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SCIENTIFIC NOTE

EVALUATION OF PARTIALLY SUBMERGED STICKY TRAPS ON LAKE SPILLWAYS FOR ADULT BLACK FLY (DIPTERA: SIMULIIDAE) SURVEILLANCE AND ARBOVIRUS DETECTION

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ABSTRACT. Sentinel surveillance systems demonstrate an improved ability to supplement monitoring data and anticipate arbovirus outbreaks (i.e., sentinel avian species). Management complications can arise during unpredictable or unseasonal disease detections, especially in rural areas where resident distribution is patchy. Using spillways near residential lake communities as static surveillance locations, we tested a novel partially submerged sticky trapping technique and screened wild populations of adult female black flies (Diptera: Simuliidae) for West Nile virus (WNV) and eastern equine encephalitis virus (EEEV). Trap site selection criteria considered the density of immature black fly colonization on spillway surfaces and the number of positive detections of arboviral targets in nearby *Culex* mosquito populations. On average (±standard error), sticky traps captured 134 (±33) adult black flies over a 24-h period, with 1 trap capturing as many as 735 individuals. Although we detected positive cases of WNV from 20 *Culex* mosquito trapping sites within 16 km (approx. flight radius) of the selected lake spillways, mixed pools of adult female *Simulium vittatum* complex and *Simulium decorum* were all negative for both arboviruses. This study yielded an application for partially submerged sticky traps to collect adult female black flies. Its potential uses for monitoring the infection rates of more well-documented *Simulium* parasites are discussed.

KEY WORDS Eastern equine encephalitis, New Jersey, *Simulium*, sticky traps, West Nile virus

Fluxes in the regional composition and behavior of arthropod-borne viruses (arboviruses) caused by modern anthropogenic changes continue to pose uncertain and potentially dire human health risks throughout the globe (Wasay et al. 2015). Conventional trapping methods for monitoring arbovirus activity in North America involve a variety of techniques including gravid traps, the Centers for Disease Control and Prevention (CDC)-CO_2 light traps, and resting boxes, which display varying capture and arbovirus detection efficiencies (Williams and Gingrich 2007). Overall, exploiting the innate behavioral cues of bloodsucking female insects is a common and effective practice in vector control. Concurrent with trapping efforts, opportunistic or planned surveillance of sentinel avian species are reported as reliable early-warning systems for regional arbovirus activity (Komar 2001).

Model sentinel organisms are commonly used to characterize potential adverse human health risks to disease and chemical pollution (Reif 2011). In this capacity, sentinel organisms provide vital data to predicting arbovirus emergence and informing impactful management decisions. The sensitivity of positive arbovirus detections is restricted by a number of biological and ecological determinants including host-seeking behavior, virulence and viral load, and available habitat (i.e., containers for some *Culex* mosquitoes). Static locations that attract highly mobile sentinel organisms may provide greater sensitivity and sample a larger geographic area; however, exploratory studies focusing on this topic are limited to herd animals and domestic or wild bird populations (Racloz et al. 2006, Chaintoutis et al. 2014).

In Sussex County, NJ, most residents live in or around recreational lake areas where most positive arbovirus detections occur (Lockburner and Risley, unpublished data). Ample standing water and container habitat puts lake communities at greater risk of arbovirus exposure. Sussex County contains over 200 dams/spillways, and many are surrounded by neighborhoods. Lake spillways control the release of flowing water, prevent damage to dams from flooding conditions, and vary in design based on lake capacity and surface area. Primarily controlled by a metal, concrete, or wooden sluice, flowing nutrient-rich water consisting of plankton and fine particle organic material (FPOM) promotes the colonization of filter-feeding invertebrate communities, such as hydropsychid caddisflies and black fly larvae (Richardson and Mackay 1991). Available autochthonous material exiting lentic surface waters can promote dense populations of filter-feeding invertebrates, specifically black fly larvae, with some accounts reporting greater than a million individuals per square meter on spillway surfaces (Wotton 1987).

As adults, certain black fly species are a nuisance to human populations and pose serious threats to livestock due to their tenacious biting behavior.

