drill a 20 mm hole into the top of the 9 L basin and place the funnel spout into the hole to position it directly above the sample tray.

1.9. Using a drill with a hex-head driver bit, secure the plastic basin covering the pet feeder to a 500 mm piece of treated pine fence paling (**Figure 1**, item 9) with galvanized hex-head screws (**Figure 1**, item 14).

1.10. To stabilize the entire trap for hoisting into the air via ropes, attach a wooden stake (17 x 17 x 1200 mm, **Figure 1**, item 10) to the piece of treated pine fence paling (**Figure 1**, item 9) with an angle bracket and tie wire (**Figure 1**, items 13, 15 and 16).

[Place **Figure 1** here]

2. Trap deployment

NOTE: The traps were attached to trees 6 m above the ground (directly below the experimental or control lights) to capture flying insects (**Figure 2**). Emptying and collection of traps were done by three people on a single day. Further days can be sampled if required by lowering the trap to remove the collected samples, resetting the pet food dispensers, and placing the trap back in position every three days based on the sampling regime.

[Place **Figure 2** here]

2.1. Once at the sampling location, remove the automated pet food dispenser (**Figure 1**, item 8) from beneath the plastic basin.

2.2. Open up the automated pet food dispenser (**Figure 3A**) and place foil dishes containing soapy water or a preservative of choice in each food tray (**Figure 3B**, here 20 mL of propylene glycol was used as a preservative).

2.3. Follow the directions provided with the automated pet food dispenser to set the food tray rotation times. First, set the clock time, then program each pet food dispenser tray.

NOTE: The automated pet food dispenser rotates food trays (1–6) at pre-programmed times. The 6-meal bowls can be set to open at any time of the day or night, with the trays rotating in sequential order. For this study, the aim was to sample nocturnal and diurnal insects separately. Tray 1 sampled from 8 PM on the first night and then moved to tray 2 at 7 AM the following morning, followed by tray 3 at 8 PM, tray 4 at 7 AM, tray 5 at 8 PM and tray 6 at 7 AM. A delay function allowed for a one-day delay in sampling because it took 2 days to set up all sites, thereby ensuring sampling commenced on the same day/time at all sites.

[Place **Figure 3** here]

2.4. Place the automated pet food dispenser back underneath the plastic basin and secure the basin to the timber fence piece with the galvanized hex-head screws (**Figure 1**, item 14).

2.5. Attach a rope to the top of the trap with a karabiner (**Figure 1**, item 17). With the use of a ladder, hoist the trap into position and secure it beneath experimental lights by the karabiner.

2.6. Attach a second wooden stake (17 mm x 17 mm x 1200 mm, **Figure 2**B) to the tree (or lamp post) with an angle bracket to stabilize the trap in high winds.

2.7. The trap sits on top of the stake; secure it with two large cable ties (**Figure 2B**).

2.8. To collect insect samples, lower the traps with a rope. Remove the automated pet food dispenser from beneath the plastic basin.

2.9. Remove the lid of the pet food dispenser (**Figure 3A**) and lift the aluminum trays out to pour the contents into pre-labeled sample vials (**Figure 3B**).

REPRESENTATIVE RESULTS:

The traps were trialed in a survey of flying insects attracted to experimental lighting at four bushland reserves across Melbourne, Australia. Sites consisted of either remnant or revegetated bushland surrounded by residential housing and averaged 15 km apart (range 3–

24 km) and 45 ha in size (range 30–59 ha). A total of sixteen traps were installed, four at each site, with and without experimental lights (3 lights and 1 control per site), and surveyed for 3 days and 3 nights from 30 March to 2 April 2021. Installing the traps took a team of two people 2 days, but by utilizing the delay function on the pet food dispenser, sampling commenced at the same time and day for all traps.

