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SCIENTIFIC NOTE

AN INEXPENSIVE SYSTEM TO INVESTIGATE THE DAILY RHYTHMS OF INSECTS

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ABSTRACT. Insects' daily rhythms occur in response to their surrounding environment. Recognizing the daily rhythms of pathogen vectors can be helpful in developing effective, safe, and sustainable management strategies to control vector insects and reduce the spread of pathogens. However, studying the daily rhythm of insects often requires costly or labor-intensive trapping, and few tools are available to quantify daily rhythms in the field. We developed a simple collection system to study the flight activity of mosquitoes and biting midges using a contained, programmable, rotating, automatic pet feeder. A diverse assemblage of nuisance and vector species were collected with our system, including mosquitoes of the genera *Aedes*, *Anopheles*, *Culex*, and *Deinocerites* and biting midges (Ceratopogonidae) such as the coastal pest *Culicoides furens*. Surprisingly, mosquitoes and biting midges were less active during crepuscular periods (1800-2100h; 0600-0900h) than during dark periods (2100h-2400h; 0300h-0600h). A number of urban and agricultural pest insects were captured, including Coleoptera, Hymenoptera, Isoptera and Lepidoptera. This study shows that relatively inexpensive products can be adapted to study the daily rhythms of flying vectors and nuisance arthropods, with implications for vector-borne disease transmission and control. The collection system could also be used with flight intercept or pitfall traps, permitting study of the circadian activity patterns of a diverse array of arthropods.

KEY WORDS Circadian rhythm, flight activity, vector control, vector-borne disease

Daily rhythms influence the biology and behavior of pathogen vectors and their interactions with vertebrate hosts, therefore shaping the severity and transmission of infectious diseases (Rund et al. 2016). Daily rhythms have been demonstrated in the flight activity (Jones et al. 1967, Taylor and Jones 1969), oviposition (Sumba et al. 2004), and blood-feeding (Fritz et al. 2014) in pathogen vectors, including medically important mosquito species such as *Aedes aegypti* (L.), *Anopheles gambiae* Giles, and *Culex pipiens* L.

Some *Aedes* spp., such as *Ae. aegypti,* are considered to be more active during the day, while most *Anopheles* spp. are generally active at night, both due to their biting behavior (Baik et al. 2020). Daily rhythms in *Ae. aegypti* suggest that their cyclical activity of oviposition and sugar-feeding is controlled by an endogenous rhythm that is triggered by a change from light to dark (Taylor and Jones 1969).

Recognizing the daily rhythms of pathogen vectors can be helpful in developing management strategies to control these vectors and reduce the spread of pathogens. The daily rhythm of blood-feeding insects can be used to predict when infectious bites will occur, allowing practices to minimize contact between blood-feeding insects and their hosts (Fritz et al. 2014). The daily rhythms of insects can also be used to time control measures such as insecticide applications to maximize their efficacy. Insecticide applications to control mosquitoes are usually performed during dawn and dusk since these are the times when most mosquito activity occurs (Cilek et al. 2008). However, some pesticides experience rapid degradation in the environment, which can limit the efficacy against the target species if applications are timed incorrectly but can be useful to minimize non-target effects in beneficial insects when applications are timed correctly (Giunti et al. 2022).

In this article, we report the development of an inexpensive, automated collection system to study the daily rhythms of vector and nuisance arthropods. While a few novel systems have been designed to separate light trap catches (Wrenn 1980, Taylor et al. 1982) or pitfall trap samples (McMunn 2017) into discreet time periods, to our knowledge, the collection bottle rotator (Model 1512, John W. Hock Co., Gainesville, FL) remains the only commercially available trap to collect and automatically separate flying insects, particularly mosquitoes, into predetermined time-period samples. While the collection bottle rotator is effective, the cost of the rotational system is over 1,300 US dollars (2023) and does not include the cost of the light trap, battery, and other materials (support tripod, battery charger, attractants). Field-trapping costs often impose limitations on insect surveillance (Braz Sousa et al. 2020) and finding alternatives to costly and labor-intensive traps is of interest to the scientific community (Batista et al. 2018). Based on these considerations, the present design has been simplified from other designs available in the literature by easing its construction, reducing costs, incorporating existing commercial products (automatic pet feeder), and allowing the collection system to be adapted to diverse traps.

