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Larviciding *Culex* spp. (Diptera: Culicidae) Populations in Catch Basins and Its Impact on West Nile Virus Transmission in Urban Parks in Atlanta, GA

Joseph R. McMillan,[1] Rebekah A. Blakney,[2] Daniel G. Mead,[3] Sarah M. Coker,[3] Levi T. Morran,[1,4] Lance A. Waller,[1,5] Uriel Kitron,[1,2] and Gonzalo M. Vazquez-Prokopec[1,2,6]

[1]Program in Population Biology, Ecology, and Evolution, Emory University, Atlanta, GA, [2]Department of Environmental Sciences, Emory University, Atlanta, GA, [3]Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA, [4]Department of Biology, Emory University, Atlanta, GA, [5]Department of Biostatistics and Bioinformatics, Emory University, Rollins School of Public Health, Atlanta, GA, and [6]Corresponding author, e-mail: gmvazqu@emory.edu

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Abstract

In urban environments, road-side catch basins are common larval habitats of *Culex* spp. (Diptera: Culicidae) mosquitoes and important targets of larval control in areas subject to West Nile virus (WNv) transmission. We quantified the impact of larviciding basins on *Culex* spp. populations and WNv infection prevalence by treating basins in and around urban parks in Atlanta, GA, using Mosquito Dunks and Bits (active ingredient, a.i., *Bacillus thuringiensis* subsp. *israelensis*, Dunks—10.31%, Bits—2.86%) and Altosid 30-Day Briquets (a.i., S-methoprene 8.62%) in two separate seasons. Treatments were coupled with WNv surveillance using gravid traps and aspiration of adults resting in basins. Larviciding led to >90% reductions in *Culex* spp. larval and pupal collections (Dunks/Bits) and >90% pupal mortality (Briquets) in treated sites during treatment periods; however, we did not observe significant reductions in *Culex* spp. collections in gravid traps (general linear mixed-effects model [GLMM] result, $P > 0.1$) or in adults collected resting in basins (GLMM, $P > 0.5$). In addition, WNv infection prevalence in *Culex* spp. mosquitoes was similar between treated and untreated sites (GLMM, $P > 0.05$). Larval control remains important for controlling WNv in Atlanta; however, at the scale and frequency applied in our study, larval control alone may not lead to meaningful reductions in adult populations and WNv infection prevalence. A greater understanding of the annual dynamics of *Culex* spp. breeding and the importance of basins as *Culex* spp. larval habitats are needed to meaningfully affect WNv in cities such as Atlanta.

Key words: *Culex*, methoprene, *Bacillus thuringiensis* subsp. *israelensis*, mosquito control, pathogen surveillance

West Nile virus (WNv) is a mosquito-borne zoonotic pathogen transmitted among birds by *Culex* spp. (Diptera: Culicidae) mosquitoes (Hayes et al. 2005). The primary WNv vectors include members of the *Culex pipiens* L. complex (Diptera: Culicidae), distributed as *Culex pipiens pipiens* Linnaeus in northern latitudes and *Culex quinquefasciatus* Say in southern latitudes, both of which are often found as larvae in road-side catch basins in urban environments. Road-side catch basins are subterranean containers used to mitigate precipitation run-off from impermeable surfaces and are designed with a catchment that collects and reduces sediment and debris from entering underground water transportation systems. Such catchment reservoirs accumulate water that is often eutrophic and is an optimum larval habitat for *Cx. pipiens* complex mosquitoes (Bunker 1917, Geery and Holub 1989, Rey et al. 2006). Previous research has linked the risk of WNv spillover to humans to the presence of man-made waste water management systems, such as combined sewage overflows and road-side catch basins (Ruiz et al. 2004, Calhoun et al. 2007, Vazquez-Prokopec et al. 2010). Thus, catch basins represent an abundant and common target of vector control interventions, which often rely on the application of larvicides (Anderson et al. 2011, Harbison et al. 2014b).

Larvicides are ideal for treating permanent and semipermanent water sources (Centers for Disease Control and Prevention 2013), such as those located within catch basins. Product formulations and distribution methods (i.e., pellets, briquets, oils) each affect the efficacy of the larvicide in the aquatic habitat (Harbison et al. 2018). There is also evidence that mosquito larval populations within catch basins can persist despite repeated larvicide applications (Harbison et al. 2014c, 2015). Despite the breadth of knowledge regarding the efficacy of larvicides in catch basins, whether larviciding basins leads

to significant reductions in adult *Cx. pipiens* complex populations or reductions in WNv infections in mosquitoes has received little attention in the scientific literature (Bellini et al. 2014). This is an important knowledge gap to address because some municipalities in the United States, such as those in the metropolitan counties of the City of Atlanta, GA, perform only seasonal larval control in conjunction with countywide WNv surveillance.

Vector control in the Atlanta metropolitan area is most intensely implemented in Dekalb and Fulton Counties, the most populated and epidemiologically relevant for WNv transmission (Vazquez-Prokopec et al. 2010). Despite differences between Counties in human resources for vector control (Dekalb relies on Board of Health staff and Fulton contracts with Clarke Mosquito Control) and insecticides utilized (Dekalb uses Altosid products [active ingredient, a.i., S-methoprene], whereas Fulton uses Natular [a.i., spinosad] and Vectolex [a.i., *Bacillus sphaericus*]), both Counties actively treat road-side catch basins from late June through October in an attempt to control WNv transmission. On detection of WNv-positive mosquito pools, interventions are expanded to include neighborhood outreach campaigns near the detected infection(s), (re)larviciding of basins within a buffer distance around each case, and in Fulton County, barrier spraying with Flit 10EC (a.i., 5% permethrin). In addition, neither County has operational budgets that allow for monitoring larvicide efficacy in basins or insecticide resistance in larval or adult populations.

Given the lack of epidemiological information about the population-level impact of larviciding, we performed two experiments in separate years in and around two previously identified urban foci of enzootic WNv transmission within the City of Atlanta, GA, with the objective of quantifying the impact of larviciding catch basins on *Culex* spp. adult female abundance and WNv infection prevalence.

Materials and Methods

Experimental Design

We conducted two separate experiments designed to test for the effect of larvicides applied: 1) during the epidemic period of WNv (July–September) and 2) at the beginning of the *Culex* spp. breeding season in Atlanta, GA (March–May). In experiment 1, the timing of larvicide applications was similar to those of Fulton and DeKalb Counties; experiment 2 could be considered a proactive experiment because control was implemented in an attempt to suppress mosquito populations before the WNv epidemic period began. A different larvicide product was used in each experiment: Mosquito Dunks and Bits (Summit Chemicals, Baltimore, MD, a.i. *Bacillus thuringiensis* subsp. *israelensis* strain BMP 144, Dunks—10.31%, Bits—2.86%) were used in experiment 1, whereas Altosid 30-Day Briquets (Central Life Sciences, Schaumburg, IL, a.i. S-methoprene, 8.62%) were used in experiment 2. These products were used following label instructions and mimicked the types of larval control products applied by DeKalb and Fulton Counties.