The traps operated under variable weather conditions (6.7–29.5 °C, nighttime minimum, and daytime maximum temperatures; 17–46 km/h maximum wind gusts), including rain, without any failures or rain flooding the collecting trays. A total of 488 flying insects were captured over the three sampling days, with 374 from nocturnal sampling and 114 from diurnal sampling. All non-flying taxa (Arachnida, Isopoda, Myriapoda, and Formicidae) were excluded. To evaluate the efficiency of the traps, divide the total number of arthropods collected (488) by the effective surface area (23 cm x 61 cm) of each trap (1403 cm^2) by the number of trap-days (16 traps x 3 days) they were operated (48)[23]. This yielded a value of 0.007 insects/cm^2/trap-day, which is within the range of other studies using flight intercept traps (**Table 2**). The difference between traps placed under lights and those not under lights (i.e., controls) was also examined, as lit traps would effectively become an active light trap and should therefore have increased capture rates (**Table 2**). Hence, the traps appear to be as effective as traditional flight intercept traps but with the added benefit of sub-sampling at user-defined time periods.

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic diagram of trap construction. (1 and **2)** 610 mm x 230 mm x 8 mm polycarbonate sheets; **(3)** 230 mm x 305 mm x 8 mm polycarbonate sheet; **(4)** 24 cm diameter plastic funnel; **(5a–b)** 20 mm angle brackets; **(6a–b)** M4 x 15 mm screws, washers & nuts; **(7)** 38 cm diameter 9 L plastic basin; **(8)** automated pet food dispenser; **(9)** 150 mm x 12 mm treated pine paling; **(10)** 17 mm x 17 mm x 1200 mm wooden stake; **(11)** 125 mm x 150 mm angle bracket; **(12)** 16 mm x 16 mm hex-head screws; **(13)** angle bracket; **(14)** 16 mm x 16 mm hex-head screws; **(15** and **16)** wire stabilizer; **(17)** karabiner, used for lowering and raising into position.

Figure 2: Low-cost automated flight intercept trap for sampling insects at user-defined time points. **(A)** Polycarbonate crossed baffles serve as the flight intercept area that allows for collecting insects from all four sides. The polycarbonate roof serves to direct insects downwards and protect the collected samples from the weather. The funnel beneath the flight intercept barrier servers to funnel insects that have collided with the polycarbonate barriers into the collecting trays housed within the circular basin. **(B)** Trap suspended below experimental light and secured to the tree by a wooden stake and angle bracket. The plywood box below the intercept trap contains a bat detector used to passively record echolocation calls produced by free-ranging insectivorous bats.

Figure 3: Automated pet food bowl. (A) Battery-operated 6-meal pet food bowl used to sample insects at user-defined intervals. The food bowls were programmed on alternating schedules to sample nocturnal and diurnal insects. For example, tray 1 opened at 8 PM (nocturnal day 1), tray 2 opened at 7 AM (diurnal day 1), tray 3 opened at 8 PM (nocturnal day 2), tray 4 opened at 7 AM (diurnal day 2), tray 5 opened at 8 PM (nocturnal day 3), and tray 6 opened at 7 AM (diurnal day 3). **(B)** Lid removed from automated pet food bowl to

show the six collection trays. Foil dishes containing propylene glycol as a preservative allowed for the easy removal of the collected insects.

Table 1: Design cost of automated intercept trap. The table lists the cost and source of all the components required to build the trap.

Table 2: Comparison of relative capture efficiency of various flight intercept traps. To calculate the number of arthropods/cm^2/trap-day, divide the total number of insects collected by the effective surface area of each trap by the number of trap-days they were operational[23].

DISCUSSION:

Despite the automated flight-intercept trap described by Bolliger et al. (2020)[8] being well designed and very effective at sampling at user-defined time periods, they are likely to be cost-prohibitive for many researchers. This study shows that passive trapping surveys using automated traps for sub-sampling flying insects at user-defined periods can be carried out on a modest budget. Traps were built to sample at six pre-defined time points by utilizing a commercial pet food dispenser and materials commonly available from hardware stores, without any specialized skills, for a tenth of the cost required to build a Bolliger et al. (2020)[8] trap. Professional electronic and mechanical knowledge is also required to build the Bolliger et al. (2020)[8] automated flight-intercept traps at a cost of EURO 700 (AUD 1,000) per trap. Similar quotes were obtained locally for the construction of traps based on the Bolliger et al. (2020)[8] design, with the most competitive being AUD 937 per trap.

