Evaluation of factors for rapid development of *Culex quinquefasciatus* in belowground stormwater treatment devices

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ABSTRACT: Water samples from 11 belowground stormwater treatment Best Management Practices (BMPs) were evaluated for their capacity to support rapid development of the West Nile virus (WNV) mosquito vector, *Culex quinquefasciatus*. The observed minimum development time from egg to pupa ranged from six to over 30 days. Concentrations of potential food resources (total suspended solids and the particulate organic matter in water samples) were significantly correlated to development times. In addition, the rate of immature mosquito development was both site-dependent and variable in time, suggesting that factors favorable to rapid development were strongly influenced by watershed characteristics and seasonal changes in temperature. Measured temperatures in belowground BMPs suggest that these structures may remain amenable to WNV virus activity longer each year than sites aboveground. ***Journal of Vector Ecology* 34 (2): 182-190. 2009.**

Keyword Index: Stormwater, BMP, belowground, mosquito development, *Culex quinquefasciatus.*

INTRODUCTION

Clean water regulations require that stormwater management include provisions to reduce concentrations of pollutants carried by stormwater and urban runoff through implementation of Best Management Practices (BMPs) (Braune and Wood 1999, Marsalek and Chocat 2002, Ice 2004). Although the term "BMP" is now used to describe a myriad of activities, it was adopted in the 1970s in reference to actions and practices used to reduce flow rates and constituent concentrations in runoff (WEF and ASCE 1998, Ice 2004). In urban areas, structural above- and belowground BMPs designed to capture and treat runoff can provide sites for the development of immature mosquitoes, especially when water stands for more than three days (Santana et al. 1994, Metzger et al. 2003, Metzger 2004). In response to this problem, public health agencies frequently recommend aboveground BMPs be designed and maintained to drain completely within 72 to 96 h to ensure no mosquitoes are produced (Metzger 2004, CDC 2005, CDPH 2008). This time period corresponds to the absolute minimum time any mosquito species can develop from egg to adult under optimal conditions (Gunstream and Chew 1967, Yang 1995). In contrast, draining standing water from belowground BMPs is usually not a viable option because the vast majority of these structures incorporate permanent-water features specifically designed to improve their efficiency at removing pollutants (Minton 2005). As a result, mosquito control in belowground BMPs is restricted almost exclusively to insecticide treatment (Metzger 2004).

Belowground BMPs may be attractive alternatives to large aboveground structures in urban and suburban developments because their "small footprint" reduces land use. However, these structures share many features with traditional belowground stormwater infrastructure known to promote mosquito production, including sources of organically enriched standing water, exclusion of predators, and relatively stable year-round temperatures (Hazelrigg and Pelsue 1980, Strickman and Lang 1986, Su et al. 2003). *Culex quinquefasciatus* Say, is a competent vector of human diseases including West Nile virus (WNV) and has the potential to thrive in these structures (Metzger et al. 2008). With their function to capture and concentrate water runoff pollutants in permanent water debris sumps, belowground BMPs might further benefit *Cx. quinquefasciatus* by providing favorable nutritive conditions for rapid larval development.

The objective of this study was to examine the capacity of belowground BMPs to support rapid egg-to-pupa development of *Cx. quinquefasciatus*. The implications of larval habitats found within these belowground structures and their potential contribution to WNV activity are discussed.

MATERIALS AND METHODS

Study design

A variety of belowground stormwater treatment BMPs were selected in urban, suburban, and commercial areas of Los Angeles, Riverside, and San Bernardino counties, California. Sites were chosen based on several criteria, including safe access, presence of permanent standing water, and previously documented mosquito production. The intent was to sample water captured within belowground BMPs originating from different land uses and, depending on the BMP design, from debris sumps and water quality treatment zones. A total of 11 BMPs were selected, representing five different technologies: a non-proprietary multi-chambered treatment train (MCTT) and proprietary StormFilter®, CDS®, Vortechs®, and VortSentry® (CONTECH Construction Products Inc. West Chester, OH). Descriptions of these technologies can be found elsewhere (Caltrans 2004, Minton 2005).

