

The BG-Counter: A New Surveillance Trap that Remotely Measures Mosquito Density in Real-Time

by Catherine Pruszyński

Anyone who has ever done mosquito surveillance knows how labor intensive it can be. Whether it's landing rate counts (LRC) or setting CDC light traps, the most time-consuming aspect is having someone physically go out to site to survey for mosquitoes in the area. When you finally get the collection information to operations you may have already missed your window for control. In this modern technologically advanced era of self-driving cars and Jeopardy champion robots, is it too much to ask for some of that rapid-response automation to trickle down into this tedious sector of mosquito science?

Consider our dilemma: the Florida Keys Mosquito Control District (FKMCD) has 266 human landing rate count stations throughout Monroe County, FL, and each station is visited daily by one of our sixteen field inspectors. We estimate they each spend approximately 2 hours per day just driving to landing rate count sites where they count for one minute the number of mosquitoes that land on them. They'll check a rain gauge while they're there, then get back into the truck to drive to the next count station. Some of our furthest stations are on offshore islands that require a boat to visit, or a 45-minute walk into the hardwood hammocks of the Florida Keys Wildlife Refuges. We conduct this daily surveillance to comply with the Chapter 5E-13.036 Mosquito Control Program Administration rules of the Florida Department of Agriculture and Consumer Services (FDACS); in order to send a spray truck or schedule an aerial adulticide mission, we need proof of a quantifiable increase in the number of mosquitoes. The rule permits mosquito control districts to use various standardized trapping methods, but that can become even

more burdensome when factoring in the acquisition of an attractant like carbon dioxide in dry ice, replacing batteries, and identifying and counting trap contents.

After analyzing the cost of labor spent on daily landing rate counts, it was clear to FKMCD administration that an alternative was necessary. Eliminating LRC stations altogether was out of the question, and replacing them with

conventional trapping methods was not a practical solution. We needed a trap that could be remotely operated, was self-powered, and could transmit catch data back to our office computers. This meant it would have to be able to differentiate between mosquitoes and other insects. Our director, Michael Doyle, composed a Request for Bid to find a tech-savvy group that could produce this cutting edge mosquito technology.



Figure 1: First prototype of the BG-Counter, with CO₂ tank and battery.