The Bolliger et al. (2020)[8] paper failed to recognize any of the older entomology literature and stated, “there were no current time-interval sampling devices for insects”. This is not the case, as time-interval or sub-sampling devices have been used in a number of studies since 1934[14]. However, these older devices were large and most often operated as single units (see Figure 1. In Steinbauer, 2003[5]); hence up-scaling to a number of devices for replication that could be mounted at height (i.e., 5–6 m) would be difficult.

The new trap design described here was as effective as other flight-intercept traps (**Table 2**) despite trapping occurring immediately following a full moon, with lunar illumination known to reduce catches[24] and in the austral autumn when insect activity is beginning to decline[25]. Capture rates would be expected to increase during more favorable seasons and weather conditions. Each collection tray has a 330 mL capacity to accommodate most applications, but it would be beneficial to test during swarming events to ensure collection trays do not overfill. These traps can be used for both passive and active sampling of flying insects and will have a broad range of applications in studies that require greater temporal resolution in flying insect collection than was previously possible.

With major insect declines being reported worldwide[26,27], the key roles insects play in ecosystem services and trophic interactions have generated ecological concern[28] and debate[29]. Our current understanding of these declines is insufficient to identify the drivers, and to date, there have been modest attempts at understanding spatial, temporal, and taxonomic factors[30]. One area of growing concern is the role that ALAN has as a driver of insect activity, community composition and decline[31,] and nocturnal species are especially impacted by changes in natural light cycles. To correctly survey insect responses to ALAN, nocturnal synchronous sampling at defined time periods (i.e., nighttime only) across a number of replicated sites and treatments cannot be accurately performed using manual traps without high labor intensity, the trap described here provides a novel and low-cost solution to address these research questions.

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DISCLOSURES:
None

SUPPLEMENTARY FILES:
Data are available from the Dryad Data Repository: http://doi.org/10.5061/dryad.gqnk98sp1