Water sampling

Using plastic buckets attached to nylon rope, approximately 30 liters of water was collected from each belowground BMP for off-site trials. Water samples were collected from debris sumps in proprietary devices and three serial chambers targeting progressively smaller particulates in the MCTT: a debris sump, a settling basin (waters treated by sorbent pillows and tube settlers), and a media chamber sump (waters filtered through a sand and peat moss media layer). Observations related to water quality (e.g., presence of oil films, floating trash and debris, water coloration) and the presence or absence of adult and immature mosquitoes were noted.

June to September 2007. Water was collected twice per month from four BMPs. Sites were located in a Department of Transportation Park and Ride facility in the city of San Dimas, Los Angeles County (MCTT), a commercial parking lot in the city of Ontario, San Bernardino County (CDS®), and a suburban neighborhood street (VortSentry®) and turf grass community park (Vortechs®) in the city of Perris, Riverside County.

October 2007 to May 2008. Water was collected monthly from the four aforementioned BMPs and a Vortechs® unit located in a commercial parking lot in Ontario. Six additional BMPs located in commercial parking lots in Ontario (two CDS® and two Vortechs®) and in the city of Rancho Cucamonga (two StormFilter®), San Bernardino County, were sampled only once during this time period. The exact site locations of BMPs are available from the authors.

Mosquito development

Culex quinquefasciatus were reared under both field and laboratory settings in containers filled with water samples collected from the studied BMPs. Surrogate rearing sites were utilized in lieu of BMPs because it was not safe or practical to rear mosquitoes within the confines of belowground BMPs (i.e., concerns with working in confined spaces, the relatively large distance between sites, and the potential for the escape of rapidly developing mosquitoes). Minimum development time, defined as the time from egg raft deployment (Day 0) to the appearance of the first pupa, was recorded for each container.

Field trials. Development trials were conducted twice per month from June to September 2007 inside a concrete flood control channel underpass (6 m wide by 6 m tall by 40 m long) in Ontario. This location was chosen because it shared some characteristics with belowground BMPs, specifically concrete structure, buffered air temperature, and absence of direct sunlight. Clear, uncovered plastic containers (60 x 51 x 33 cm deep; Iris® USA Inc., Pleasant Prairie, WI) were used to rear immature mosquitoes. Two replicate containers were each filled with approximately 15 liters of water collected from one of the three proprietary BMPs and each of the three MCTT serial chambers, for a total of six different pairs. A container filled with tap water aged 24 h was used as a control for unknown constituents in BMP water that might affect immature development. The control container and one container in each pair was supplemented with ca. 3 cc of dry food diet (ground alfalfa pellets and brewer's yeast (Twinlab®, American Fork, UT) at 3:1 v:v) on Day 0 and every two days thereafter for the duration of the trial. Preliminary studies showed this to be the maximum amount of food that could be added to this volume of water without creating a potentially suffocating film on the water surface.

Culex quinquefasciatus egg rafts (<24 h-old) were collected from alfalfa-infusion-baited egg traps left overnight at the field site. A single egg raft was placed in each container on Day 0 within a plastic straw modified to form a small floating triangle. The plastic straw triangle served to distinguish the deployed egg raft from additional egg rafts that were deposited overnight in the containers by resident area mosquitoes. Containers were inspected daily to remove newly-laid egg rafts, observe the development of immatures, and document the minimum development time. When the first pupa was observed in a container, a sample of five to ten larvae was collected and identified in the laboratory to confirm them as *Cx. quinquefasciatus*.

Laboratory trials. Development trials were conducted indoors once per month from October 2007 to May 2008 at average water temperatures of 20.3° C and 26° C. White enamel trays (approximately 30.5 x 18 x 5 cm deep) were used to rear immature mosquitoes. Average water temperature of 26° C was maintained using three 100-watt heat lamps with continuous light, whereas 20.3° C was the average ambient air temperature within the laboratory with approximately eight h of light per day. One to three trays were filled with approximately two liters of water collected from BMPs; at least one tray per temperature. One to three trays filled with tap water aged 24 h and supplemented with a "pinch" of dry food (previously described) were used at each temperature as a control for unknown constituents in BMP water that might affect immature mosquito development. Water samples from four proprietary BMPs and each of the three MCTT serial chambers were tested monthly, whereas water samples from six additional proprietary BMPs were tested only once.