Each experiment followed a similar design: 1) a subsample of catch basins in all sites were surveyed weekly for larval, pupal, and adult resting abundance, and gravid traps were set weekly within 200 m of the sampled basins in an attempt to link larval productivity to adult population collections; 2) larvicides were applied weekly (Dunks and Bits, experiment 1) or biweekly (30-Day Briquets, experiment 2) in all basins and storm drains within the experimental boundary during a specified temporal interval; 3) all *Culex* spp. adult female mosquitoes collected from catch basins and gravid traps were tested for WNv infections using Vec-Tests and reverse transcription polymerase chain reaction (RT–PCR); and 4) larval, pupal, and adult collections as well as mosquito WNv minimum infection rate (MIR) estimates before (when data were available), during, and after larvicide application periods were compared using general linear mixed-effects models (GLMMs). We chose to use GLMMs to analyze all data due to potential positive correlations between repeated spatial and temporal measurements (i.e., weekly sampling of the same catch basins or same locations with gravid traps all placed within the same park). All GLMMs comparing catch basin data also accounted for the amount of precipitation recorded in Atlanta 24 h prior to catch basin sampling (precipitation data collected from the National Oceanic and Atmospheric Administration's weather station at Atlanta's Hartsfield-Jackson International Airport). All GLMMs were implemented using the 'glmer' function in the R package 'lme4' (R Development Core Team 2008, Bates et al. 2015).

Experimental Locations

Treatment and nontreatment sites were chosen in the City of Atlanta after an initial survey of catch basins around public parks in DeKalb and Fulton Counties in May and June of 2015. We chose parks as our spatial unit for control and surveillance because there are less access restrictions for sampling mosquitoes in public parks and because prior work showed that Atlanta parks are areas of high mosquito abundance and WNv transmission (Levine et al. 2016). Site selection criteria included 1) areas within parks suitable for gravid trap placement, 2) catch basins either within or surrounding parks with water and *Culex* spp. larvae, and 3) park locations within hot-spot areas of high WNv transmission (Vazquez-Prokopec et al. 2010). Four urban parks within Atlanta met our criteria (Fig. 1). Our treatment sites were as follows: Grant Park (GP; both experiments, 134 basins and drains identified in an area of 0.37 km^2, basin density—362 km^{-2}) and Springvale Park (SVP; experiment 2 only, 56 basins and drains identified in an area of 0.21 km^2, basin density—267 km^{-2}). Our untreated sites were Phoenix Park (P3; both experiments, 52 basins within 200 m of the sampled basins [buffer area 0.27 km^2], basin density—207 km^{-2}) and Inman Park (IMP; experiment 2 only, 52 basins within 200 m of the subsampled basins [buffer area 0.28 km^2], basin density—186 km^{-2}). Residents near each treatment site were informed of our experiments and WNv surveillance methods by contacting local homeowners' associations.

WNv Surveillance Methods

In each site, regardless of experiment or designation as a treatment or control site, we performed the same surveillance techniques. A subset of catch basins (Fig. 1, 7 [experiment 1] or 10 [experiment 2] in GP, 10 in P3, 8 in IMP, and 9 in SVP) were surveyed weekly for larval, pupal, and resting adult mosquito abundance. Each basin's interior was aspirated with a handheld Prokopack aspirator (Vazquez-Prokopec et al. 2009) for up to 5 min to collect resting adult mosquitoes. Then, three 300-ml water samples were collected with a dip cup attached to an extendable rod and visually examined for larvae and/or pupae. If fourth instar larvae and/or pupae were identified, samples were stored individually in 500-ml Whirl-Packs (Nasco, Fort Atkinson, WI). Each basin's water depth was then measured with a meter stick attached to a rope. CDC gravid trap collections took place weekly in all sites within 200 m of sampled catch basins (four traps in GP, three in P3, four in IMP, and three in SVP). Traps were baited with an infusion of dog food, hay, and tap water following (Chaves et al. 2009) and set on evenings with ≤ 30% chance of precipitation; traps were set after 5 p.m. and retrieved the following morning before 12 p.m. All collections were returned

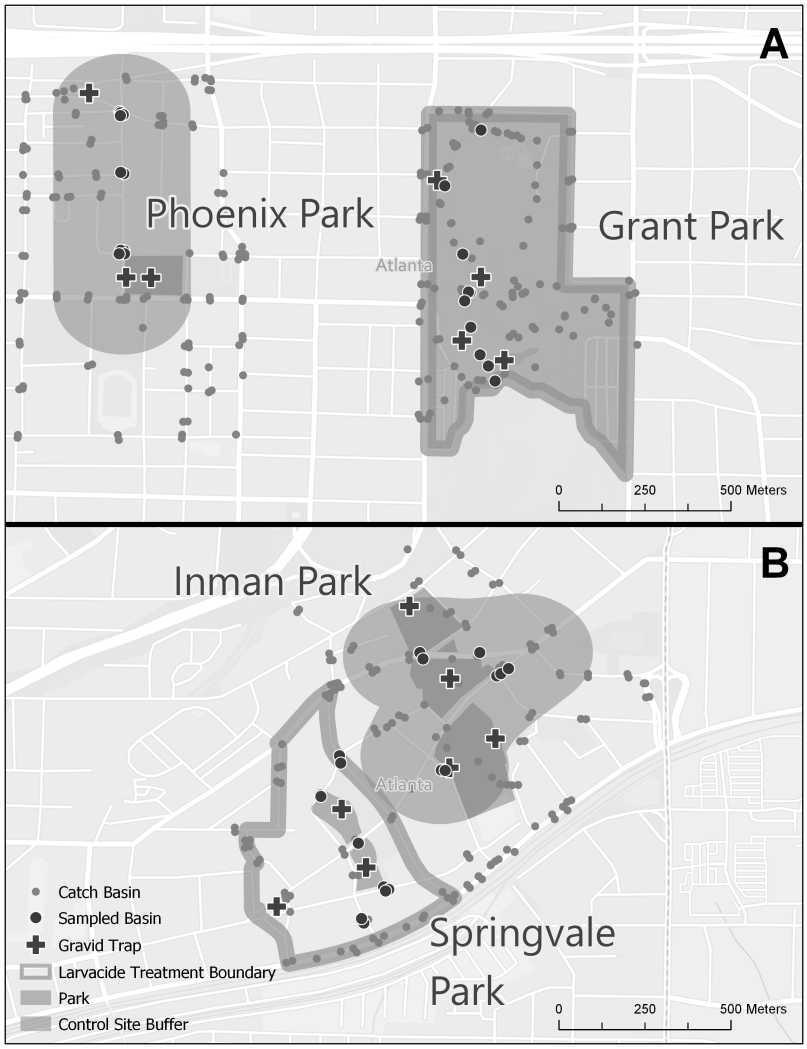

Fig. 1. Experimental site map.

to the Emory University laboratory where adult female mosquitoes from gravid traps and basins were identified to species following a dichotomous key (Darsie and Ward 1981). Collected pupae were counted, placed in a BioQuip emergence chamber (Bioquip Products, Rancho Dominguez, CA) and held at ambient lab temperature until emergence. After all pupae emerged, adults were euthanized at –20°C and then were counted and identified to sex; females were identified to species following a dichotomous key. The number of pupae unable to emerge as adults was also recorded.

All female mosquitoes identified to at least the genus level were pooled for WNv infection testing by date, collection method, collection site, and genus/species with up to 25 individuals per pool; adults emerged from pupae were not tested for WNv. Pools were tested for WNv using previously described virus isolation techniques (Levine et al. 2016). In brief, pools were homogenized with a tissuelyzer at 18 cycles/s and then centrifuged at 9,000 rpm for 10 min. Then, 100 µl of supernatant was aliquoted onto a well of a 12-well plate with 2-d-old Vero E6 cell culture monolayers. Wells were incubated at 36°C and checked daily for visual evidence of cytopathic effects. Suspected WNv infections were screened using Vec-Tests and confirmed using RT–PCR following (Levine et al. 2016). WNv MIRs per 1,000 individuals were estimated using the PooledInfRate plugin for Microsoft Excel (Biggerstaff 2005).