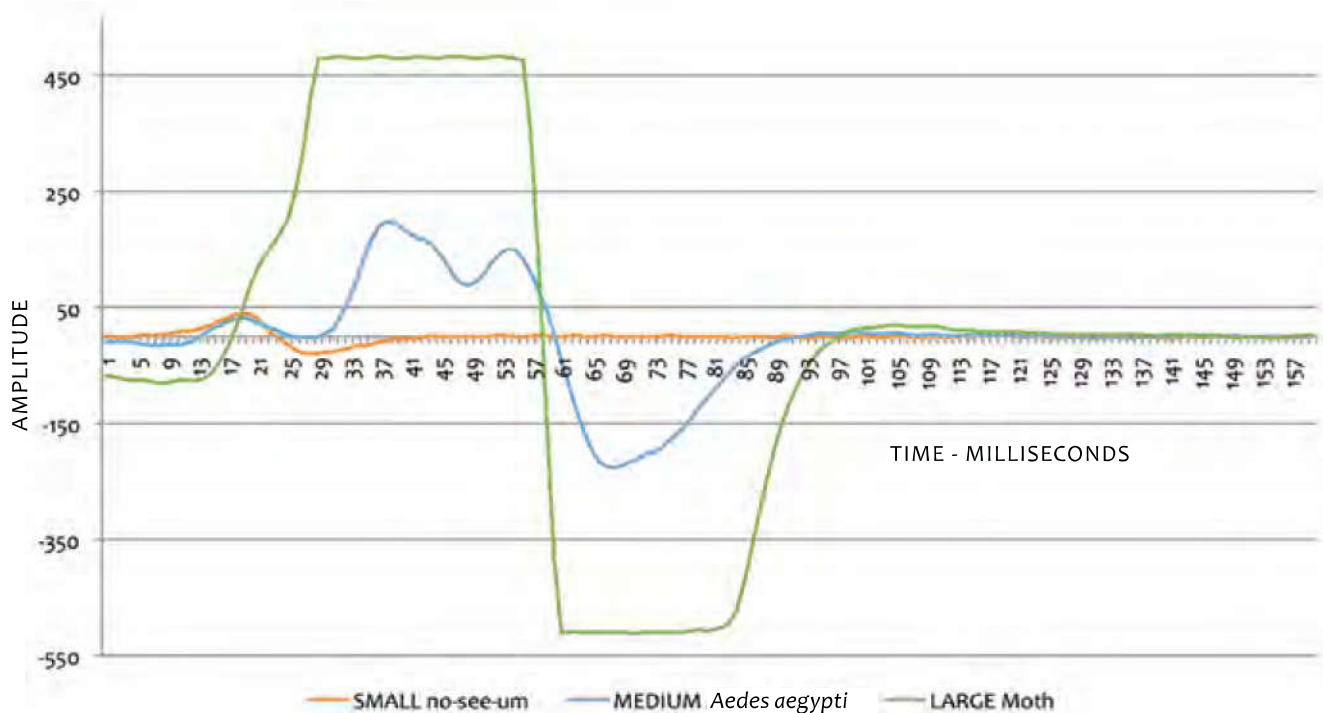


Figure 2: Wavelength signature profiles of three different insect sizes: small (no-see-um, *Culicoides* species), medium (mosquito, *Aedes aegypti*) and large (moth).

Biogents AG (Regensburg, Germany) and onVector Technology (Sunnyvale, CA) jointly took up the challenge of designing the trap and delivered the first prototype to our office in March 2015. The trap's base was a BG-Sentinel trap®, the attractant was carbon dioxide (CO₂) gas with a programmable release, and the ventilator was powered by a 12-volt car battery recharged by solar panel; see Figure 1. These met the first two bid requirements of attracting mosquitoes similar to conventional methods and being self-powered. The real magic lay in the trap's ability to count objects as they entered the trap.

A strip of infrared LED lights line the inside of the entrance of the trap, creating a barrier across the surface of the trap entrance. When an insect nears the trap, it gets sucked into the entrance by the ventilator, breaks the infrared barrier and is counted as an event. The trap's internal software measures the amount of light displaced, which equates to insect size. Over several collections, a classification algorithm can be devised based on the significantly differentiated signature wavelengths produced by

insect types as they enter the trap; see Figure 2. For instance, all medium-sized mosquitoes produce the same signature light disruption when they pass through the infrared barrier. The same goes with larger lepidopterans. Small insects like no-see-ums will produce the same wavelengths as small droplets of rainwater, which makes them indistinguishable from one another to the software. But since this is a mosquito trap and not a *Culicoides* trap, this wasn't terribly inconvenient for us.

We field tested the first prototype in a variety of locations in order to calibrate the mosquito algorithm. The first few trials collected some mosquitoes, but to really put the counting software to the test, we needed a location that was producing thousands. That was easy to find in June on Rockland Key, a small neighborhood 3 miles east of Key West, which abuts US Navy property that is off limits to FKMCD. One of our regularly set CDC light traps had collected over 5,000 mosquitoes in one night, with 98% being *Aedes taeniorhynchus*. The following morning, the area inspector had a LRC of

42 mosquitoes per minute. We sent a spray truck through the neighborhood that night, and the next day the inspector still had a LRC of 21 mosquitoes per minute. It was time to put the BG-Counter to the test.

