FIELD EVALUATION OF EMERGENCE TRAP DESIGN FOR MONITORING MANSONIA PRODUCTION FROM WATER LETTUCE (PISTIA STRATIOTES)

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ABSTRACT

Larvae of the mosquito species Mansonia titillans and Mansonia dyari attach to the roots of floating aquatic plants, primarily water lettuce (Pistia stratiotes) and water hyacinth (Eichhornia crassipes), to obtain oxygen and avoid predators. Surveillance for these species involves a robust monitoring program that identifies Mansonia habitat and production sites. This report evaluates floating emergence trap efficiency for Mansonia surveillance and identification of production sites. Three trap designs were utilized in the evaluation trials, including standard passive emergence traps, modified (active) emergence traps containing a CDC-light trap with and without standard incandescent bulbs. Overall, the active emergence trap with light resulted in the collection of a significantly higher number of emerging mosquitoes.

Key Words: Mansonia, emergence trap, water lettuce, water hyacinth

There are approximately 25 species of the genus Mansonia Blanchard known throughout the world, two of which are found in the state of Florida (Rojas-Araya et al. 2020). Mansonia dyari (Belkin, Heinemann and Page) and Mansonia titillans (Walker) larvae procure oxygen and avoid predation by attaching their siphon to the roots of floating aquatic plants, specifically, water lettuce (Pistia stratiotes L.) and water hyacinth (Eichhornia crassipes (Mart) Solms), respectively (Slaff and Haefner, 1985). This unique behavior renders traditional surveillance methods difficult. These species are fierce biters, most active during sunset, and travel 1300 meters on average from their emergent habitat (Verdonschot and Besse-Lototskaya 2014). Mansonia species are potential vectors for filarial nematodes, including dog heartworm (Dirofilaria immitis (Leidy)) (Bemrick and Sandholm 1966) and lymphatic filariasis (Wuchereria bancrofti (Cobbold)) (Ughasi et al. 2012). In addition, there have been instances where wild-caught females were found to be infected with West Nile virus (Unlu et al. 2010), St. Louis encephalitis virus (Beranek et al. 2018), and Venezuelan equine encephalitis virus (Sudia et al. 1971).

Controlling Mansonia involves a strong integrated approach by reduction of the aquatic host plants, as well as targeting the immature and adult stages of the mosquitoes (Rojas-Araya et al. 2021). Vector control agencies that focus on Mansonia control should adopt a monitoring program that identifies Mansonia habitat and production sites. Because of their intimate relationship with host plants, identifying Mansonia production sites and surveying for larval distribution can prove difficult (Service, 1993). Plans for the control of Mansonia species can be based off the trapping of newly emerged adults using emergence traps, providing a measure of mosquito production and an estimation of emergence from a given habitat.

Until recently, there was no aquatic plant or Mansonia production site monitoring program in place by the Collier Mosquito Control District (CMCD), located in Collier County Florida. In an effort to investigate whether the presence of water lettuce significantly contributes to CMCD's mosquito abundance, we began developing a monitoring program for Mansonia larval habitat, adult production, and adult abundance. Sampling adult emergence using the typical passive emergence trapping methods proved difficult, with small capture rates due to a combination of predation, length of time spent in the emergence chamber, and loss of emerged mosquitoes from catch container transfer. Due to CMCD's large-scale monitoring program, aspiration of adult mosquitoes from emergence traps to increase capture efficiency was not feasible as the process can be time consuming and labor intensive. Based on the above considerations, new sampling tools capable of overcoming the known constraints in Mansonia monitoring are continually needed. This study aimed to compare capture efficiency of the original pyramidal passive emergence trap design (Slaff et al, 1984) to two modified active emergence traps.

Three trap designs were utilized in the evaluation trials, including passive emergence traps (Figure 1A),...
modified (active) emergence traps containing a CDC miniature light trap (CDC-light trap) (John W. Hock Company, Gainesville, Florida, USA) baited with and without a standard incandescent bulb (Figure 1B). Traps were built in-house using PVC pipe, mesh screen and foam pool floats. The PVC pipe was used to construct a 0.37 m² (4 ft²) base and pyramidal structure reaching a height of 0.5 m (1.64 ft). Foam pool floats were attached to the base for flotation and mesh screen comprised the walls. Weatherproof ammunition boxes holding 6-volt batteries were used to power the CDC-light traps. To avoid battery flooding caused by rapidly rising water and trap placement, 0.37 m² (4 ft²) floating platforms were designed to keep the batteries above water. The floating platforms were constructed of a rectangular PVC pipe frame, mesh screen to increase surface area, and foam pool floats (Figure 1B). For passive traps, a funnel was placed atop the apex of the pyramid structure with a modified catch container (Figure 1A), which consisted of a 1.5-qt plastic funnel directed to a 2 qt plastic container and lid outfitted with a 10.16 cm (4 in.) plastic circular louver and wire mesh for ventilation. Active traps were designed for CDC-light traps to be seated with a catch container hanging down inside the trap enclosure. The incandescent bulbs were removed from three CDC-light traps for use in the active emergence trap without light. No carbon dioxide or any other attractants were used.

The area used for the trap evaluation trials was routinely mapped using a DJI Mavic Pro Platinum unmanned aerial vehicle (SZ DJI Technology Co., Ltd., Nanshan, Shenzhen, China) to create orthomosaic maps, computationally inspect habitat, and pinpoint trap placement through the Drone Deploy mapping software (Drone Deploy Inc., San Francisco, California, USA) (Figure 1C). Traps were placed at 9 locations at least 3 m apart along the perimeter of the pond located in Ave Maria, Florida, containing full surface coverage of water lettuce (Figure 1C). The type of trap placed at each location was determined by creating a blocked randomization list using a pseudo-random number generator (Sealed Envelop Ltd., 2021). The evaluation was conducted over three weeks in April 2021, with three trap periods each lasting a duration of four days. The randomization list for trap type placement was regenerated for each trap duration.

