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Environmental Health Insights

Supplementary Issue: Disease Vectors

Identification of Larvicide-Resistant Catch Basins from Three Years of Larvicide Trials in a Suburb of Chicago, IL

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ABSTRACT: The tens of thousands of catch basins found in many urban areas are a primary target of local vector control agencies for seasonal application of extended-release larvicides. A concern with using larvicides in these structures is that active ingredients can be hampered by high flows, debris, and sediment, all of which are common to these structures. As such a certain proportion of basins may be "resistant" to larvicide treatments due to site specific characteristics that may promote these and other factors that hinder larvicide action and/or promote mosquito infestation. Analyses from three years of larvicide efficacy trials suggest that over a quarter of basins in the study area may not be receiving adequate protection from a single dose of larvicide that is routinely applied. Implications of increasing the dose and/or toxicity of larvicide treatments are discussed further.

KEYWORDS: larvicide, catch basin, mosquitoes, West Nile virus

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Introduction

In urbanized areas throughout the world, catch basins (subsurface vaults designed to capture urban storm water runoff) are common habitats for the aquatically confined immature stages of vector mosquito species.[1–7] In the United States, West Nile virus (WNV) vector species in the *Culex pipiens* complex are found consistently within these structures, making catch basins an important focus of local vector control programs.[8–11] In some urbanized regions, including the Chicago metropolitan area, these structures may even be the most prevalent source of these species.[12,13] As a result, significant resources are required for mosquito reduction in these basins. For example, the city of Chicago monitors and applies larvicides to up to 200,000 storm water catch basins annually to reduce the potential exposure to WNV by reducing mosquito populations.[14] Likewise, the North Shore Mosquito Abatement District (NSMAD), serving the predominately suburban area (205 km^2 [79 sq mi]) just north of Chicago, treats about 60,000 catch basins each year.

Owing to the expense and effort required to apply treatments to thousands of individual basins, local mosquito abatement districts in the Chicago metropolitan area utilize specially formulated extended-release catch basin larvicides. These include products such as Natular™ XRT tablets (Natular: Clarke Mosquito Control Products, Inc., St. Charles, IL) and FourStar™ Briquets (FourStar: FourStar Microbial Products LLC, Sag Harbor, NY) that are formulated and marketed to be effective in catch basins for up to 180 days. In many temperate climates, this time period can encompass an entire mosquito season, and the prospect of applying only a single

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dose of a larvicide for each of a vector control program's many basins is attractive logistically and financially.

A number of studies, however, have found that the duration of effective treatment using these larvicides varies and may be shorter than the 180 days promoted. For the FourStar formulation, the City of Columbus' Public Health Vector Control Program reported approximately 100 days of effective treatment in their basins during the 2012 season,[15] while Harbison et al.[16] noted approximately 56 days of reduced mosquito production compared to untreated basins. Studies examining Natular in catch basins found it to be effective but the length of its control varied between 35,[10] 56,[16] and at least 98 days.[11] A shorter period of larvicidal effectiveness may greatly increase the need to apply a second dose. For example, NSMAD technicians begin the season in May by applying a single dose of an extended-release larvicide to catch basins in the southern, more densely populated half of the District. The southern half has a high density of catch basins and this work continues into the month of June. Technicians then begin treating catch basins in the northern and more suburban half of the District. By the time all northern basins have received treatments, typically in August, another round of larvicide applications to these structures begins again in the southern portion of the District.

Factors that can potentially contribute to a decreased period of efficacy of catch basin larvicides are stated in these pesticides' product labels and include the presence of debris or sediment and high rainfall or strong water flow. All of which may reduce the dispersion and the residual life of the active ingredient/s. Unfortunately, by nature of their design and function to capture runoff, such adverse conditions are common to, if not to be expected in, catch basins. These phenomena may be a particular concern when the maintenance of structures is sporadic. Therefore, when water is heavily polluted, catch basin larvicide product labels will suggest two doses of a larvicide per application. Besides the timing of application and dose of larvicides, certain site-specific abiotic and biotic factors can affect the presence of larvae in these structures. For example, larger rainfall events or catch basins prone to higher volumes and/or intensity of runoff may "flush" mosquitoes out of basins resulting in decreased larval abundance in the following days.[17–19] Jackson et al.[4] found catch basins surrounded predominately by pavement were less likely to hold larvae than those with nearby trees or grassy areas. Indeed, Gardner et al.[20] observed certain environmental features, including ammonia and nitrates in basin water, and the area of all shrubs of height <1 m surrounding the catch basins were positively associated of high larval abundance, whereas pH and area of flowering shrub were negatively associated with larvae. To the contrary, Geery and Holub[21] were unable to find an association with larval abundance and pH, but did suggest a positive association with the presence of floating organic debris in the structures.