The trap has no manual 'on' switch, as it is controlled through a webpage maintained by Biogents. The main page details the location of the trap and the trap identification code, and enables changes to the trap schedule; see Figure 3. The trap schedule can be programmed differently for every day of the week in half-hour intervals to enable the operation of CO₂ release, the ventilator, and the counter itself. The page also displays the trap's collection in real time with a bar graph depicting the number of large events, small events, and medium mosquito-probable events occurring over time. The page will also give you the option of downloading the data in an Excel spreadsheet. This spreadsheet includes even more information collected by the trap, including temperature, humidity, ambient light, battery voltage, and cell reception.

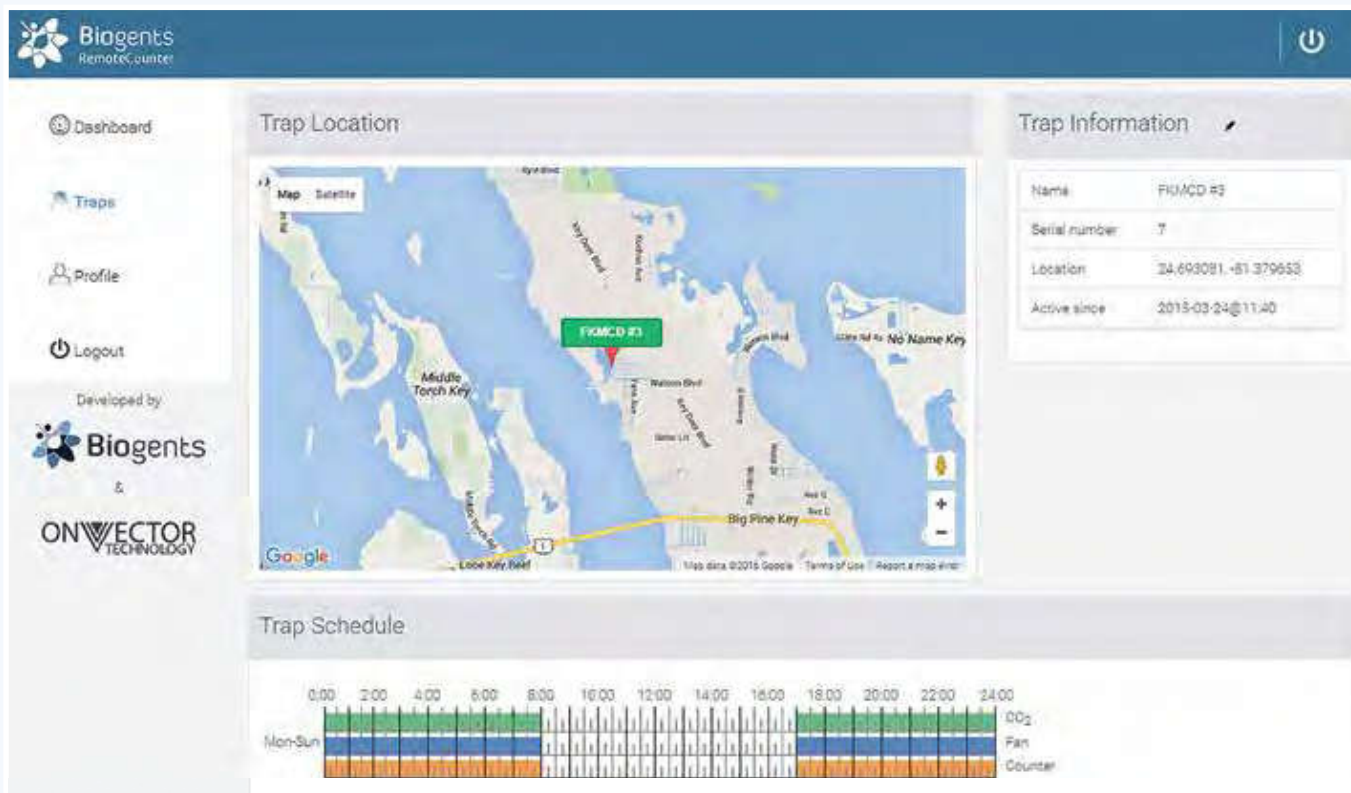


Figure 3: Screen shot of BG-Counter webpage depicting trap location, trap information, and trap schedule.