The collection system was developed to collect night-flying insects into predetermined time-period samples, consisting primarily of a light trap and programmable

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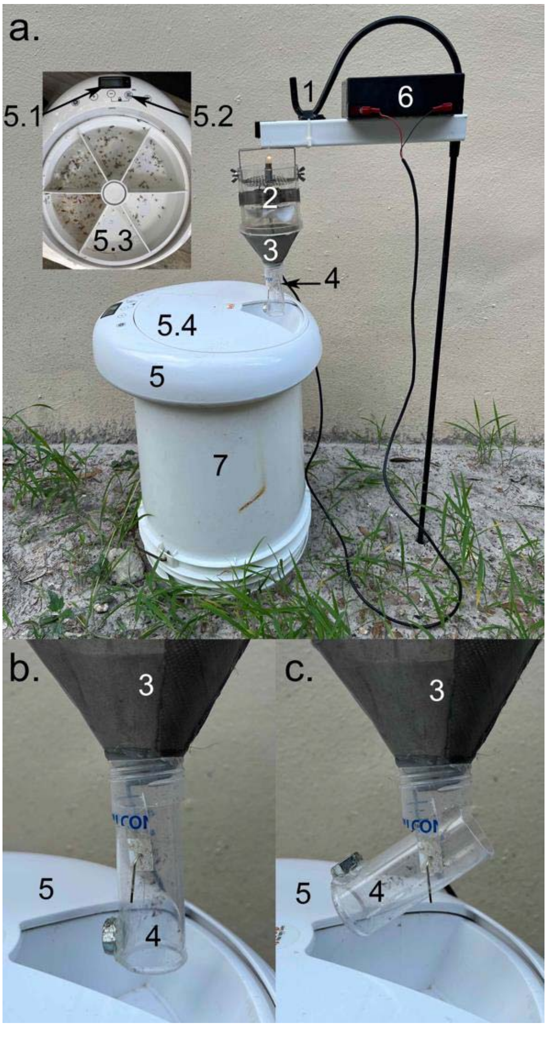

Fig. 1. (a) Schematic of the collection system: (1) shepherd hook ($10.98), (2) CDC miniature light trap, 6 VDC ($131.00), (3) stainless steel funnel, (4) section of the 50 ml Falcon tube, (5) rotating, automatic pet feeder ($54.99), (5.1) panel and timer, (5.2) settings buttons, (5.3) 3-ounce compartments with detergent solution, (5.4) top cover, (6) 6V rechargeable battery ($17.99), (7) reused plastic bucket to support the pet feeder; (b) close-up photo of the 6-cm portion of the 50ml Falcon tube modified to pivot around metal pin; (c) close-up photo of the portion of Falcon tube at the time of rotation. Not to scale. Dollar amounts in USD.

rotating pet feeder (Fig. 1). The trap used was a Centers for Disease Control and Prevention (CDC) miniature light trap (Model 2836 BQ, BioQuip, Rancho Dominguez, CA) baited with an incandescent yellow light bulb and powered by a 6-volt rechargeable battery that was replaced every day. The CDC miniature light trap included a stainless steel (316 stainless steel with 0.0145" opening, 53% open area; McMaster-Carr) funnel at the outflow in place of a collection chamber to deliver trapped insects directly into individual 3-oz (~85.2 ml) compartments of a programmable 6-compartment rotating automatic pet feeder (Model QPETS, Shenzhen Qpets supply Inc). Each compartment was filled with water and a drop of detergent so that insects would not be repelled by surface tension (Fig. 1). To ensure that insects were directly delivered into the detergent solution, a 6-cm portion of 50 ml Falcon tube was fastened to the funnel that positioned the outflow approximately 0.5 cm from the surface of the detergent solution. The section of Falcon tube pivoted around a metal pin so as to avoid obstructing the movement of the pet feeder compartments.

The collection system was initially operated daily for 2 weeks to make adjustments as needed and ensure that it functioned properly. Following the adjustment period, insects were sampled using the system for 10 nights between September 23 and October 17, 2022. Samples were collected into 3-h periods starting 1 h before sunset, at 1800h, and finishing approximately 1 h after sunrise, at 0900h. The trap was placed in a coastal hardwood hammock at the Florida Medical Entomology Laboratory in Vero Beach, Florida.

Samples were retrieved from the pet feeder with a turkey baster and transferred into 50 ml Falcon tubes. In the laboratory, specimens were identified to order, and insects of the order Diptera were further categorized as mosquitoes (Culicidae), biting midges (Ceratopogonidae: *Culicoides*), gall midges (Cecidomyiidae), and moth flies (Psychodidae). Biting midges and mosquitoes were identified to species level by external morphology using published keys (Blanton and Wirth 1979, Darsie and Ward 2004) where possible.