Experiment 1: 2015 WNv Transmission Season

Experiment 1 approximates the approach of larval control by DeKalb and Fulton Counties: larvicides were applied during the historical epidemic period of WNv. Larval and adult weekly surveillance per the above methods began in GP beginning in April. From 17 July to 2 September (CDC weeks 29–35), 134 catch basins and storm drains in GP were treated weekly with Mosquito Dunks (a.i., *B. thuringiensis* subsp. *israelensis* 10.31%) and Bits (a.i., *B. thuringiensis* subsp. *israelensis* 2.86%) applied as one Dunk and one tablespoon of Bits per basin; Bits were applied to provide a quick kill at the time of application, whereas Dunks were applied to provide sustained release of *B. thuringiensis* subsp. *israelensis* in the larval habitat. Larvicides were applied in basins whether they contained water or not to ensure full coverage within the treatment area in case of rain. The manufacturer's reported duration of Mosquito Dunks is 30 d, though a single study with other *B. thuringiensis* subsp. *israelensis* products in catch basins noted significant declines in efficacy after 1 wk (Anderson et al. 2011); in our study, we assumed a residual duration of the larvicides to be only 1 wk. Collections in P3 were considered an untreated control to collections in GP; however, we did not start sampling mosquito populations in P3 until June 2015. Because GP and P3 were not sampled equally during the 2015 surveillance season, GLMMs comparing collections between GP and P3

Table 1. Summary weather, catch basin, gravid trap, and WNv sampling information from experiments 1 and 2

			Experiment 1			Experiment 2	
			2015 WNv transmission season			2016 WNv transmission season	
Experimental periods			Before (weeks 21–28) (Ref. Period GP only)	During (weeks 29–36)	After (weeks 37–44)	During (weeks 12–25)	After (weeks 26–39)
Average temperature (°C)			26.1 (2.11)	26.8 (1.94)	19.1 (3.05)	21.1 (5.25)	27.5 (1.99)
Average precipitation (mm)			6.16 (10.9)	3.82 (8.38)	4.65 (10.7)	1.91 (5.81)	2.85 (7.05)
Water column depth	Treated	GP	28.7 (8.09)	25.3 (8.02)	27.1 (9.42)	21.8 (11.9)	17.8 (10.8)
		SVP	NS	NS	NS	18.3 (9.06)	19.8 (10.8)
	Control	P3 (Ref. Exp. 1)	NS	25.5 (10.8)	29.5 (12.1)	11.5 (11.1)	10.8 (11.7)
		IMP (Ref. Exp. 2)	NS	NS	NS	15.4 (10.3)	18.2 (12.6)
Culex spp. fourth instar larvae	Treated	GP	7.59 (11.8)	**0.86 (2.07)**[f]	**6.09 (11.5)**[β]	11.0 (20.9)	**27.3 (27.7)**[β]
		SVP	NS	NS	NS	3.76 (12.6)	4.69 (14.8)
	Control	P3 (Ref. Exp. 1)	NS	6.24 (38.2)	0.63 (1.33)	8.2 (19.7)	7.85 (16.8)
		IMP (Ref. Exp. 2)	NS	NS	NS	15.9 (37.4)	12.1 (25.4)
Pupae	Treated	GP	5.25 (9.44)	**0.80 (4.18)**[f]	5.68 (15.5)	10.1 (34.2)	**14.6 (20.6)**[β]
		SVP	NS	NS	NS	2.81 (8.77)	3.50 (16.9)
	Control	P3 (Ref. Exp. 1)	NS	0.95 (2.67)	1.37 (3.90)	2.66 (5.65)	1.61 (4.25)
		IMP (Ref. Exp. 2)	NS	NS	NS	8.63 (19.2)	3.30 (10.5)
Culex spp. adults resting in basins	Treated	GP	17.6 (24.1)	**6.45 (10.5)**[f]	**7.27 (14.4)**[fβ]	**10.7 (13.1)**[β]	**14.9 (15.4)**[β]
		SVP	NS	NS	NS	1.90 (3.39)	4.08 (5.85)
	Control	P3 (Ref. Exp. 1)	NS	3.82 (10.2)	1.20 (2.43)	4.67 (12.8)	4.84 (9.26)
		IMP (Ref. Exp. 2)	NS	NS	NS	6.57 (12.1)	6.88 (9.20)
Culex spp. female gravid trap collections	Treated	GP	141.7 (127.2)	**43.7 (42.0)**[f]	**27.8 (21.5)**[f]	107.9 (124.2)	**95.6 (79.6)**[β]
		SVP	NS	NS	NS	83.5 (84.8)	138.8 (99.0)
	Control	P3 (Ref. Exp. 1)	NS	30.6 (18.2)	48.6 (36.7)	69.6 (69.1)	122.2 (137.0)
		IMP (Ref. Exp. 2)	NS	NS	NS	66.5 (56.5)	160.8 (127.5)
WNv infection prevalence	Treated	GP	8/1,148	**25/449**[f]	0/475	0/1,560	42/1,480
		SVP	NS	NS	NS	0/514	24/647
	Control	P3 (Ref. Exp. 1)	NS	5/232	0/281	0/557	**24/759**[β]
		IMP (Ref. Exp. 2)	NS	NS	NS	0/710	72/1,113

Cell values presented as average (± SD) collection from all catch basin and gravid trap sampling events in each site during each experimental period. Experiment 1 was conducted in 2015 from CDC weeks 21 to 44 and consisted of weekly applications of Mosquito Dunks (Summit Chemicals, Baltimore, MD, a.i., 10.31% *Bacillus thuringiensis* subsp. *israelensi*) and Bits (a.i., 2.86% *B. thuringiensis* subsp. *israelensi*) in catch basins in GP from CDC weeks 29 to 36. Experiment 2 was conducted in 2016 from weeks 12 to 39 and consisted of biweekly applications of Altosid 30-Day Briquets (Central Life Sciences, Schaumburg, IL, a.i., S-methoprene, 8.62%) in catch basins in GP and SVP from CDC weeks 12 to 26. Bolded cells signify statistically significant differences from the reference variable. NS (not sampled).

Symbols indicate statistical significance at the $P = 0.05$ level and reference the Site or Experimental Period the cell value is compared with.

[f]Collections significantly different from the before larvicide application period of the experiment.

[β]Collections significantly different from the reference control site listed in the table.

were restricted from 17 July (CDC week 29) to 3 November (CDC week 44), which spans the eight during-treatment weeks (inclusive of a 1-wk residual effect of last larvicide application) and eight after-treatment weeks. GLMMs comparing collections within GP included data collected from eight before-treatment weeks beginning 20 May (CDC week 21).

Because *B. thuringiensis* subsp. *israelensis* products such as Mosquito Dunks and Bits kill larvae, we chose larval, pupal, and resting adult (male and female) *Culex* spp. collections in catch basins as the primary end points for evaluating larvicide efficacy. When comparing collections between GP and P3, we utilized GLMMs for each experimental period with P3 modeled as the reference Site; for GLMMs comparing GP collections only, larvicide treatments were modeled as a period-specific categorical variable (1, before; 2, during; and 3, after) with Period: before-treatment modeled as the reference period. We used Poisson-error GLMMs to compare larval, pupal, and resting adult collections in basins and negative-binomial error GLMMs to compare female *Culex* spp. gravid trap collections. To compare WNv MIRs, we first rounded MIR estimates to the nearest whole number and then implemented Poisson-error GLMMs on the transformed variables. In each GLMM, 'Week' of collection and either 'catch basin ID' or 'gravid trap location' were modeled as random effects.