The trap was programmed on location from an iPhone to start the ventilator and counter and continuously release CO₂ a half-hour after it was set to avoid a dilution effect from human presence. From then on, the trap collection was monitored from the webpage because it was possible and exciting, so why not? The following morning after the CO₂ had been programmed to turn off (the ventilator stayed on to keep the catch inside the net), the trap collection bag was retrieved from the location and stored in a freezer until identification.

The trap software counted 717 medium-sized mosquito events that night, with the highest collection (102 mosquitoes) occurring between the interval of 8:50 and 9:00 pm; see Figure 4. This is typical for *Ae taeniorhynchus* activity. Painfully monotonous truck trapping has shown that the height of *Ae taeniorhynchus* flight activity occurs between 45 and 75 minutes after sunset (Pruszyński 2014). When the collection was counted by hand, 827 total mosquitoes were counted, giving

the trap an 86.7% accuracy. The trap was set again the following night with the same parameters. The same trend emerged, with most activity occurring an hour after sunset, except there was an unusual spike in activity between 7:00 and 7:30 pm (sunset was at 8:18 pm); see Figure 5. The graph shows a bell curve of 68 mosquito events in that half-hour period, with a steep decline to single digit collections again until after sunset. A short interview with the homeowner revealed that he had come out around that time to check out the 'TV and satellite dish' left in his yard. It will be a fascinating future study to measure the influence of a human attractant next to one of these BG-Counter traps.

More trials were conducted to evaluate the accuracy of Prototype 1, including a few specifically surveying for *Ae aegypti*. After all, the BG-Sentinel trap is now the standard for container species mosquitoes like *Ae aegypti* and *Ae albopictus* (Williams *et al* 2006; Wright *et al* 2015; Krockel *et al* 2006). The trap was set in a Key Largo boat

yard that is a known hotspot for *Ae aegypti*; see Figure 6. It was programmed to run for 22 hours, releasing CO₂ in half-hour intervals. The trap was also affixed with a brand new BG human lure that contains a mixture of odors designed to attract anthropophilic mosquitoes. About 30 ft away from the BG-Counter, a BG-Sentinel 1.0 trap, with a cooler of 3 lbs of dry ice as the attractant, was also set. The BG-Sentinel 1.0 collected 87 female and 131 male *Ae aegypti* (55%) and 175 female *Ae taeniorhynchus* (45%). The BG-Counter collected 112 females and 135 male *Ae aegypti* with only 4 female *Ae taeniorhynchus* and 1 female *Culex quinquefasciatus*. That means 98% of the mosquitoes collected in the BG-Counter were *Ae aegypti*, compared to 55% collected in the BG-Sentinel 1.0 trap. We are not exactly sure why there was a greater draw for *Ae taeniorhynchus* to the BG-Sentinel trap compared to the BG-Counter, but we suspect the dry ice (because of its higher CO₂ emission rate) or the new BG human lure influenced mosquito behavior. Future experiments will explore these