Trap collections were brought back to the laboratory after each four-day trap duration. Insects collected from the traps were cold anesthetized and adult mosquitoes were identified by morphology (Burkett-Cadena 2013). Consistent with previous emergence-trap data collected by CMCD (data not shown), *Ma. dyari*, *Anopheles crucians* complex (*An. crucians* (Weidemann), *An. bradleyi* (King)), *Culex nigripalpus* (Theobald), and *Uranotaenia sapphirina* (Osten Sacken) were the most prevalent species collected (Figure 2A). Passive emergence traps and active emergence traps without light consistently produced fewer mosquitoes per trap duration with 2.61 ± 2.55 and 4.11 ± 3.01 (Mean ± SD) mosquitoes collected in total on average, respectively (Figure 2A). Both trap designs captured *Ma. dyari*, *Anopheles crucians* complex and *Cx. nigripalpus*, while *Ur. sapphirina* was not detected in either the passive or active-without-light trap designs. The combination of light and fan (active with light) facilitated the largest number of mosquitoes caught per trap duration, collecting a total of 23.94 ± 6.68 mosquitoes on average (Figure 2A). The active emergence trap with light collected a significantly higher number of mosquitoes than the passive emergence trap (t = 5.17, df = 4, p = 0.006) and the active emergence trap without light (t = 4.69, df = 4, p = 0.009). Interestingly, *Ur. sapphirina* was only collected in active emergence trap with light. There were no significant differences in catch rates by week.

Using our emergence-trap data, we estimated the relative production of *Mansonia* mosquitoes per acre of water lettuce with each trap design for the trapping duration. Production per acre was estimated by using the number of mosquitoes produced in 0.37 m² and converting to number of mosquitoes produced in 1 acre. Estimates from data obtained through the passive emergence trap and active emergence trap without light were low, with 20,570 ± 20,641 and 9,680 ± 5,834 (Mean ± SD) *Mansonia* mosquitoes per acre, respectively. The active emergence trap with light estimated significantly more *Mansonia* mosquitoes per acre with 173,029 ± 51,889 compared to estimations determined using the passive emergence trap (t = 4.73, df = 4, p = 0.009) and active emergence trap without light (t = 5.42, df = 4, p = 0.006).

Active emergence traps with light were the most reliable trap design evaluated in this study, catching the highest number and a more diverse collection of emergent mosquitoes. In addition, *Ur. sapphirina* were only found in the active emergence trap with light. Due to placement of traps and identical emergence trap bases, the total number of mosquitoes and species emerging should not have been impacted by the presence or absence of a trap light or fan. The light of the active emergence trap likely attracts the emerged mosquitoes to the fan, therefore catching more adults than the passive emergence trap or active emergence trap without light. Using the mounted CDC-light trap on an emergence trap base eliminates labor-intensive and time-consuming collection methods. In previous passive trap surveys, freshly emerged adult *Culex* mosquitoes spent -1 day in the emergence enclosure before reaching
Emergence trap design for Mansonia mosquitoes

Figure 1: Emergence trap design and placement for evaluation. (A) Passive emergence trap design. (B) Modified (active) emergence traps containing a CDC miniature light trap (CDC-light trap), and (C) Orthomosaic map depicting the 9 trap locations.

Figure 2: Trap collections and estimates of production. (A) Average number of mosquitoes collected in the three emergence trap designs for all three trap durations. (B) Estimates of Mansonia production per acre of water lettuce by trap type. Data is represented as Mean ± SD, * denotes p < 0.05.
the catch container (Walton, 2009). However, our studies indicate that the passive trap design results in extensive loss of emerged mosquitoes and reduced attractiveness to *U. minitans* species. It is important to note that the light emitted by the CDC-light trap may create a sampling bias by attracting more larvae under the floating emergence trap. Larvae of several mosquito species have been shown to be attracted to aquatic light traps (Service et al. 1983, Beehler & Webb 1992, Hribar & Hribar 2006). Further research is required to understand the attractiveness of the above-water incandescent light of the active emergence trap for *M. dyari* larvae and if this results in an increase in catch rates.

In addition, the construction and utilization of the floating battery platforms further increase our emergence trap efficiency when running emergence traps on a 4-day period. During our rainy season (July-October), water levels at trap sites can drastically change in that time causing the battery to flood without the battery platform and leaving gaps in our surveillance data. The floating platforms allow movement with the flux of water level and lessen strain on the wires. The addition of the floating platforms also allows for overall better trap placement. In several surveillance sites, water lettuce is present beyond other submerged aquatic plants several meters off the bank. Previously, trap placement was limited by the length of wires from battery to the trap. Now traps have been repositioned to areas fully covered by water lettuce.

Overall, our active emergence trap design in combination with floating battery platforms has enhanced our efficiency in collecting mosquito species associated with aquatic plants. Through this trapping method, CMCD has been able to map and define mosquito species derived from aquatic weed habitat. This monitoring program has allowed CMCD to define the scope and need of an aquatic weed control program in Collier County. By identifying production sites and estimating *M. dyari* production per acre of water lettuce, we can now target these areas for mechanical removal or herbicide application of aquatic weeds or for larvicide application for immature stages of *Mansonia* species.

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