Therefore, site characteristics of catch basins and their surrounding landscapes – whether they may promote infestations of mosquitoes, hinder larvicide effects, or both – could render some basins essentially unaffected by or "resistant" to seasonal larvicide applications. Such a phenomenon was informally noted by NSMAD during routine catch basin larvicide efficacy trials beginning in 2011. During the summer months of 2011, 2012, and 2013; a proportion of the same 60 catch basins (40, 60, and 30 curbside catch basins, respectively, by year) were monitored weekly within the same small 0.25 km^2 suburban area of the NSMAD operational area. Monitoring was performed by removing the manhole grate of each structure and taking two dip samples using a standard 350 mL dipper. Each year basins were either treated once with an extended-release larvicide formulated for use in catch basins or left untreated. The average number of mosquitoes per dip in untreated and treated basins was then used to inform NSMAD staff on larvicide efficacy. During monitoring, it was noted that certain basins appeared to hold consistently higher numbers of mosquitoes regardless of treatment status, possibly indicating that these particular structures required two or more doses of treatment per application. It was these observations that motivated the current study. Therefore, our objective was to estimate the percentage of basins that were "resistant" to larvicides during these 2011–2013 NSMAD efficacy trials. In particular, we sought to determine whether the NSMAD may be under-dosing a significant number of these structures. It was neither our intent to establish the duration of larvicide effectiveness of Natular and FourStar in this current study, as this has been reported elsewhere,[10,11,15,16] nor was this study meant to evaluate the overall effectiveness of the NSMAD as this agency performs other important services to promote and protect public health aside from monitoring and treating catch basins including, but not limited to, reducing, monitoring, and treating aboveground sources of mosquito larvae, monitoring and testing pools of adult mosquitoes for WNV, spraying adulticides when the risk of WNV transmission is high, and giving educational outreach to the communities it serves.

Methods

During the months June–September of 2011, 2012, and 2013, NSMAD evaluated three different larvicides formulated for use in catch basins. Each year's evaluation was performed by monitoring groups of catch basins within the same small 0.25 km^2 suburban area near the center of the District. Prior to the 2011 trials, 60 basins were identified in this study area for weekly monitoring that was used as study basins during the subsequent 2011–2013 trials. During each weekly monitoring event (two dip samples), the number of larvae and pupae collected by each dip for each basin was recorded. Approximately 1–2 minutes was given after removing the catch basin grate and between dips to allow for resettling of larvae and pupae. All monitoring was performed by the same inspector.

Over the first two years of trials, one to two groups of basins received a single application of an extended-release larvicide at the beginning of the trial, with the remaining basins

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left untreated as controls. During 2011, a group of 20 basins received Natular™ XRT tablets while 20 were left untreated.[11] During 2012, 20 basins received Natular tablets, while 20 were treated with FourStar™ Briquets and 20 were left untreated.[16] Weekly pooled dip samples from treated and untreated basins of the 2011 and 2012 trials and the associated methodology have been reported elsewhere.[11,16] Finally, during 2013, a group of 30 basins were monitored weekly. During the 2013 season, each basin was treated once with a single dose of FourStar when its average weekly dip samples reached 12 mosquitoes or more and subsequently treated with an application of CocoBear™ larvicide oil (CocoBear, Clarke Mosquito Control Products, Inc., Roselle, IL) each week that the threshold was again surpassed. During 2011 and 2013, the basins were monitored for 14 weeks and for 15 weeks during 2012. Further description of the study area and specific methods can be found elsewhere.[11,16]

Natular, a formulation of 6.25% spinosad (a mixture of the neurotoxins spinosyn A and D), kills larvae both through contact and ingestion.[10,22–24] FourStar, a formulation of 6% *Bacillus sphaericus* and 1% *Bacillus thuringiensis israelensis*, kills larvae through the ingestion of toxins released by these bacteria.[10,25] CocoBear is a formulation of 10% mineral oil that leaves a thin film on the surface of treated water potentially killing larvae and pupae via contact or suffocation. Because this film tends to break down quickly, particularly when the water surface is disturbed, this larvicide is considered to be effective for a much shorter duration than the extended-release larvicides.