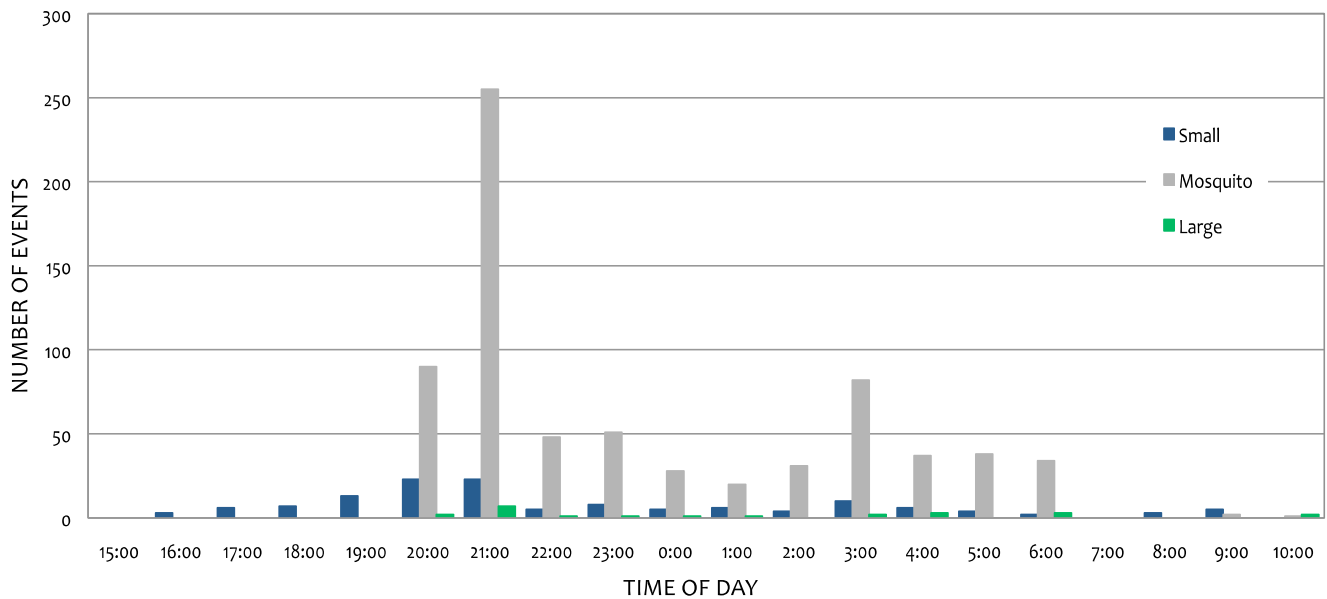


Figure 4: Chart of Capture graph from BG-Counter website for Rockland Key, FL on trap night June 17-18, 2015.