This collection system successfully trapped diverse insects, including groups with species of agricultural, medical, urban, and veterinary importance. The cost of the collection system was 214.96 US dollars (Fig. 1), which was at least 1,000 US dollars less expensive than the alternative. Insects from nine orders were collected with this collection system, including (in order of abundance) Diptera, Hymenoptera, Isoptera, Lepidoptera, Coleoptera, Collembola, Hemiptera, Odonata, and Trichoptera. Three-quarters of the total specimens collected over the 10 nights belonged to the order Diptera, including mosquitoes (730 total, 62.1%), moth flies (Psychodidae, 261 total, 22.2%), gall midges (Cecidomyiidae, 156 total, 13.3%), and biting midges (28 total, 2.4%). Wasps and ants (73 total, 4.9%), termites (51 total, 3.4%), moths (37 total, 2.5%), and beetles (24 total, 1.6%) were commonly sampled.

Biting midges, mosquitoes, and moths had a similar pattern of activity, where activity was greater between 2100h and midnight, and 0300h and 0600h, and lower numbers were captured in crepuscular periods and from midnight to 0300h (Fig. 2a). Seven species of mosquitoes from 5 genera were collected (Fig. 2b), most of which were *Deinocerites cancer* Theobald (78.6%), followed by species of *Culex* (*Melanoconion*) (9.7%), *Aedes taeniorhynchus* (Wiedemann) (6.5%), *Culex nigripalpus* Theobald (2.2%), *Anopheles*

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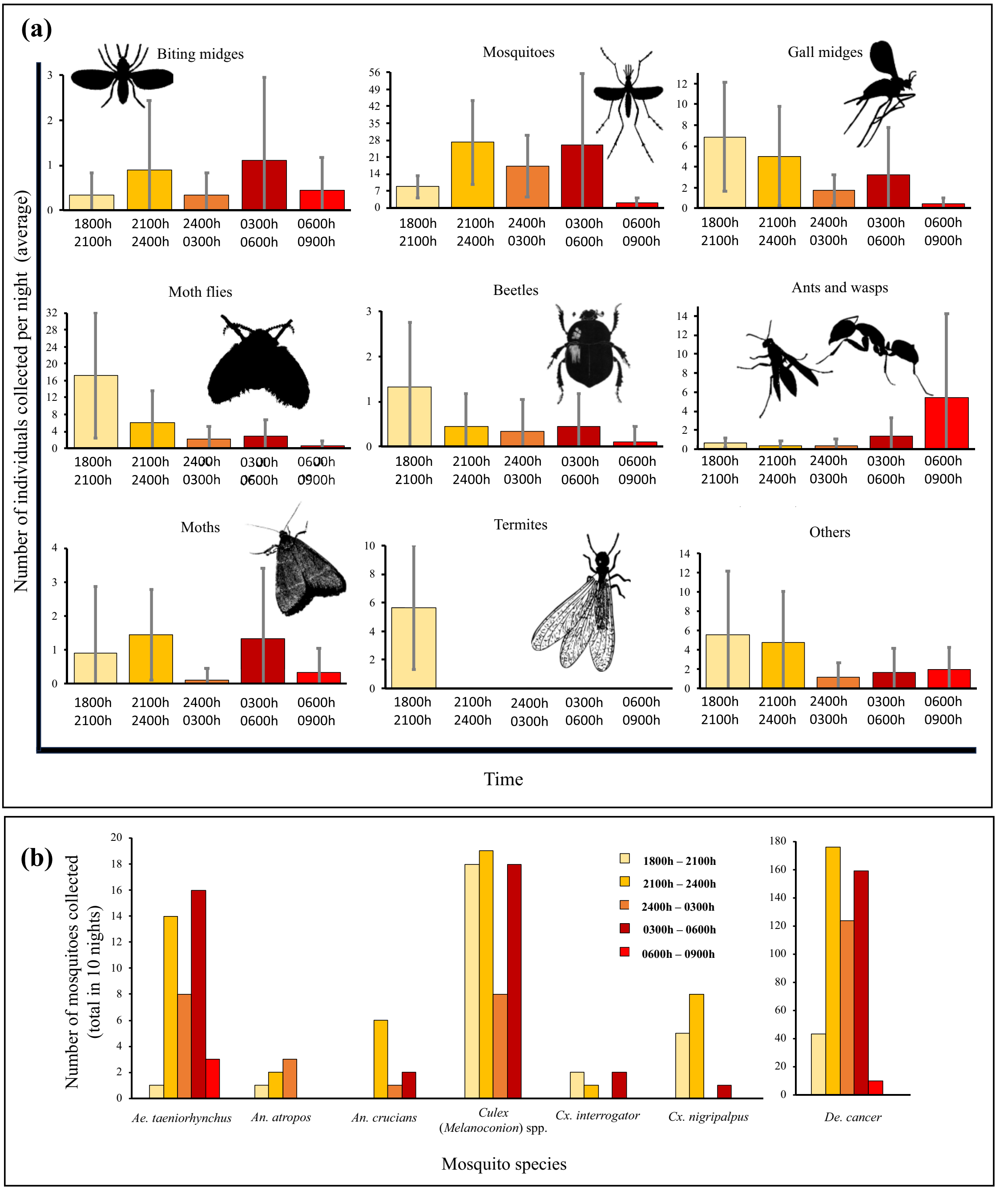