Several black fly species display competent feeding plasticity, targeting mammal and bird hosts. Adult females will fly long distances to search for a blood meal (≥16 km) and are cited as determined feeders, making them poor disease vectors (Adler et al. 2004). Using lake spillways as collection sites, we sought to evaluate partially submerged sticky traps for collecting adult black flies and test the ability of adult female black flies to detect West Nile virus (WNV) and eastern equine encephalitis virus (EEEV) in areas of high arbovirus activity (i.e., positive detections in *Culex* mosquitoes).

Surveys were conducted June to July in 2017 on 40 preselected lake spillways to assess black fly density and species assemblage. These lakes were selected due to previous complaints by residents issued to the Sussex County Mosquito Control and the number of residents living in the lake community. Using a modified rapid black fly population assessment outlined by Palmer (1994), a coarse measure of black fly larvae density was performed on four 4 × 4 cm squares on colonized lake spillway surfaces and cobbles below the lake outlet. An estimate of overall black fly larvae and pupae abundance was obtained by visual assessment by percentage cover in each 4 × 4 cm square. Based on a semilogarithmic scale, the percentage cover score can be converted to approximate abundance (Palmer 1994). We collected a subset of black fly larvae (individuals greater than 5 mm in length) and pupae from 1 of the 4 preset 4 × 4 cm squares to determine dominant and secondary species. Lake spillway surveys were conducted by the 1st author, negating the potential for error from multiple observers. Larvae and pupae were identified using the dichotomous keys in Adler et al. (2004). Species were confirmed by the Vector Management Laboratory with the Pennsylvania Department of Environmental Protection.

Eighteen spillways harbored immature black fly populations. The species occurrences for each of the 18 spillways were as follows: *Simulium vittatum* Zetterstedt complex (17), *Simulium decorum* Walker (13), and *Simulium tuberosum* Lundström (1). *Simulium vittatum* complex was also the most abundant black fly sampled among the active spillways. Black fly larvae scores on spillways were significantly higher than pupae ($t = 4.15$, df = 19, $P = 0.001$). Spillway height was the best predictor of black fly larvae abundance (pairwise correlation; $r = 0.422$) with a significant positive correlation (β ± standard error [SE] = 0.212 ± 0.09, $t = 2.23$, $P = 0.035$). On average (±standard deviation), we observed immature black fly colonization on 46.1% (±29.5%) of the spillway surface area. Algal and plant growth on spillway surfaces varied substantially (e.g., filamentous algae, *Hydrilla verticillata* [L.F.] Royle, matted decomposing submerged macrophytes, iron-oxidizing bacteria) with most sites featuring thick mats of brown algae.

Adult female black flies were sampled August to September in 2017 at lake outlets identified as "at risk" based on lake spillway surveys and higher positive arbovirus detection. Following the modified methodology of Hunter and Jain (2000), partially submersed sticky traps were deployed to target adult female black flies. Three strips of Black Flag® Fly Paper (Spectrum Brands Inc., Middleton, WI) were fixed to the spillway using a large binder clip and collected within 24 h of deployment. Each strip was 83 cm long × 5 cm wide, and approximately 76 cm of the strip was submerged in the current. Submersed sticky traps mimicked trailing pieces of vegetation, which are ideal ovipositioning sites for gravid females.

Twenty-four-hour sticky trap deployments proved to be an effective means of capturing adult black flies in abundance (Fig. 1). However, we did observe high variance in trapping numbers depending on trap spillway location (i.e., the placement of the trap on the spillway) and the selected lake (Table 1). Among the 9 lake spillways sampled, a cumulative total of 3,616 individuals were captured, which averaged to 134 (± 33) adult black flies per sticky trap. Observations on other insect taxa captured by sticky traps were also made. A lower abundance of diverse insect taxa was also collected using these methods, which included: nonbiting midges (Diptera: Chironomidae), water striders (Heteroptera: Gerridae), fungus gnats (Diptera: Mycetophilidae), net-spinning caddisflies (Trichoptera: Hydropsychidae), dark-winged fungus gnats (Diptera: Sciaridae), and broad-shouldered water striders (Heteroptera: Veliidae).