One-half of a single *Cx. quinquefasciatus* egg raft (< 24 h-old) collected from egg traps left at the field location overnight (previously described) was placed in each tray. Containers were inspected daily to observe the development of immatures and document the minimum development time. To prevent overcrowding, all but ten larvae were removed using a fine mesh net after second instars were noted in a tray. When the first pupa was observed in a container, a sample of five larvae was collected and identified to confirm them as *Cx. quinquefasciatus*.

Temperature data

Water surface temperatures were recorded hourly using floating HOBO® pendant data loggers (Onset Computer Corp., Bourne, MA) for the duration of all field and laboratory trials. A logger was kept in one container during field trials and in one tray at each temperature during laboratory trials. In addition, a logger fastened with steel framing wire was placed within the serial chambers and debris sumps of each of the original four BMPs (MCTT, CDS®, Vortechs®, and VortSentry®) from June 2007 to June 2008 to document water temperatures within these structures. Daily high, low, and mean outside air temperature data was provided by weather stations of the NOAA National Weather Service Forecast Office located at March Air Reserve Base (Moreno Valley, CA) and the Ontario International Airport (http://www.weather.gov/climate/index.php?wfo=sgx). All sampled BMPs were within 19 km of one of these stations.

Suspended particulate matter

Total suspended solids (TSS) and ash-free dry mass (AFDM) of particulate matter in water samples collected from BMPs was measured once per month at the University of California, Riverside, Aquatic Entomology Laboratory. Between 350 ml and one liter of sampled water was filtered through a 427 μm screen and then vacuum aspirated through a glass microfiber filter (pore diameter = 1.5 μm; Whatman® 934-AH™; Whatman Inc., Clifton, NJ) previously pre-ashed in a 500° C oven for one h and weighed. This range (1.5 to 427 μm) was chosen so as to include suspended particle sizes commonly ingested by mosquito larvae (Clements 2000). Filters holding aspirated material were then dried at 100° C overnight and weighed to the nearest μg for total TSS. Weighed filters were again placed in a 500° C oven for three h to combust any organic material, allowed to cool, and weighed to the nearest μg to determine AFDM (difference in weight between TSS and three h combusted filters) (APHA 1998).

Data analysis

Statistical analyses were conducted using Stata™ 9.2, (StataCorp LP, College Station, TX). Three variables (mg/L TSS, mg/L AFDM, and mean water temperature) were compared to the minimum observed development time using Spearman's rank correlation test. Wilcoxon rank sum tests were used to run pair-wise comparisons of TSS and AFDM among the five BMPs sampled monthly. These tests were also used to compare the minimum development time among the aforementioned BMPs and aged tap water with supplemental food under field and controlled 26° C conditions.

RESULTS

Mosquitoes (adults, immatures, or both) were observed in all proprietary BMPs at least once over the course of this 12-month study (33 of the 79 [42%] water sampling events) and during all months except February and March. Of the three proprietary BMPs sampled from June 2007 to May 2008, mosquitoes were observed most often within the CDS®, with 11 out of 16 (69%) positive site visits. Tight-fitting aluminum covers prevented mosquito entry into the MCTT.

Temperatures recorded inside the concrete flood control channel underpass during field trials and in belowground BMPs were buffered when compared to aboveground air temperatures, providing some support that the surrogate field site held environmental conditions approximating those found in belowground BMPs (Table 1). Comparisons of temperature data recorded inside BMPs against aboveground air temperatures revealed that average yearly temperatures inside BMPs were generally warmer with a much smaller range (Table 2), and mean monthly belowground temperatures usually remained cooler during warm summer months and warmer during winter months (Figure 1).