Fig. 2. Insect collections per time period: (a) all insect groups; (b) species of mosquitoes. Bars represent the average number of specimens collected per group with the collection system described in this article. Error bars represent the standard deviation. Collections were done in Vero Beach, FL, USA.

crucians Wiedemann (1.2%), *Anopheles atropos* Dyar and Knab (0.9%), and *Culex interrogator* Dyar and Knab (0.8%) (Fig. 2b). Over the 10 nights sampled, *Ae. taeniorhynchus* and *De. cancer* were the only mosquitoes collected across the five time periods (Fig. 2b). *Culicoides furens* (Poey), a biting nuisance pest in coastal areas of eastern North America (Koch and Axtell, 1979), comprised 82.1% of biting midges. The remaining biting midges (17.8%) were *Culicoides edeni* Wirth and Blanton, a vector of blood parasites including, but not limited to *Haemoproteus* spp. (Sloyer et al. 2019). The total of biting midges observed between

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0300h and 0600h was 4 times more abundant than that of those collected between 1800h and 2100h (dusk) (Fig. 2a), contrary to the notion that these insects are mainly crepuscular (Koch and Axtell 1979, Garvin and Greiner 2003). However, environmental variables that may have affected the temporal flight activity of biting midges were not considered for this study.

Moth flies, gall midges, and beetles were more active between 1800h and 2100h (Fig. 2a). Gall midges were mostly collected between dusk and midnight, from 1800h to 2100h (39.8%), and 2100h to midnight (28.8%) (Fig. 2a). Patterns of activity for termites, and ants and wasps were unique. Termites (*Neotermes* spp.) showed a clear trend for time of activity, being collected solely between 1800h and 2100h (Fig. 2a). *Neotermes* spp. are common in Florida, and alates swarm at dusk or at night (Scheffrahn and Su 2002). The activity of ants and wasps was left-skewed with nearly three-quarters of ants and wasps collected early in the morning between 0600h and 0900h (Fig. 2a). It is suspected that their activity was even greater later in the day, in periods of time that were not sampled for this study.

We demonstrate here that an inexpensive and simple collection system can be utilized to study the temporal activity of vector and nuisance arthropods in the field, including mosquitoes and biting midges. This contained, collectable, and programmable collection system offers a simplified alternative for studying the flight activity of insects in field settings. The daily rhythm of pathogen vectors is typically studied in laboratory settings that require insect colonization (Yee and Foster 1992, Eilerts et al. 2018), which is challenging for some species of medical importance such as biting midges (McGregor et al. 2022). Additionally, daily rhythm studies have suggested that laboratory and field experiments can generate contrasting results because, in nature, insects are exposed to numerous physical and social factors that may be absent in the laboratory (Yerushalmi and Green 2009). The rotational automatic pet feeder could be adapted to a variety of traps for studying the temporal biology of arthropods. For example, an automated pitfall trap was developed for studying the circadian rhythms of surface-active arthropods, particularly ants (McMunn 2017). The rotational automatic pet feeder could be enclosed in a protective case and buried underground to use with pitfall traps or could be combined with flight intercept traps (Batista et al. 2018). Additional attractants such as octenol or carbon dioxide could be added to this collection system for attracting host-seeking females (Pombi et al. 2014).

We experienced difficulties identifying mosquito species in the subgenus *Melanoconion* due to collection into the detergent solution, causing the loss of scales and setae that are important for species-level identification. However, specimens of all other groups were adequately preserved for identification up to a month after collection. The results discussed in this article are not provided to draw conclusions on the time of natural activity of these insects but to demonstrate the potential of this simple and inexpensive automated collection system to study the daily rhythms of arthropods.

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