Adult samples were enumerated and separated into pools (max. 50 adults per pool) and stored at −70°C until analyzed. Specimens used in our analyses were still alive upon being transferred into the freezer for storage. Preference was given to living specimens to improve chances of arbovirus detection. Twenty pooled black fly samples from 9 different spillways were submitted for WNV and EEEV screening to the Arboviral Detection Services Laboratory at the Cape May County Department of Mosquito Control. The unbalanced number of pools submitted per lake spillway was due to adult female black fly abundance, which varied among trap sites (Table 1). Arboviral detection methods can be provided upon request.

Between August 21 and September 21, some 31 positive WNV detections were isolated from mixed female *Culex* mosquitoes collected from gravid traps. Among the 31 positive WNV detections, 20 gravid trap sites were within 16 km of a spillway heavily occupied by immature black flies. A total of 939 adult female black flies were screened for WNV and EEEV from 9 different lake spillways found in 7 township municipalities in Sussex County, NJ. Mixed pools of adult female *S. vittatum* complex and *S. decorum* were all negative for both arboviral targets (Table 1).

As a byproduct of this exploratory study, we found partially submerged sticky traps were very effective

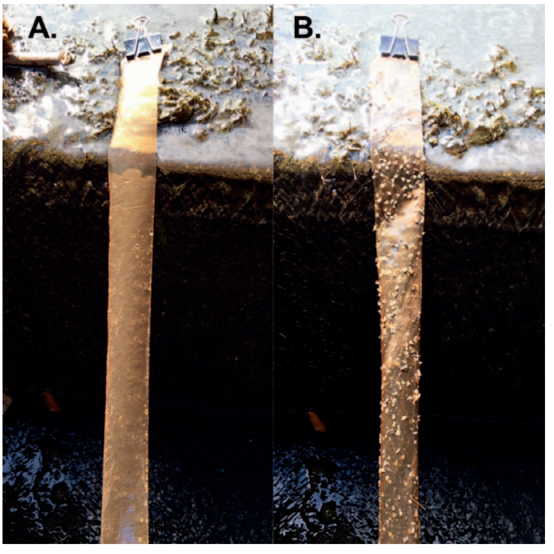

Fig. 1. Photos displaying the effectiveness of partially submerged sticky trap methods (A) before and (B) after 24-h deployment.

at capturing adult female black flies. Moreover, we discovered spillways were indeed black fly "hot-spots" in Sussex County. Now, avenues for black fly reduction are being discussed and will ultimately improve the quality of life for lake community residents, pets, and domestic livestock. Although *S. vittatum* complex and *S. decorum* are not as problematic for humans, these species are becoming increasingly more abundant in nearby states and will swarm near humans.

The role of nonmosquito, hematophagous dipterans in transmitting predominantly mosquito-borne viruses (i.e., WNV) continues to be investigated with outcomes varying taxon-to-taxon (Gancz et al. 2004, Sabio et al. 2006). In North America, simuliid-borne diseases are well documented in birds (e.g., leucocytozoonois) and wild mammal populations (e.g., filarial nematodes, vesicular stomatitis virus, and tularemia). Few studies have demonstrated the ability of nonmosquito dipterans to replicate mosquito-borne viruses (i.e., WNV); however, detection in wild nonmosquito dipteran populations has been previously documented (Chotkowski et al. 2008, Johnson et al. 2010). These studies include biting midges (Ceratopogonidae), louse flies (Hippoboscidae), and stable flies (Muscidae) (Doyle et al. 2011). There are data suggesting black flies may play a role in transmitting disease more relevant to human health risks. Anderson et al. (1961) successfully isolated EEEV from 2 ornithophilic black fly species, *Eusimulium johannseni* Malloch and *Simulium meridionale* Riley, from an experimental flock of infected turkeys. Our results indicate wild black fly populations using lake spillways (i.e., *S. vittatum* complex and *S. decorum*) are likely poor vectors of WNV and EEEV. We hypothesize species with more ornithophilic feeding preferences may produce different results; however, their emergence patterns may not coincide with peak arbovirus detections in the region. *Simulium vittatum* complex and *S. decorum* likely target mammals such as deer, cattle, and horses. Among other studies surveying the natural infection of potential dipteran arbovirus vectors, Reeves and Milby et al. (1990) screened 9 *Simulium* species from sampling efforts in southern California, which yielded negative detections for all arboviral targets (e.g., western equine encephalomylitus virus, Saint Louis encephalitis virus, Turlock virus) from 2,339 specimens. A more recent study tested a small sample size of engorged *Simulium bivittatum* Malloch for WNV after feeding on horses in southern California and found no positive detection (Gerry et al. 2008). While there is a paucity of data in the literature explicitly testing the transmission potential of black flies, especially from species known to seek multiple blood meals, our results corroborate with previous findings.