Experiment 2: 2016 WNv Transmission Season

Experiment 2 was designed to suppress mosquitoes during the growth period of *Culex* spp. populations in early summer. In 2016, WNv surveillance in all sites began the week of 20 March (CDC week 12). Larvicide treatments also began that week, and all 134 catch basins and storm drains in GP were treated with Altosid 30-Day Briquets (a.i., 8.62% S-methoprene), applied as 1 Briquet per basin. Briquets were also applied in 56 catch basins and storm drains within the SVP treatment site (Fig. 1). Because much of the space within the SVP site is private land, larvicide applications were restricted to basins and drains along the public right-of-way. The switch in products between experiments was made to better reflect the treatment of basins with insect growth regulator products as used by both DeKalb and Fulton Counties. In both GP and SVP, Briquets were applied biweekly from 20 March to 24 May (CDC weeks 12–21) in basins whether they contained water or not to ensure full coverage within the treatment area in case of rain. No larvicides were applied in GP or SVP the week of 16 May due to precipitation.

Previous publications with other methoprene products report up to 4-wk efficacy depending on the formulation and mode of application (Butler et al. 2006, Harbison et al. 2018). Though these reports utilized different products, we applied 30-Day Briquets biweekly, which should have mitgated any loss of efficacy associated with a once per 30 d application. Therefore, once Altosid applications ceased, we assumed the larvicide's residual efficacy to be 4 wk. The sites P3 and IMP received no larvicides during the 2016 surveillance season and are considered untreated controls to GP and SVP. The experimental period for experiment 2 was considered to be from 20 March to 30 September (CDC weeks 12–39), which spans 14 during-treatment weeks (inclusive of a 4-wk residual effect of last larvicide treatment) and 14 after-treatment weeks.

Methoprene products such as Altosid interfere with a pupae's ability to develop into an adult; therefore, pupal mortality (i.e., the proportion of collected pupae unable to emerge as adults) and adult collections in basins were our primary endpoints for evaluating larvicide efficacy. As in experiment 1, GLMMs comparing collections between sites were implemented for each experimental period.

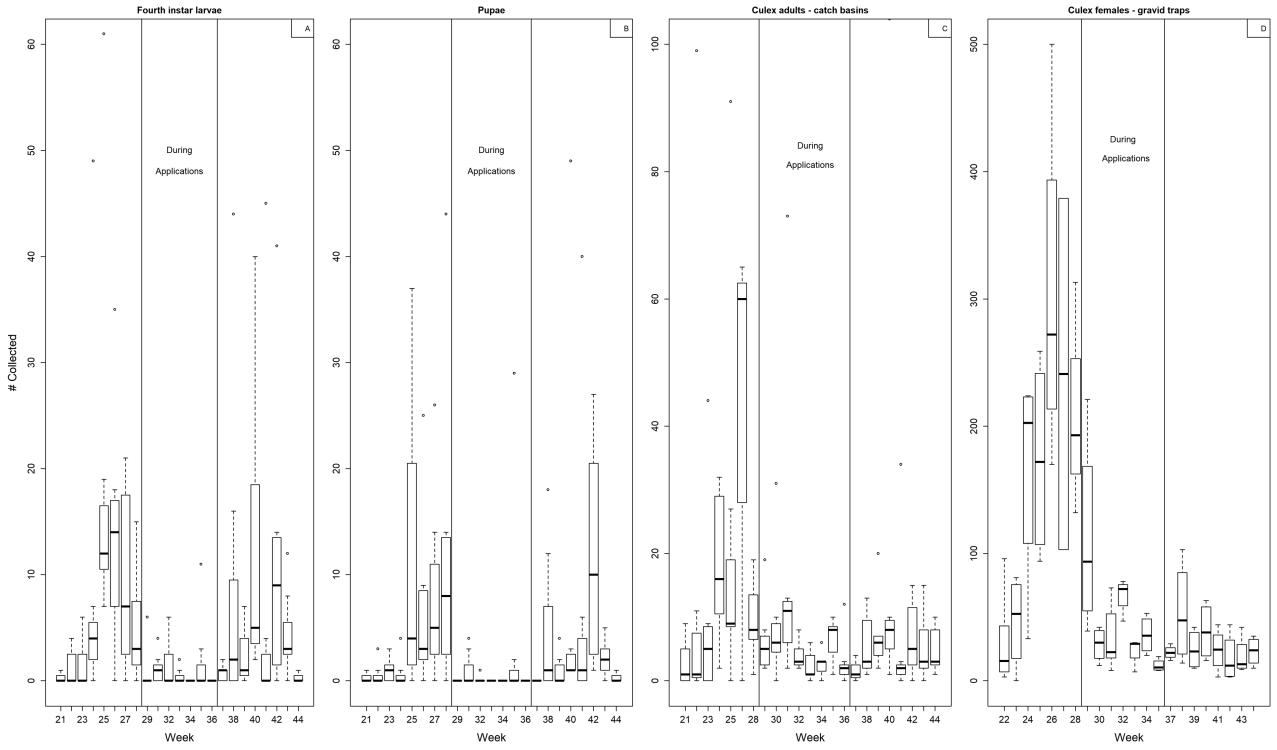

Fig. 2. Weekly *Culex* spp. fourth instar larvae collections (A), pupal collections (B), *Culex* spp. adults resting in basins (C), and *Culex* spp. females in gravid traps within 200 m of sampled catch basins (D) in GP during experiment 1, which was conducted during the 2015 WNv transmission season in Atlanta, GA. GP was treated with Mosquito Dunks Mosquito Dunks (Summit Chemicals, Baltimore, MD, a.i., 10.31% *Bacillus thuringiensis* subsp. *israelensi*) and Bits (a.i., 2.86% *B. thuringiensis* subsp. *israelensi*) in catch basins in GP from CDC weeks 29 to 36. Solid vertical lines in each plot distinguish between the before, during, and after larvicide application periods of the experiment.

In all GLMMs, IMP was modeled as the reference Site with 'Week' and either 'catch basin ID' or 'gravid trap location' modeled as random effects. We used Poisson-error and binomial-error GLMMs to compare catch basin collections and observed pupal mortality, respectively. We used negative-binomial error GLMMs and Poisson-error GLMMs to compare *Culex* spp. female collections in gravid traps and transformed MIR values, respectively.

Results

Average daily temperatures, precipitation, and weekly catch basin and gravid trap collections during each period of experiment 1 and experiment 2 are listed in Table 1, and GLMM tables for comparisons between treated and untreated sites during each experiment are available in Supp Results (online only).

Experiment 1 Results

Average daily temperature throughout experiment 1 was 24.0°C, and daily precipitation occurred 73 times averaging 4.90 mm per event. There were no significant differences in basin water depth in either site between the larvicide application periods (Table 1), and no basins went dry during the experiment. Due to equipment failures, catch basin data on larvae and pupae in weeks 30 and adults in week 26 from GP were not collected. Personnel limitations led to no catch basin sampling in P3 on weeks 32 and 40. In addition, no gravid traps were set in either park during week 36 due to personnel limitations.