The number of months (six to eight) that minimum temperatures inside BMPs were above 14.3° C (the threshold temperature for WNV replication and infection of mosquitoes in the region: Reisen et al. 2006, Nielsen et al. 2008) was greater than that for air temperatures recorded by weather stations (one and two months) from June 2007

Table 1. High/low temperatures (°C) recorded inside a concrete flood control channel underpass (field study site), inside belowground storm water treatment Best Management Practices (BMPs), and by the nearest weather station, June 25 to September 30, 2007.

	Ontario International Airport	Field Study Site	BMPs
June	35.00 / 15.56	31.06 / 19.09	30.76 / 21.57
July	38.33 / 15.56	28.85 / 18.71	32.19 / 21.76
August	42.22 / 16.11	27.27 / 18.23	30.96 / 20.52
September	43.33 / 11.63	30.66 / 13.84	31.78 / 19.38

Table 2. Mean (high/low °C) annual temperatures recorded inside four belowground stormwater treatment Best Management Practices (BMPs) and by the nearest weather station, June 25, 2007 to June 30, 2008.

Site	Mean Temperature °C	High / Low range °C
Ontario International Airport	19.0	43.3 / 1.7
CDS®	20.5	32.9 / 10.1
MCTT debris sump	18.5	28.2 / 9.3
MCTT settling basin	19.8	29.0 / 9.7
MCTT media chamber sump	20.1	26.6 / 8.0
March Air Reserve Base	17.2	42.2 / -4.4
VortSentry®	20.2	31.8 / 8.6
Vortechs®	17.8	32.1 / 10.3

Table 3. Mean (high/low) egg to first pupa development time in days at 26° C in water collected from five belowground stormwater treatment Best Management Practices (BMPs) and in aged tap water with supplemental food, June 25, 2007 to May 31, 2008.

Water	Mean Days for Development[a] (high/low range)
VortSentry® (n =9)	8.67 AB (11 / 7)
Vortechs® (n =10)	8.20 AB (11 / 7)
CDS® (n =10)	8.70 AB (11 / 7)
MCTT debris sump (n =11)	9.27 A (15 / 7)
MCTT settling basin (n =10)	8.5 AB (12 / 7)
MCTT media chamber sump (n =10)	9.40 A (16 / 8)
Vortechs®[b] (n =16)	7.56 B (10 / 6)
Tap water with food (n =25)	6.20 C (8 / 6)

[a] Means with different letters indicate significantly different rank sums (P < 0.05).

[b] Site added in October 2007.