Black flies will exploit nearly all types of flowing freshwater habitats to oviposit, depositing eggs while landed or midflight. The limitations of the described partially submerged sticky trap method should be

Table 1. A summary of partially submerged sticky trap adult black fly captures and resulting from screening mixed adult female *Simulium vittatum* complex and *Simulium decorum* pools for West Nile virus (WNV) and Eastern equine encephalitis (EEEV) in 2017.

Lake ID	Mean (±SE) captured adults[1]	Total captured adults[1]	Number of pools (adults per pool)	WNV and EEEV results	Date sampled	WNV date range (+ detections)[2]
SL	94 (±49)	281	2(50, 50)	NEG	9/13	8/24–9/21 (6)
FP	4 (±2)	12	1(12)	NEG	9/13	8/24–9/21 (6)
LM	306 (±115)	918	2(50, 50)	NEG	9/13	8/22–9/18 (7)
JL	82 (±14)	247	2(50, 50)	NEG	9/13	8/22–9/18 (7)
CL	127 (±42)	380	3(50, 50, 50)	NEG	8/23	8/21–9/11 (4)
PL	365 (±202)	1095	3(50, 50, 50)	NEG	9/06	8/21–9/11 (4)
NL	151 (±71)	453	3(50, 50, 50)	NEG	8/22	8/28–9/18 (2)
HL	42 (±12)	127	3(50, 50, 27)	NEG	8/22	8/28–9/18 (2)
LL	34 (±10)	103	2(50, 50)	NEG	9/13	8/28 (1)

[1] Three partially submerged sticky traps per spillway over a 24-h period.
[2] The date range and number of positive WNV detections from *Culex* mosquitoes within a 16-km radius of the sampled spillway.

considered while interpreting the results of this study. Sticky trap strips were intended to resemble trailing pieces of vegetation, a commonly described substrate for depositing eggs. Species accounts of black fly ovipositioning behavior are rarely documented with observations from only 44 species, about 17%, in North America (Adler et al. 2004). Among those species, 17 are confirmed to oviposit on substrates while landed and are mostly from the genus *Simulium*; however, there are some that do not follow this behavioral pattern. For example, *Simulium jenningsi* Malloch, a major human pest in New Jersey (Carle 2010), are thought to drop eggs into flowing water midflight (Adler et al. 2004). The species of interest and their ovipositioning behaviors need to be fully considered when deploying the described trapping method for population assessments or possible arbovirus screening.

Enhanced risks to public health from abundant black fly populations on spillways are well documented (Jobin 2003). The most common means of immature black fly control in natural areas (e.g., rivers and streams) is focused applications of *Bacillus thuringiensis israelensis* de Barjac (Bti) products (LaScala and Burger 1981). As man-made structures, spillways offer a number of alternative methods for black fly control. Physical manipulations of spillway flow can alter black fly species assemblages and inhibit breeding (Jobin 2003). Previous studies have observed species-specific water velocity preferences, specializing and exploiting various lotic microhabitats (Roberts and Okafor 1987). High water velocity from spillways will clear plant debris used by gravid females to oviposition, thus reducing egg laying. Conversely, periodic cessation in water release will cause immature black flies to desiccate and will alleviate adult feeding behavior near spillways. Roberts and Okafor (1987) observed significant effects after 1 to 2 days, which allowed immature black flies to dry out in the sun. Depending on the local climate and egg incubation periods, longer dry spells would control black fly breeding (e.g., greater than 10 days). Moveable spillway sluices may also prevent higher densities, where operators or local residents can shift or redirect the water flow intermittently. Further investigation of black fly control and disease surveillance strategies using spillways may provide insights on future management strategies in areas plagued by simuliid-borne diseases.

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