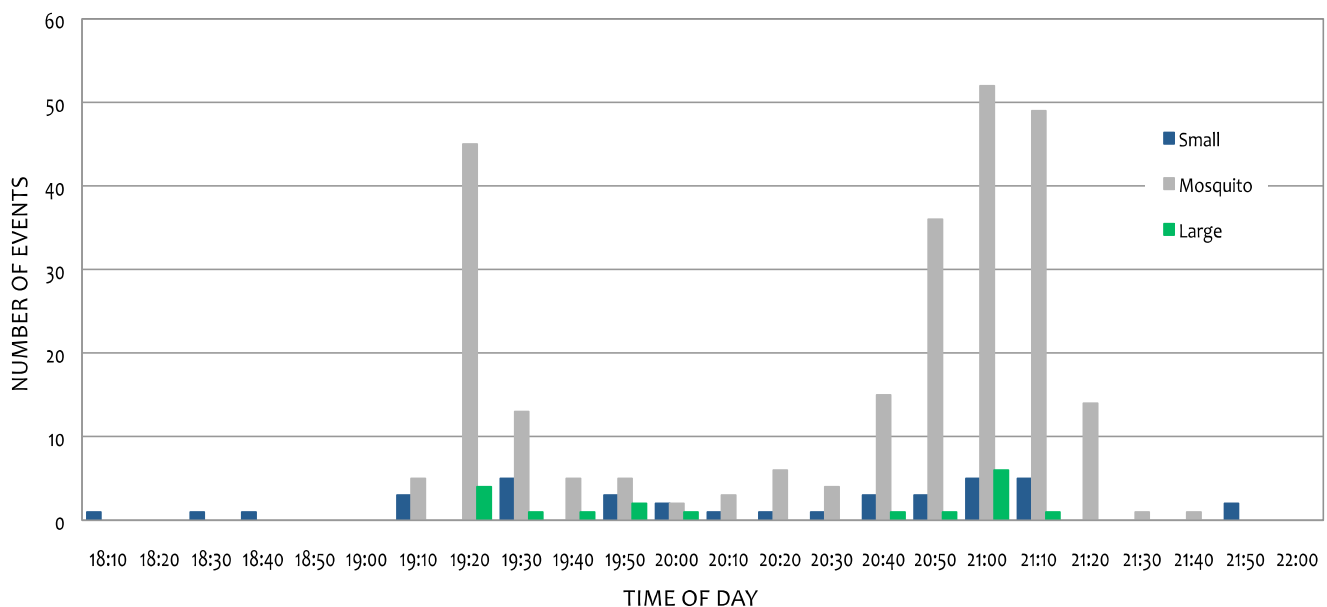


Figure 5: Chart of Capture graph from BG-Counter website showing unusual spike in mosquito activity at Rockland Key, FL trap site on June 18, 2015.

hypotheses, but it is very interesting to speculate that while the BG-Counter cannot yet identify mosquitoes to species, perhaps it could be manipulated to attract and repel different species.

The accuracy for our experiments using Prototype 1 showed 79% (SE=3.92%, n=5) when compared to hand counts, and most were underestimations of the number of mosquitoes collected

in the trap. This counting error was due to the frequent data transmissions occurring every 10 minutes. As the software collects data, it spends 45 seconds every 10 minutes sending data to the webpage. However, during transmission, the trap is unable to count the events that transpire during those 45 seconds. Therefore, 45 seconds worth of data was lost every 10 minutes. That adds up to a lot of missing data over

a trap night. Luckily for us, Biogents and onVector Technology were just putting the finishing touches on Prototype 2, which promised to eliminate this problem.

We received Prototype 2 in October 2015, and began field testing. The trap was more streamlined, with fewer wires and clunky hardware pieces than the first version, and accuracy



Figure 6: Prolific *Aedes aegypti* mosquito habitat at a Key Largo boat yard.

was much improved. We found it had a 93% (SE=2.23%, n=13) accuracy compared to hand counts. This improvement was due to changes in the transmission interval and refinement of the identification algorithm. The user can now determine when the data is be transmitted to the webpage. If the user wanted it every 15 minutes that was still a possibility, but they would incur a loss of data during transmission. However, if they'd rather have it on the hour or every 2 hours, the more time between transmission intervals the fewer data are lost. The data still appear in 15-minute resolution, so the counter can still produce specific mosquito activity throughout the trapping period. There was one trap night with heavy rain that resulted in an outlier of 65% accuracy. It was a very rainy evening culminating in only 8 total mosquitoes, while the trap only counted 5. As someone who has had difficulty herself in identifying insects from rain-soaked trap bags, I think this one outlier can be dismissed.

Other improvements to the prototype

centered on powering the trap for extended periods of time. It now has the capability of running on house current as well as solar charged battery. The trap can be put into 'hibernation mode' so it uses less power throughout non-trapping periods. The solar panel has an extended cable that will facilitate panel placement for maximum sunlight exposure. These enhancements allow the trap to move towards the 'set it and forget it' solution desired to free the inspectors from laborious landing rate counts. Even FDACS is on board; they consider the BG-Counter an acceptable trapping method for mosquito surveillance!

After field-testing and discussing improvements and changes to Prototypes 1 and 2, we are eager to see the final product from Biogents AG and onVector Technologies in April 2016. It will be equipped with software that can read an attachable rain gauge. While determining accuracy will be our first endeavor, we look forward to finally calibrating the trap for its designated purpose, which will

require comparisons to human LRCs and counts from CDC light traps. The trap certainly has potential for other research and operational projects, and we at FKMCD are excited to have been a part of the creation and design of this innovative and state-of-the art mosquito surveillance trap.

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