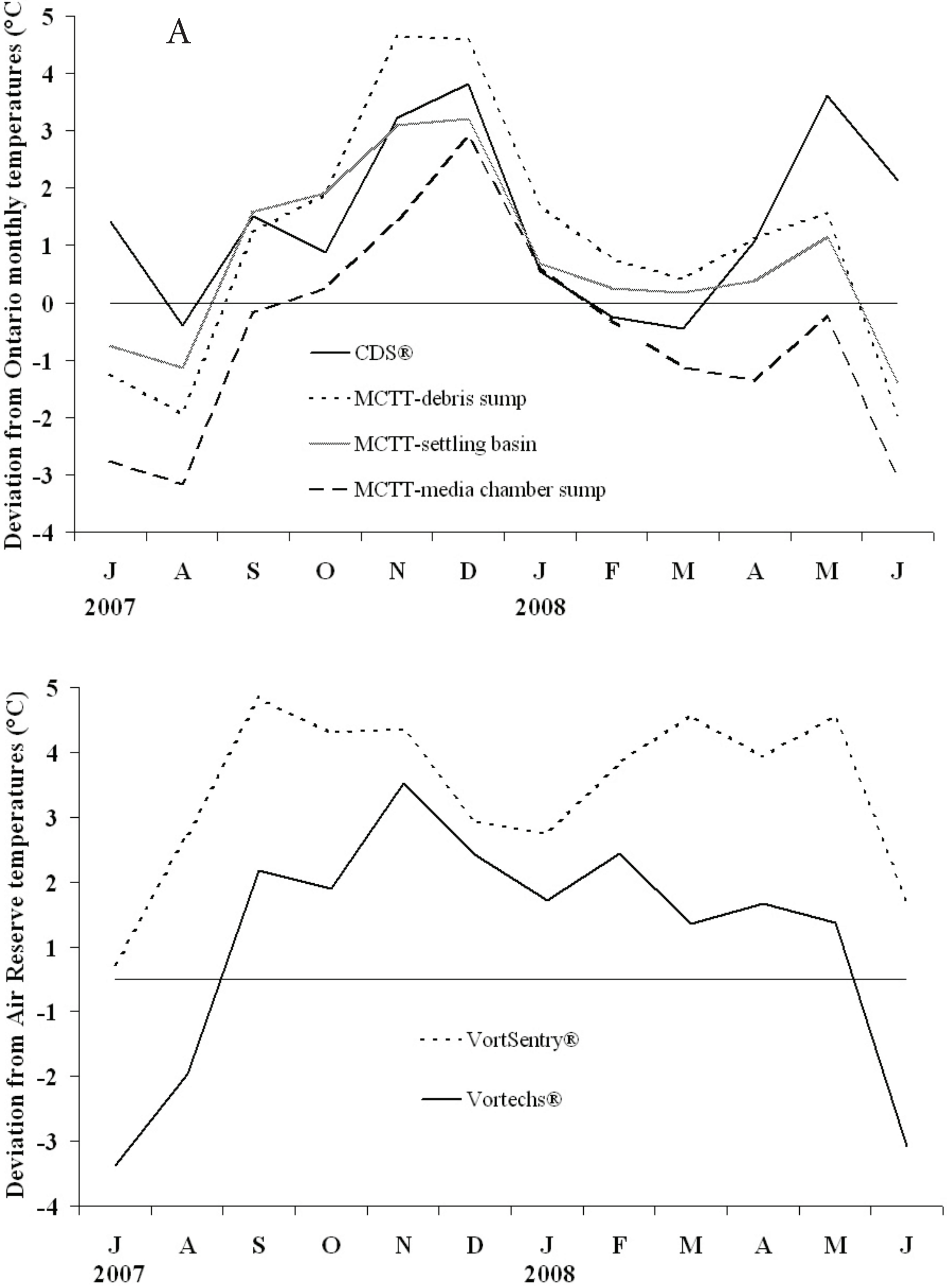

Figure 1. Deviation of mean monthly temperatures recorded in belowground stormwater treatment Best Management Practices (BMPs) from aboveground temperatures, July 1, 2007 to June 30, 2008: A) a CDS® unit and three serial chambers of a multi-chambered treatment train (MCTT) compared to Ontario International Airport, and, B) a VortSentry® unit and a Vortechs® unit compared to March Air Reserve Base.

Table 4. Mean (high/low) weight (mg/liter) of total suspended solids (TSS) and ash-free dry mass (AFDM) in water sampled from debris sumps and serial treatment chambers of five belowground stormwater treatment Best Management Practices (BMPs), June 25, 2007 to May 31, 2008.

BMP	TSS mg/liter[a]	AFDM mg/liter[a]
VortSentry® (n =15)	28.93 D (43.65 / 6.20)	14.82 C (34.54 / 3.27)
Vortechs® (n =15)	19.23 CD (57.83 / 6.20)	11.84 ABC (52.33 / 1.1)
CDS® (n =15)	14.08 BC (36.57 / 4.14)	9.49 ABC (32.33 / 1.69)
MCTT debris sump (n =15)	21.87 CD (88.03 / 2.74)	9.23 BC (31.13 / 1.23)
MCTT settling basin (n =15)	10.02 AB (20.57 / 4.3)	6.59 AB (36.14 / 1.60)
MCTT media chamber sump (n =15)	7.69 A (20.33 / 2.44)	4.61 A (15.3 / 1.27)
Vortechs®[b] (n =8)	128.94 E (355.48 / 16.17)	55.99 D (147.77 / 6.58)

[a] Means within each column with different letters indicate significantly different rank sums (P < 0.05).

