

SCOPE:

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I have been working on circadian/daily biological rhythms of mosquitoes since 2009, with 14 publications on the topic. Below I cover some of the ways I could work with SLCMAD to detect changes in the temporal biology of mosquitoes that may affecting optimum fogging time-of-day as well as attempt to understand the underlying mechanisms. In my previous work, I have explored how light, temperature, humidity, nutrition, and endogenous circadian rhythms (the biological clock) all shape temporal patterns of activity/ biology in mosquitoes.

I propose a first visit to kick-off my involvement with the project. I propose this would include:

1. Providing training and troubleshooting on the use of locomotor activity monitors (LAM's) to monitor mosquito temporal biology in the laboratory. With this equipment, the mosquitoes can be placed under various conditions (e.g. different light, nutrition, and temperature regimes) and determine when the mosquitoes are flight-active. We could also work on set up of an appropriate lightning system for the assay.

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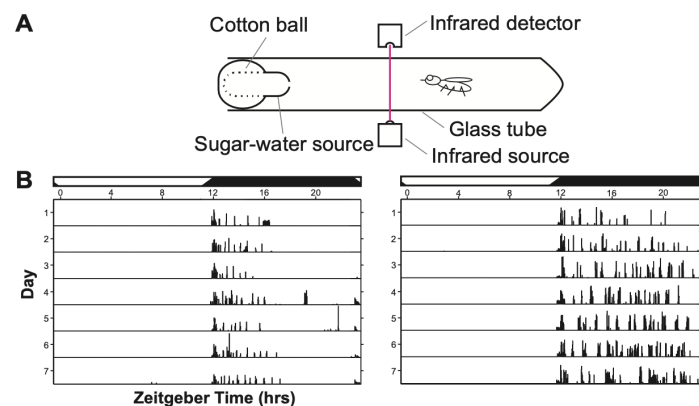
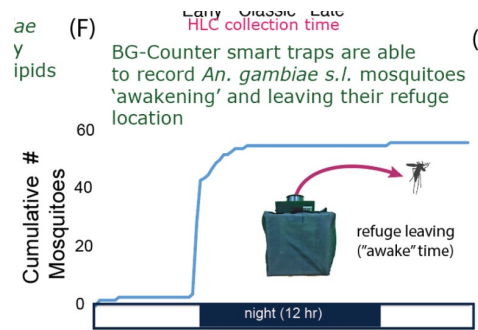


Fig. 1. Locomotor flight activity recording of *An. gambiae* mosquitoes. (A) Cartoon of the LAM 25 unit used to record locomotor flight activity. (B) Representative actograms of the flight activity of a single male (left) and female (right) *An. gambiae* mosquitoes monitored over 8 days. Day and night are indicated by horizontal white/black bars. Zeitgeber time (ZT) with ZT 12 being time of lights off, and ZT 11 time of the start of the 1 hr dusk cycle.

2. Advising/trialing the modification of BG-Counters your district already owns to monitor the time mosquitoes “wake up” (i.e. leave their refuge and thus presumably are susceptible to fogging)



- Advising on how semi-field enclosures (such as the one SLCMAD is building) and alternatives to rotator traps could be used to answer circadian questions (such as I have done in Africa)

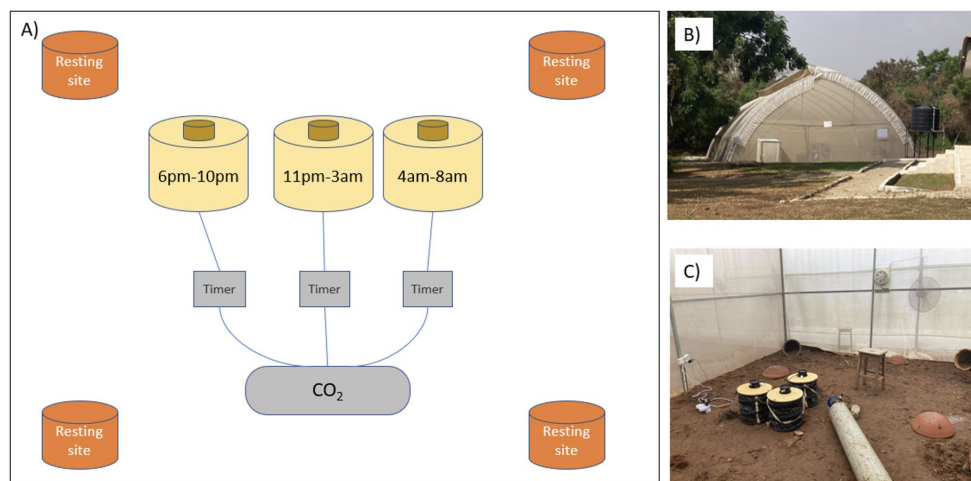


Figure 1. The semi-field behavioural assay set-up. Schematic diagram of the set-up (A), featuring four resting sites for mosquitoes, and traps baited with human odour which were programmed to turn on at 6pm-10pm, 11pm-3am and 4am-8pm respectively. These windows correspond to approx. ZT12-ZT16, ZT17-21, and ZT22-ZT2 respectively, where Zeitgeber time (ZT) defines the hours since lights on and ZT0/24 is dawn. All traps were linked to a CO₂ source, which was split to three timers ensuring

- Working with staff and partners on discussions on other areas of my relevant experience in mosquito temporal biology that include the list below. Some future areas I might help SLCMAD with include:
 - Light pollution and mosquito behavior/timing
 - Gene expression rhythms and time-of-day effects in detoxification [of insecticide]
 - Olfactory rhythms in mosquitoes
 - Mosquito nutritional status driving biting time

COST:

One week initial visit to SLCMAD: As a faculty member at Notre Dame, Notre Dame charges \$3,887 for 1 week of effort of my time which I propose for my initial kick-off meeting. This is assuming I can stay at the SLCMAD dorm facility and my plane ticket (~\$600, round trip, from South Bend) is purchased by the district.

Any follow up consultations/ data processing/ etc. would be at \$97.18 per hour for follow up data processing, etc. as needed and requested by SLCMAD and could be billed quarterly by Notre Dame.

I propose the district acquire in advance of my visit:

- Two locomotor activity monitors LAM25 and required accessories from TriKinetics.
 - 2 X LAM 25 @ \$1,275
- One PSIU9 control/power interface @ \$350.
- Glass tubes (75 @ ~ \$1.25 each)
- Consumables:
 - 2mL flat bottom tubes
 - Dental cotton wool
- A controlled lighting system. At simplest, this is an on/off electrical timer - with LED lights *not incandescent*. A more ideal system would have dawn/dusk transitions. Using an off-the-shelf dawn/dusk lighting system built for terrarium/ aquarium could keep this cost to under \$500.

This system could be built into an incubator(s) or into a light-proof box.