Within GP, which was treated weekly with Mosquito Dunks and Bits from CDC weeks 29 to 36, larval and pupal collections were significantly lower in the during-treatment period compared with the before-treatment period (Fig. 2A and B, GLMM results: Larvae, Est. −2.23, $P < 0.001$; Pupae, Est. −3.07, $P < 0.01$). There were no significant differences in larval and pupal collections in GP in the after-treatment period compared with the before-treatment period (Fig. 2A and B, Larvae, Est. −0.04, $P = 0.94$; Pupae, Est. 0.003, $P = 0.99$). Collections of adults (males and females) resting within GP basins were also significantly lower in the during-treatment period compared with the before-treatment period (Fig. 2C, During-treatment, Est. −0.93, $P < 0.01$); however, adult collections in basins during the after-treatment period were also significantly

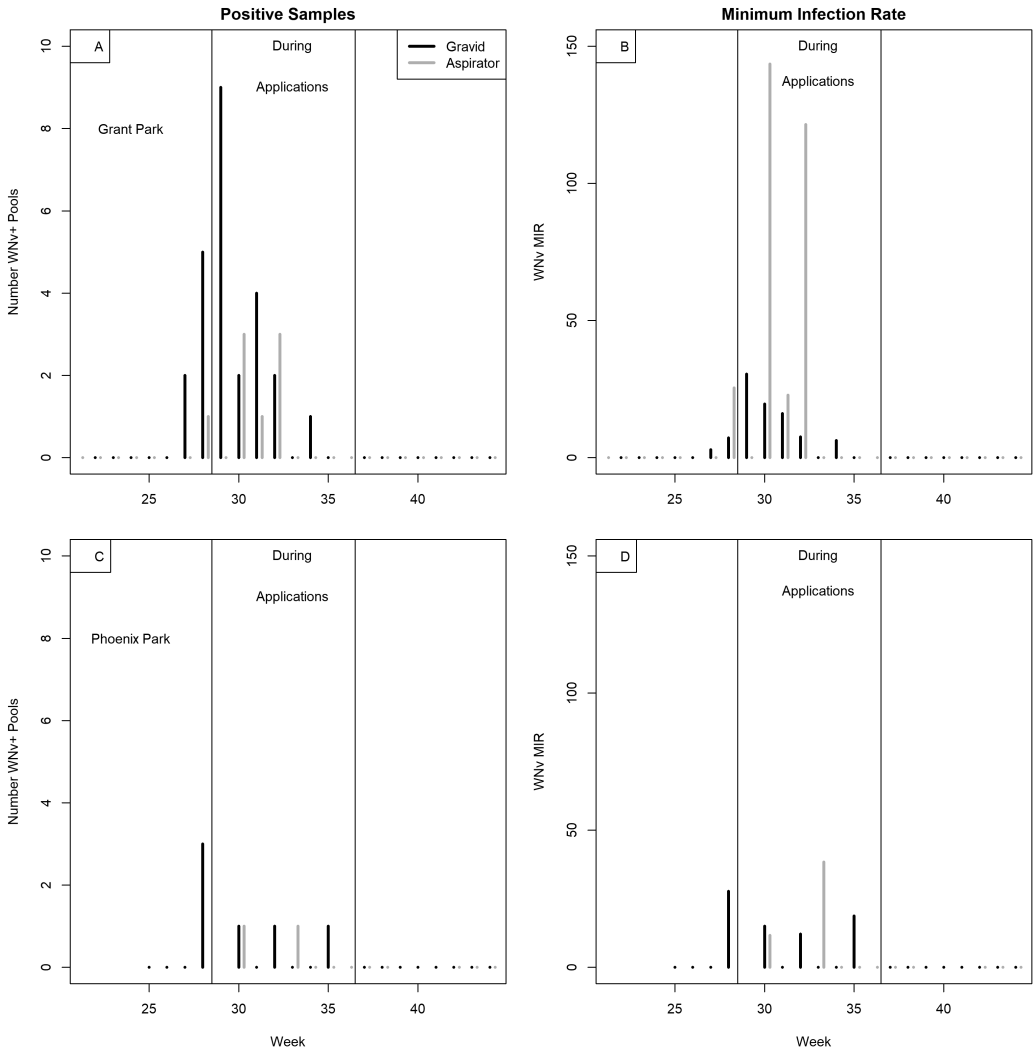

Fig. 3. The number of WNv-positive pools (A and C) and resulting minimum infection rates (B and D) from gravid traps and resting adult collections in catch basins in GP (A and B) and P3 (C and D) during experiment 1, which was conducted during weeks 20–44 of the 2015 WNv transmission season in Atlanta, GA. GP was treated with Mosquito Dunks (Summit Chemicals, Baltimore, MD, a.i., 10.31% *Bacillus thuringiensis* subsp. *israelensi*) and Bits (a.i., 2.86% *B. thuringiensis* subsp. *israelensi*) from weeks 29 to 36. Solid vertical lines in each plot distinguish between the before (GP only), during, and after larvicide application periods of the experiment.

lower compared with the before-treatment period (Fig. 2C, After-treatment Est. −0.72, $P < 0.05$). The same pattern was observed for *Culex* spp. female collections in gravid traps within GP (Fig. 2D, During-treatment, Est. −1.26, $P < 0.001$; After-treatment, Est. −1.57, $P < 0.0001$). WNv infection prevalence in GP was highest during the larvicide treatment period (Fig. 3A and B, Est. 4.20, $P < 0.05$), and WNv-positive samples were detected from both gravid trap and females resting in catch basins from CDC weeks 27 to 35.

Comparing collections in GP with collections in P3, which received no larvicide treatments, there were no significant differences in larval and pupal collections between the two sites in the during-treatment period (Fig. 4, GP: Larvae, Est. 1.24, $P = 0.37$; Pupae, Est. −0.16, $P = 0.93$); larval, but not pupal, collections in GP were significantly higher than collections in P3 in the after-treatment period (Fig 4, GP: Larvae, Est. 2.64, $P < 0.01$; Pupae, Est. 2.03 $P = 0.07$). Resting adult collections in GP were significantly higher than those in P3 in both the during- and after-treatment periods (Fig. 4, GP: During-treatment, Est. 1.71, $P < 0.05$; After-treatment, Est. 2.37, $P < 0.001$). *Culex* spp. female collections in gravid traps were similar between GP and P3 in the during-treatment period (Fig. 4, GP: During-treatment, Est. 0.17, $P = 0.63$), yet were significantly lower in GP compared with P3 in the after-treatment period (Fig. 4, GP: After-treatment, Est. −0.61, $P < 0.05$). Though more WNv-positive samples were collected in GP compared with P3 (Table 1, Fig. 3), the WNv detection period was similar between sites (Fig. 3), and there were no significant differences in WNv MIR estimates from gravid trap collections between GP and P3 (GP: During-treatment, Est. 2.83, $P = 0.16$).

Experiment 2 Results

The average daily temperature during experiment 2 was 24.3°C, and daily precipitation occurred 53 times throughout the experiment, averaging 2.38 mm per event. During 2016, the southeastern region of the United States experienced a drought, and water depth in basins was generally lower in 2016 compared with 2015 (Table 1). Despite the drought, there were no significant differences in average basin water depth between sites in both periods of experiment 2 (Table 1, Supp Table 3 [online only]). The drought, however, did affect the frequency of basins drying out, and basins going dry occurred more frequently in the after-treatment period, increasing from 11.5% of sampling events during-treatment to 18.3% of sampling events after-treatment. Overall, basins were dry more frequently in P3 compared with the other sites with 39.5% of all basin samples in P3 resulting in no water detected. All basins were aspirated to collect resting adults regardless if the basin was dry or not. If a basin was dry, larval and pupal collections were recorded as zero. Due to personnel limitations, no catch basin collections took place in IMP and SVP during weeks 20 and 22, and no gravid traps were set in these two parks during week 22.