[b] Site added in October 2007.

to June 2008. During this time period, the CDS® and the VortSentry® stayed the warmest, recording temperatures below this threshold only from December 2007 to March 2008.

Field Trials. Mosquitoes reared in containers supplemented with food developed more rapidly from egg to first pupae (seven to nine days) than did mosquitoes reared in containers containing water collected from BMPs (10 to 39 days) (z = -8.011, P<0.0001). The mean temperatures during development trials ranged from 17.1 to 23.5° C (mean = 21.1° C).

Laboratory trials. At 26.0° C, the minimum development time of mosquitoes reared in water collected from each of the five BMPs ranged from six to 16 days (Table 3). The most rapid development (six d) occurred in 21 of 25 trials of aged tap water with supplemental food and in five of 16 trials of water collected from the Vortechs® added to the study in October 2007.

The laboratory room temperature ranged from 18.5 to 22.2° C (mean = 20.3° C) during development trials. At this temperature, the minimum development time of immature mosquitoes raised in water collected from each of the five BMPs varied from nine to 62 d. The most rapid development (nine d) occurred in four of seven of trials of aged tap water supplemented with food, two of seven trials of water collected from the MCTT media chamber sump, and one of seven trials of water collected from the previously noted Vortechs®.

Suspended particulate matter. The mean concentrations of TSS and AFDM (mg/liter) measured in water collected monthly from five BMPs are summarized in Table 4. For all 11 BMPs, the TSS and AFDM in water ranged from 2.44 to 547.22 mg/liter and 1.23 to 147.77 mg/liter, respectively. Development time was significantly associated with TSS (r_S = -0.247, P < 0.01; *n* = 152) and AFDM (r_S = -0.183, P = 0.02; *n* = 152), and with water temperature (r_S = -0.752, P < 0.001; *n* = 152). During trials in March 2008, eggs failed to develop in water in two Vortechs® replicates and thus were omitted from the correlation analysis.

DISCUSSION

Belowground stormwater infrastructure for the catchment and conveyance of runoff is well-documented globally to provide habitats amenable to certain mosquito species, including those capable of transmitting the causative agents of disease (Dhillon et al. 1985, Dua et al. 2000, Kay et al. 2000, Tesh et al. 2004, Anderson et al. 2006, Giraldo-Calderon et al. 2008). Recent requirements stemming from water quality regulations have forced tremendous changes to previously acceptable stormwater and urban runoff management strategies. In particular, actions or practices must be implemented to mitigate environmental and public health consequences (both known and speculated) caused by pollutants carried in runoff *before* water enters traditional conveyance systems (Marsalek and Chocat 2002, Metzger et al. 2003, Copeland 2008). A burgeoning industry emerged as a result of these new requirements to meet the demand for engineered structures to improve the quality of discharge water, collectively referred to as stormwater treatment Best Management Practices (Metzger et al. 2003, Ice 2004). The development of compact belowground BMPs suitable for industrial, commercial, and residential developments has been especially active among the private sector.

From the perspective of public health agencies concerned with minimizing mosquito populations and preventing disease transmission, belowground stormwater treatment BMPs add an additional layer of infrastructure to belowground systems that are already complex and known to produce mosquitoes in close proximity to humans. The dilemma for these agencies is that, once installed, belowground BMPs can blend into the existing urban environment, hidden below manhole covers or metal plates in driveways, parking lots, and among landscaping. Data on the numbers and locations of these structures is also difficult to obtain and may not be available from a single source. As a result, many belowground BMPs remain unnoticed and the associated mosquito production unabated. When considering mosquito control activities in these structures, agencies need not only to weigh the difficulties involved with locating belowground BMPs but also concerns with access and worker safety (e.g., heavy manhole covers, confined spaces, vehicle traffic) against their potential to increase local mosquito populations and risk of disease transmission.