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Figure 1

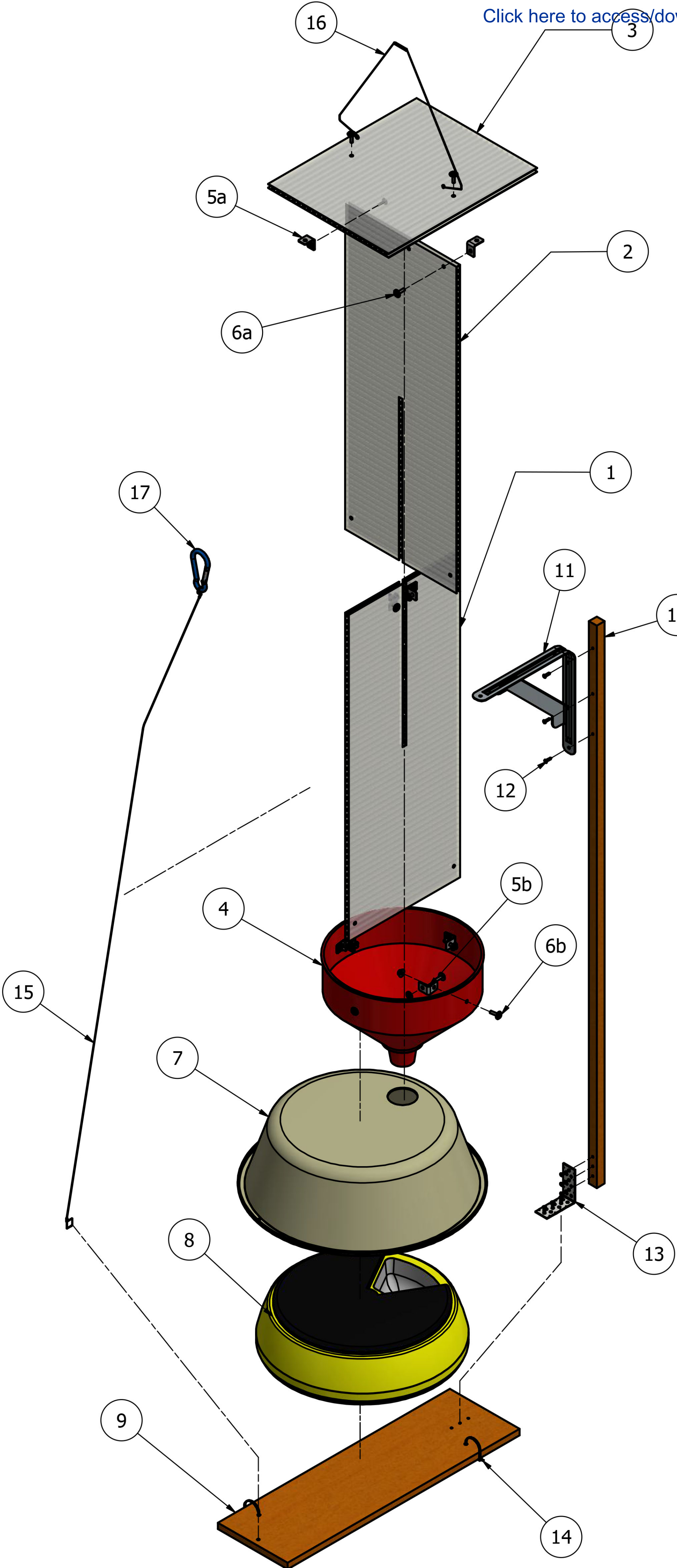

Figure 2

Figure 3

Table 1

Material	Number needed per trap	Cost AUD (per trap)
Batteries (C cell) – 10 pack	4	16.70 (6.68)
Battery operated automated 6 meal pet food bowl – each	1	59.00 (59.00)
Galvanised hex-head screws (10-16 x 16 mm) – 100 pack	5	17.54 (0.87)
Galvanised steel angle bracket (125 x 150 mm) – each	2	1.58 (3.16)
Galvanised tie wire (0.70 mm x 75 m) – per roll	~2 m	5.00 (0.13)
Plastic basin (38 cm, 9 L round) – each	1	4.50 (4.50)
Plastic funnel (24 cm) – each	1	4.99 (4.99)
Stainless steel angle bracket (20 mm) – 16 pack	8	4.73 (2.37)
Stainless steel screws & nuts (M4 x 15 mm) – 18 pack	16	3.56 (3.16)
Stainless steel washers (3/16” & M5) – 50 pack	16	4.98 (1.59)
Sunlite Polycarbonate roofing sheet (8mm x 610 mm x 2.4 m) – each	Each sheet makes 4 traps	60.00 (15.00)
Treated pine paling (150 x 12 mm) – each	1/3	1.60 (0.53)
Wooden stakes (1200 x 17 x 17 mm) – 10 pack	2	12.99 (2.60)
Total cost per trap		**AUD 104.58**

Table 1

Trap type[1]	Total captures	Trap effective surface area (cm^2)	Number of trap-days (# traps x # days)	Number arthropods/cm^2/trap-day
Novel	1,609	550	432	0.007
Window	1,241	3,721	6	0.056
Window	1,107	3,686	140	0.002
Window	3,540[#]	9,600	150	0.002
Window	30,530	26,000	2,160	0.00050
Window	428	623.7	1,860	0.0004
Multi	10,161	1,378	1,825	0.004
Multi	15,000	10,800	804	0.002
Multi	230,162	1,200	40,500	0.005
*Multi	1,360	1,680	1,548	0.0005
Multi	12	1,680	516	0.00001
*Multi	2,725	1,000	142	0.019
*Multi (A)	2,991	1,000	142	0.021
*Multi (A)	49,613	1,000	2,080	0.024
*Multi	1,625	1,000	264	0.006
*Multi (A)	449	1,403	36	0.009
Multi (A)	39	1,403	12	0.002

[1]Trap types:
Novel – not a standard window or multidirectional style trap
Window – single vane rectangular panel
Multi – multidirectional cross vane/baffle panels
Multi (A) – multidirectional automated trap.

*Denotes traps were positioned under lights
[#]Based on average catch; hence, number of trap days not multiplied by number of

Source
Carrel (2002)[23]
Chapman & Kinghorn (1955)[32]
Canaday (1987)[33]
Hill & Cermak (1997)[18]
Lamarre et al (2012)[19]
Burns et al (2014)[34]
Basset (1988)[35]
Russo et al (2011)[36]
Knuff et al (2019)[37]
Wakefield et al (2017)[6]
Bolliger et al (2020)[8]
Bolliger et al (2020)[8]
Bolliger et al (2020)[12]
This study

traps.