Comparing collections between treatment and control sites in the during-treatment period of experiment 2, there were no significant differences in larval or pupal collections from catch basins in treatment sites (GP and SVP) compared with the control reference site (IMP; Fig. 5, Larvae: GP, Est. 1.00, $P = 0.42$; SVP, Est. −1.72, $P = 0.18$; Pupae: GP, Est. 1.37, $P = 0.36$; SVP, Est. −0.93, $P = 0.36$). The biweekly application of Altosid 30-Day Briquets

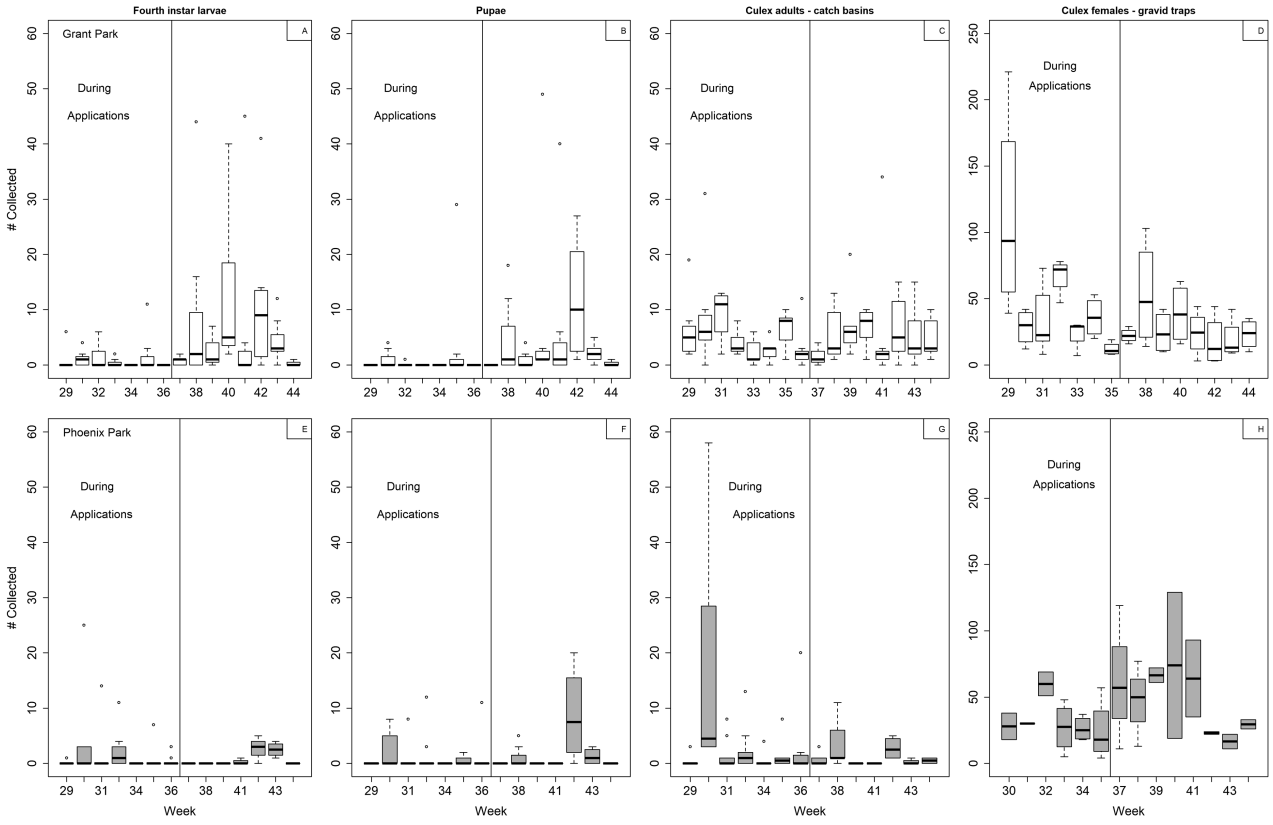

Fig. 4. Weekly catch basin and gravid trap collections from GP (white, A–D) and P3 (gray, E–H) during experiment 1, which was conducted during the 2015 WNv surveillance season in Atlanta, GA. GP was treated with Mosquito Dunks (Summit Chemicals, Baltimore, MD, a.i., 10.31% *Bacillus thuringiensis* subsp. *israelensi*) and Bits (a.i., 2.86% *B. thuringiensis* subsp. *israelensi*) from weeks 29 to 36. Solid vertical lines in each plot distinguish between the during and after larvicide application periods of the experiment. *Culex* spp. fourth instar larval collections (A and E); pupal collections (B and F); *Culex* spp. adults resting in basins (C and G); *Culex* spp. females in gravid traps within 200 m of sampled catch basins (D and H).

significantly increased pupal mortality in GP and SVP (Table 2). Despite higher rates of pupal mortality in GP, more adults were collected resting in GP basins compared with IMP in the during-treatment period (Fig. 5, GP, Est. 1.52, $P < 0.05$); no differences in resting adult collections were observed between SVP and IMP (Fig. 5, SVP, Est. −0.49, $P = 0.52$). There were no significant differences in *Culex* spp. female collections in gravid traps between the treated sites and IMP in the during-treatment period (Fig. 5, GP, Est. 0.55, $P = 0.20$; SVP, Est. 0.19, $P = 0.63$). No WNv-positive samples were detected in any site in the during-treatment period of experiment 2.

All measures of *Culex* spp. populations in sampled basins were significantly higher in GP compared with IMP in the after-treatment period of experiment 2 (Fig. 5, GP: Larvae, Est. 2.41, $P < 0.05$; Pupae, Est. 3.02, $P < 0.01$; Adults, Est. 1.24, $P < 0.05$). We also observed a long residual effect of Altosid in GP and SVP as pupal mortality remained significantly higher in these sites compared with IMP in the after-treatment period of the experiment 2 (Table 2, Supp