The water quality treatment function of the 11 belowground BMPs sampled in this study was evident from observations made during water collections. Floating trash and organic debris were consistently noted in debris sumps and the water was often aromatic, dark in color, and would sporadically have an oily sheen on the surface. To increase the range of water types examined for suspended particulate load beyond typical debris sumps of proprietary BMPs, samples were also taken from two serial chambers of an MCTT that removed progressively smaller particulates from captured runoff. Water samples from both the settling basin and media chamber sump lacked any floating trash or debris, were clearer in color, had little or no odor, and held less visible suspended material.

The rate of immature mosquito development in BMP water was both site-dependent and variable in time suggesting that factors favorable to rapid development were strongly influenced by watershed characteristics affecting the composition of stormwater runoff captured by BMPs. Those BMPs receiving water runoff with relatively high amounts of suspended material provided ample food for larval development. While the specific suspended materials found within water samples were unknown, some portion clearly fit larval nutrition requirements. This finding is supported by the significant negative correlation found between larval development time and TSS or AFDM (both potential proxies for water quality). Containers supplemented with food were included in trials to provide an environment with assumed "optimum" food availability for the most rapid growth. Results comparing the rate of larval development in supplemented and unsupplemented trials suggest food availability may be an important limiting factor to immature mosquito growth in belowground BMPs, in contrast to what has been seen in similar structures elsewhere (Subra 1981).

Another limiting factor to mosquito development connected to food availability can be larval density (Smith et al. 1995, Clements 2000). The density of immature mosquitoes observed in belowground BMPs over the course of this 12-month study varied from "few" to "many"; however, optimum densities for rapid development in these habitats is unknown. As such, a conservative approach was taken for development trials to minimize possible overcrowding of larvae that might slow growth. It is likely that the larval densities utilized in the development trials represented near optimum densities due to noted rapid development.

Although most of the unsupplemented development trials suggested food to be a limiting factor, several samples allowed for six-d egg-to-pupa growth at 26° C. Findings of previous studies (Shelton 1973, Rueda et al. 1990, Henn et al. 2008) suggested that this rate is likely close to the maximum for *Cx. quinquefasciatus*. In six of 23 (26%) unsupplemented indoor trials, the most rapid development rate for both experimental temperatures (26 and 20.3° C) occurred in waters from one particular BMP (Vortechs®). This BMP was located in the parking lot behind a home supply store and consistently received runoff from the greenhouse section of the building. Based on these results, it appears that time periods may exist in certain locations when the amount of runoff and type of suspended material collected in these structures are enough to create conditions where development is no longer limited by food.

All proprietary BMPs included in this study were produced by a single manufacturer; however, mosquitoes have been observed in the vast majority of other belowground BMPs sampled by the authors in southern California (JEH and MEM, personal observation). Our findings suggest that belowground BMPs play a role in producing mosquitoes and should not be overlooked or ignored in mosquito-borne disease surveillance and control activities. In all but two months of the study, larvae and/or adults were noted in at least one of the ten proprietary BMPs. While harboring mosquitoes during the cold months of December and January, temperatures within BMPs were more stable and remained above the threshold for WNV replication three to four times longer than air temperatures aboveground. These conditions could aid overwintering and even year-round virus activity. Studies have shown similar belowground stormwater infrastructure to not only harbor infected adult mosquitoes during the coldest months in the northeastern United States, but contribute to WNV activity throughout the year in southern regions of the country (Nasci et al. 2001, Tesh et al. 2004, Farajollahi et al. 2005).

While it may be difficult to locate and access all belowground BMPs, those likely to receive waters with high organic pollutant loads (i.e., landscape runoff, nursery runoff) should be prioritized for surveillance and control efforts. Based on temperature and development data, certain belowground BMPs could offer habitats promoting adult emergence slightly a week after oviposition during warmer summer periods. With habitat so potentially conducive to the propagation of mosquitoes and WNV, more investigation is needed on the capacity of these structures to produce and harbor WNV infected mosquitoes. As the number of belowground BMPs will likely grow, mosquito control agencies should proactively seek collaboration and cooperation with appropriate stormwater and urban runoff management agencies to aid in locating these cryptic structures and to provide recommendations for safer and more accessible designs.

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