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Dear Dr Krishnan,

Thank you for considering our manuscript for publication, and the opportunity to submit revisions. We have revised the manuscript in line with your own comments and those of the two reviewers'. Our revisions are detailed below in red.

We trust that you will find our manuscript improved and now suitable for publication.

Yours sincerely

Dr Kylie Robert
Senior Lecturer
Department of Ecology, Environment & Evolution
La Trobe University

Editorial comments:
Editorial Changes
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. Done
2. Please provide an institutional email address for each author. Done, however author 3 is not affiliated with any institution
3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). This has been changed throughout, although we note that many existing papers in JOVE do not do the same.
4. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Done
5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Done
6. Please highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader. Done
7. Lines 246-252: Please remove the Table of Materials legend from the Figure and Table Legends. It is not required. Done
8. As we are a methods journal, please ensure that the Discussion explicitly covers the following in detail in 3-6 paragraphs with citations: We believe this has been covered but not necessarily in this order hence we have made an attempt to reorganise the discussion to better reflect this
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique
9. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s) before punctuation. Corrected throughout

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Reviewers' comments:
Reviewer #1:
Manuscript Summary:
This is a clever solution to the problem of sampling aerial arthropods which allows samples to be assigned to specified time periods. This has the potential to be useful to researchers who need to leave traps in situ for periods of more than a few hours, but need to be able to distinguish between samples collected at different times eg day versus night. The authors should be congratulated on developing this useful trap.

Major Concerns:
None

Minor Concerns:
Query 2nd author affiliation?? This author is not affiliated with an institution
Aerial arthropods - why not flying insects? We have changed to flying insects throughout
As far as I know, isopods and crustaceans don't fly, and neither do arachnids although these can balloon on the wind - but would not be attracted to light. Anyway, these were excluded from the analysis. Line 195 says "All non-flying taxa (Arachnida, 196 Isopoda, Myriapoda and Formicidae) were excluded." So think it would flow better if just said flying insects throughout.
Title is a bit wordy - why not "Low-cost automated flight intercept trap for sampling of flying insects attracted to artificial light at night" (already then says it is at night so don't need the other words) We have retained "temporal sub-sampling" in the title given this is the novelty of the method
Line 48. Add https://doi.org/10.3389/fevo.2020.579193 to sampling techniques? added
Line 66. Best stick to citing reviews?
Suggest: https://doi.org/10.1111/eea.12754, https://doi.org/10.1111/icad.12447, https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.4557 One of these suggested reviews is already cited here – Owens & Lewis 2018.
Line 192 - mentions temperature range and rainfall - not wind, which will also be a factor Included maximum wind gusts
Line 256 - stick to reviews here. Wagner good. Drop Hallmann. Done
Add: https://doi.org/10.1126/science.aax9931

Reviewer #2:
Manuscript Summary:
I congratulate the authors on a well-written manuscript and the design of the trap. The authors have developed an easy to construct, low-cost automated flight intercept trap for sampling aerial arthropod attracted to artificial light at night, where the researcher can define the time-period on each collection. This design will be very useful for insect collections specific to suit the researcher's objectives and to specifically target time periods (minutes or hours) during the night.

The authors correctly highlight limitations and uses of various light traps.

Major Concerns:
The manuscript is straight forward and in general has no faults.

Minor Concerns:
I would like a paragraph discussing potential large insect collections. I imagine that if used on a nightly basis (rather than hourly) or during periods of insect swarming the dispenser trays may overfill. Is it possible to address this somewhere in the discussion? We have added a statement to address this *"Each collection tray has a 330 ml capacity that will accommodate most applications, but it would be beneficial to test during swarming events to ensure collection trays do not overfill."*

L256. I would say this point is debatable. See Saunders, M. E., Janes, J. K., & O'Hanlon, J. C. (2019, July 16). Moving on from the insect apocalypse narrative: engaging with evidence-based insect conservation. https://doi.org/10.1093/biosci/biz143. In addition to other work by Saunders. Agree and added this reference

Tables 1 and 2 did not fit on the portrait page correctly and may need adjustment prior to proof. The tables have been submitted as excel files as required by the journal.

A video showing trap construction and operation would greatly enhance the reach of this paper. A video will be produced

Well done.