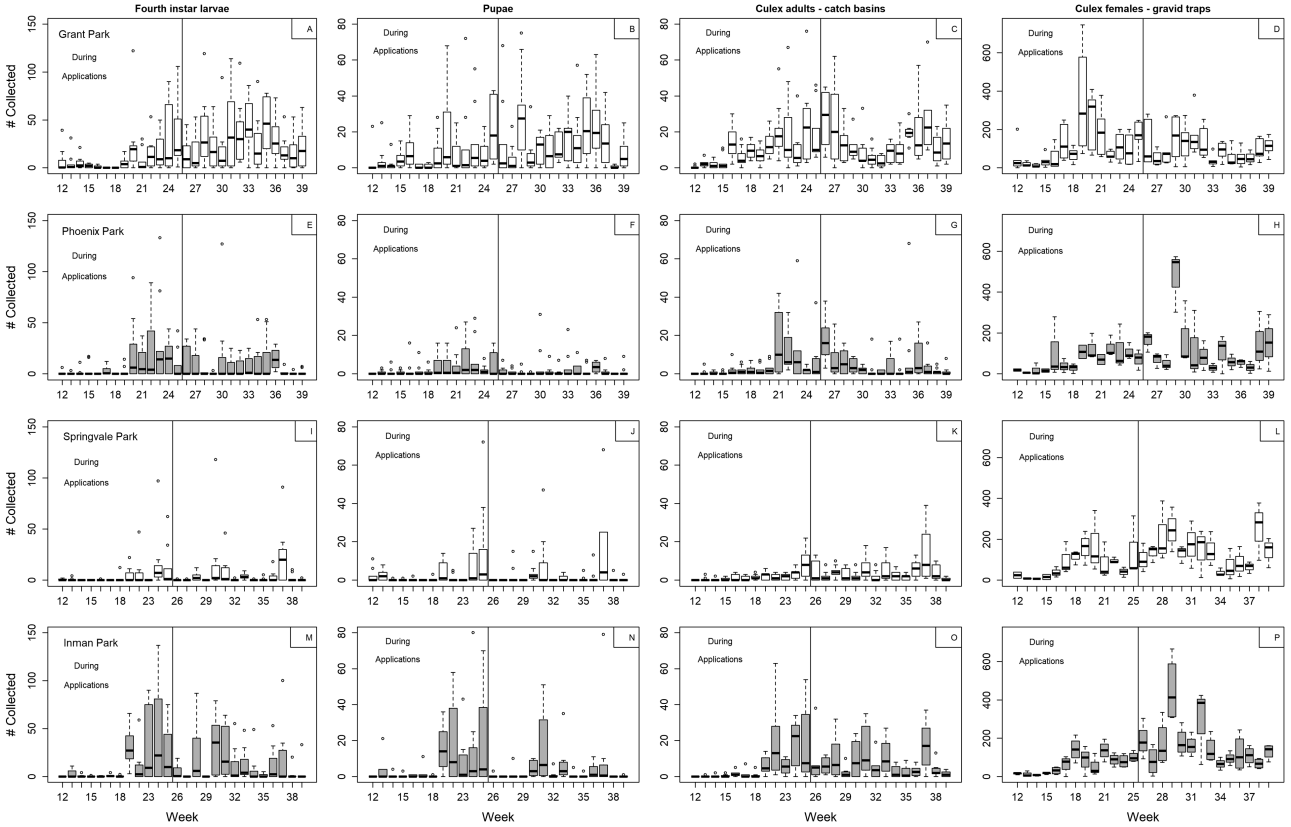

Fig. 5. Weekly catch basin and gravid trap collections from GP (white, A–D), P3 (gray, E–H), SVP (white, I–L), and IMP (gray, M–P) during experiment 2, which was conducted during the 2016 WNv surveillance season in Atlanta, GA. GP and SVP were treated biweekly with Altosid 30-Day Briquets (Central Life Sciences, Schaumburg, IL, a.i., S-methoprene 8.62%) from weeks 12 to 25. Solid vertical lines in each plot distinguish between the during and after larvicide application periods of the experiment. *Culex* spp. fourth instar larval collections (A, E, I, M); pupal collections (B, F, J, N); *Culex* spp. adults resting in basins (C, G, K, O); *Culex* spp. females in gravid traps within 200 m of sampled catch basins (D, H, L, P).

Table 2. Odds ratios that a collected pupa would be unable to emerge as an adult from sampled catch basins in treated and untreated sites during experiment 2, which was conducted during weeks 12–39 of the 2016 WNv transmission season in Atlanta, GA, and consisted of biweekly applications of Altosid 30-Day Briquets (Central Life Sciences, Schaumburg, IL, a.i.—S-methoprene, 8.62%) in GP and SVP from weeks 12 to 25

	Experimental period	
	During Altosid applications	After Altosid applications
Variable	Weeks 12–25	Weeks 26–39
Intercept	0.08 (0.02–0.34)	0.03 (0.003–0.29)
GP *treated*	46.0 (10.0–211.0)	208.8 (16.7–2718.0)
SVP *treated*	71.1 (11.9–422.7)	69.7 (5.06–959.7)
P3 *not treated*	0.77 (0.13–4.70)	2.64 (0.13–55.4)
IMP *not treated*	Ref.	Ref.
Precipitation previous 24 h	1.00 (0.95–1.04)	0.98 (0.95–1.01)

Odds ratios were generated from a binomial-error GLMM for the proportion of collected pupae unable to emerge as adults from sampled catch basins with IMP, which was not treated with any larvicide (neither was P3), as the reference for the variable for Site in each GLMM.

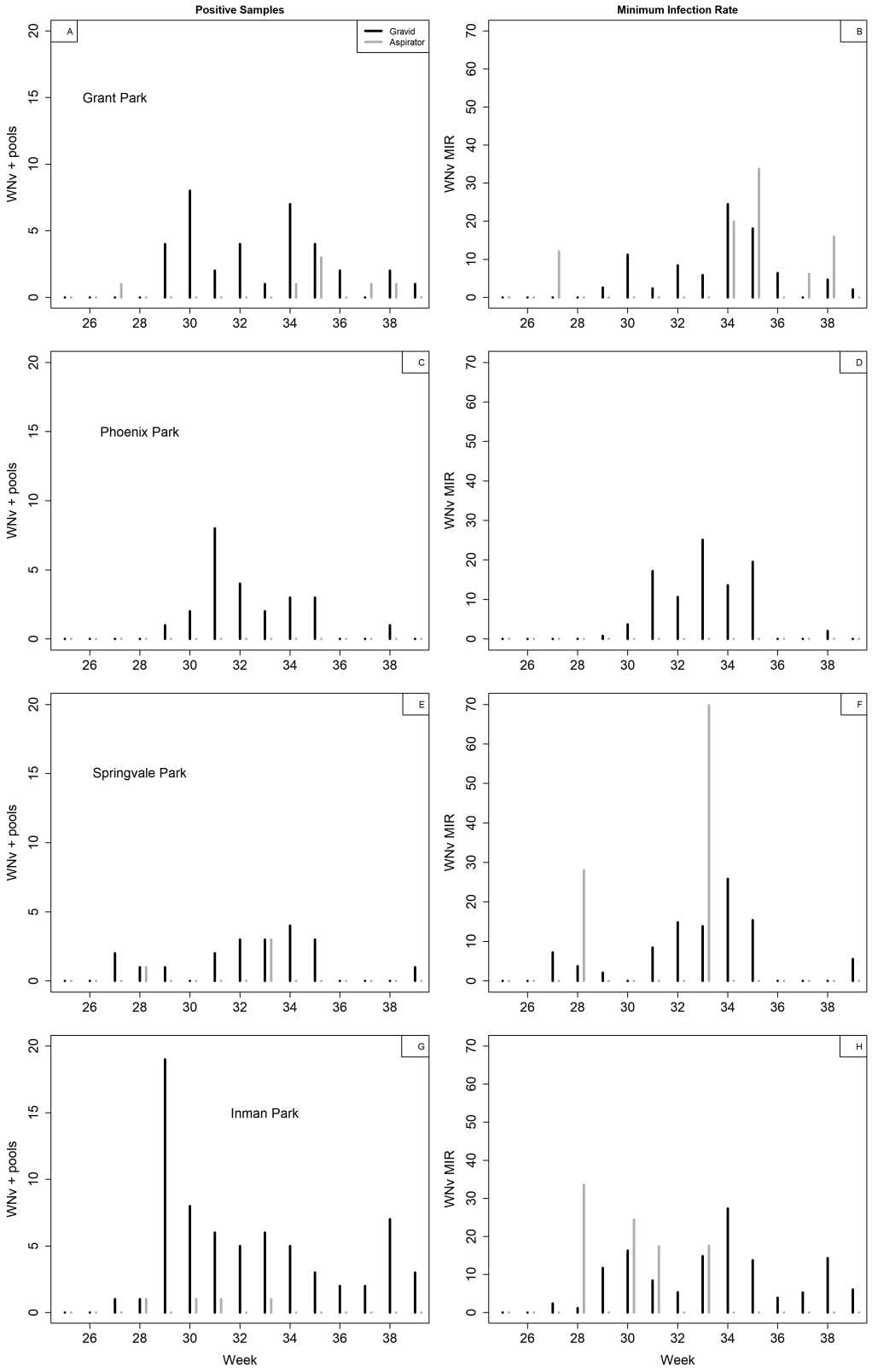

Fig. 6. The number of WNv-positive pools (left column) and resulting minimum infection rates (right column) from gravid traps and adult collections in catch basins in GP (A and B), P3 (C and D), IMP (E and F), and SVP (G and H) during experiment 2, which was conducted during weeks 12–39 of the 2016 WNv transmission season in Atlanta, GA. GP and SVP were treated biweekly with Altosid 30-Day Briquets (Central Life Sciences, Schaumburg, IL, a.i., S-methoprene 8.62%) from weeks 12 to 25. WNv-positive samples were detected in all sites from approximately CDC weeks 27 to 39.

Table 3 [online only]). Though more adults were collected in GP basins compared with IMP, there were significantly fewer female *Culex* spp. collections in gravid traps in GP compared with IMP in the after-application period (Fig. 5, GP, Est. −0.48, $P < 0.05$). WNV-positive mosquitoes were collected in gravid traps and from adults resting in basins from all sites from CDC weeks 27 to 39 (Fig. 6); no WNv-positive samples were collected from females resting in P3 basins. Peak WNv minimum infection rates from gravid traps occurred during week 33 for P3 (untreated) and week 34 for GP (treated), IMP (untreated), and SVP (treated; Fig. 6). GLMMs comparing transformed WNv MIR estimates from gravid trap collections determined that there were no significant differences in WNv infection rates in treated sites compared with the reference control site, IMP (GP, Est. −1.41, $P = 0.09$; SVP, Est. −1.66, $P = 0.06$).

Discussion

Our results confirm previous reports that larvicides are effective at controlling *Culex* spp. larval and pupal populations in road-side catch basins. However, at the spatiotemporal coverage we applied these products (e.g., periods of 8–14 wk over areas of 0.37 and 0.21 km^2), we were unable to link control of larval populations to reductions in adult female collections in gravid traps in close spatial proximity to treated basins. We also infrequently observed declines in adults resting in treated basins, indicating that catch basins are important resting sites for adult mosquitoes in urban environments. The presence of WNv-positive mosquitoes in treated catch basins and our inability to detect significant differences in WNv MIRs between treatment and control sites during either experiment indicates that our larvicide application procedures were insufficient to suppress enzootic WNv transmission in our study areas.

Reported attempts to link larval control to reductions in adult *Culex* spp. mosquito populations have yielded mixed results. Large-scale removal of invasive Amur honeysuckle plants in Mahomet, IL, was linked to reductions in adult *Culex* spp. mosquitoes in light and gravid traps (Gardner et al. 2017). A larval control study in Chicago, IL, found that gravid trap collections were temporally correlated with larval collections in near-by catch basins at a 1-wk lag; however, no link was found between changes in adult collections associated with larval control (Harbison et al. 2014a). In a study from Rome, Italy, in which mosquito control treatment sites were similar in size to ours, researchers found only a small but significant decline in adult *Cx. pipiens pipiens* collections related to larviciding coupled with adulticiding (Caputo et al. 2015). In our study, declines in *Culex* spp. larval and pupal collections (experiment 1) and increases in pupal mortality (experiment 2) were inconsistently associated with reductions in adult *Culex* spp. collections in treated sites compared with untreated control sites. The presence of larval habitats outside of and untreated sites within the boundaries of our treatment sites may have affected our ability to show a reduction in adult abundance. *Cx. pipiens* complex adults are reported to travel up to 1.5 km/night (Hamer et al. 2012), and it is likely that mosquitoes produced from far outside our treatment sites were captured within treatment sites. Despite these limitations, a strength of our approach is that the spatial extent of our experiments was prespecified, and our concentration on public parks provided a tractable system to apply control and monitor mosquito larval and adult populations. In addition, we attempted to link larval control to changes in metrics of WNv infections in mosquitoes, which is seldom investigated. The spatial extent and timing of larvicide treatments in urban environments needed to affect adult *Culex* spp. populations is unknown, and more studies, similar in design to ours (although perhaps much larger), are needed to determine whether increasing the spatial coverage of larvicides (i.e., treating more basins and more nonbasin habitats) leads to greater impacts on *Culex* spp. adult populations and WNv transmission.

Previous research has shown that larvicide product formulations can affect efficacy in basin habitats (Harbison et al. 2018), such that the products we used in our experiments may not have been ideal for control in basins. Our results show that the weekly application of larvicides led to statistically significant decreases in larval and pupal collections in experiment 1 and statistically significant increases in pupal mortality in experiment 2. However, the presence of pupae in treated basins in experiment 1 and the less than 100% pupal mortality observed in experiment 2 may represent operational failures of our treatments. Research has shown basin-specific attributes, either physical or chemical, can result in basins being 'resistant' to larvicide treatments (Harbison et al. 2015, 2016). Identifying 'resistant' basins would certainly improve the operational success of any mosquito larval control program (Harbison et al. 2015), as would using the most effective and operationally efficient larvicide formulation for treating basins. However, what remains critically untested is the link between successful operational control of larvae and successful epidemiological control of adult mosquito populations and WNv transmission.

Road-side catch basins and storm drains are ubiquitous in urban and periurban environments, and *Culex* spp. mosquitoes are known to proliferate within these man-made structures. We have provided empirical evidence that larvicides applied in catch basins may be insufficient to control WNv transmission within urban parks where enzootic transmission is active in the southeastern United States. Our findings coupled with the identified knowledge gaps pertaining to the use of larvicides to control WNv are directly relevant to the surveillance and control techniques employed by DeKalb and Fulton Counties in Atlanta, GA. The Centers for Disease Control and Prevention (2013) recommends larval control as a preventive measure to control WNv, yet both DeKalb and Fulton Counties primarily utilize larviciding catch basins to control mosquito populations; neither county has operational budgets that allow for evaluating the efficacy of their vector control measures. Therefore, each county relies on both product evaluations and the available scientific and public health literature to inform their mosquito control operations. To better inform public health policy, controlled field trials evaluating the epidemiological impact of existing larval control methods against *Culex* spp. mosquitoes are needed (Bellini et al. 2014). Without a clear measure of epidemiological impact, the choice of vector control methods and the timing for their application could lead to major control failures, particularly given the unpredictable nature of WNv outbreaks.

Supplementary Material

Supplementary data are available at *Journal of Medical Entomology* online.

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J.R.M. wrote the first manuscript draft; and all authors contributed substantially to revisions. The authors declare no conflicts of interest. Funding for this research was provided by Emory University (Project ID 00058626).

Data Accessibility

Data from this study are available from the Open Science Framework data archive at https://osf.io/7kt5n/ (McMillan 2018).

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