To determine if the larvicide treatments generally reduce mosquitoes, a two sample *t*-test was used to compare overall means of dip samples from treated and untreated basins during the three-year period. An alpha-*p* value of 0.05 was used to determine statistical significance. To then aid identification of specific basins in which larvicide treatment appeared to have less or no effect, averages with 95% confidence intervals from treated and untreated dips were calculated for each of the 60 basins. From these, basins were categorized into groups that shared similar trends, and we determined the degree to which apparently resistant basins were present. Precipitation and temperature data were collected from a nearby weather station of the NOAA National Weather Service Forecast Office located at the Chicago O'Hare Airport (http://www.nws.noaa.gov/climate/index.php?wfo=lot).

Results

All 60 basins were observed to hold mosquitoes over the three seasons of trials. The average number of mosquitoes found in each dip sample over the three seasons from treated basins was significantly less (6.6 ± 0.76 [95% CI, n = 2,120]) than those from untreated basins (10.9 ± 0.96 [95% CI, n = 2,158, t = −6.77, 4,276 df, $P < 0.0001$]). Over the three seasons a total of 20,885 mosquitoes were collected overall from all 2,158 dip samples from untreated basins and 25,378 from all 2,120 dip samples from larvicide-treated basins, however, these amounts varied by year. Of all collected mosquitoes over the three years, nearly the same percentage of late instars stages were observed from both untreated samples (8,615 forth instars and pupae [41.2%]) and untreated catch basins (10,942 forth instars and pupae [41.3%]).

We then placed 48 of the 60 basins into one of five categories based on apparent "innate" productivity relative to their response to the treatments applied (Fig. 1). Ten of the 60 were not included in this analysis as there were no treated dip samples from these basins. Two others, each having only four dip samples without treatment, were also not included in this categorization. The first category was "treatment ineffective." This included 13 (27.1%) of the 48 basins where treated samples appeared to have produced mosquitoes at a rate equal to or higher than untreated samples. In addition, the average of all dip samples from treated basins in this initial category was higher than the three years average of 6.6 mosquitoes for all treated basins (Figs. 1 and 2). In one basin, there appeared to be no difference in untreated and treated samples but both were lower than the 6.6 overall average. This basin was classified as "treatment ineffective but low infestation" (Fig. 3). In the remaining 34 basins, treatment appeared to be effective, defined by the observation that treated samples appeared to be lower than untreated samples. Eight of these "treatment effective" basins had average counts lower than the 6.6 average in both treated and untreated samples and were classified as "treatment effective – low infestation." Two other "Treatment effective" basins were identified as "treatment effective – severe infestation" (Fig. 3). In these, the averages of dip samples during treatment were less than the averages from untreated samples but were higher than the 10.9 average for all untreated samples over the three years of study (22.9 and 23.35 mosquitoes per dip, respectively).

In 2011, the average temperature and total precipitation was 20.78 °C and 3.39 cm for June, 26.11 °C and 28.32 cm for July, 23.06 °C and 11.53 cm for August, and 16.78 °C and 8.76 cm for September. In 2012, the average temperature and total precipitation was 23.28 °C and 2.29 cm for June, 27.28 °C and 9.30 cm for July, 22.94 °C and 5.26 cm for August, and 17.83 °C and 4.47 cm for September. In 2013, the average temperature and total precipitation was 20.28 °C and 15.82 cm for June, 22.89 °C and 5.64 cm for July, 22.78 °C and 4.29 cm for August, and 19.56 °C and 6.53 cm for September.

Discussion

In reviewing three seasons of monitoring data, larval abundance in catch basins appeared to be highly variable in our study area. Not surprisingly, all dip samples from larvicide-treated basins overall held significantly fewer mosquitoes than those from untreated basins. In the United States, the application of pesticides to catch basins has been a standard control measure for over a century and can be an effective way to reduce

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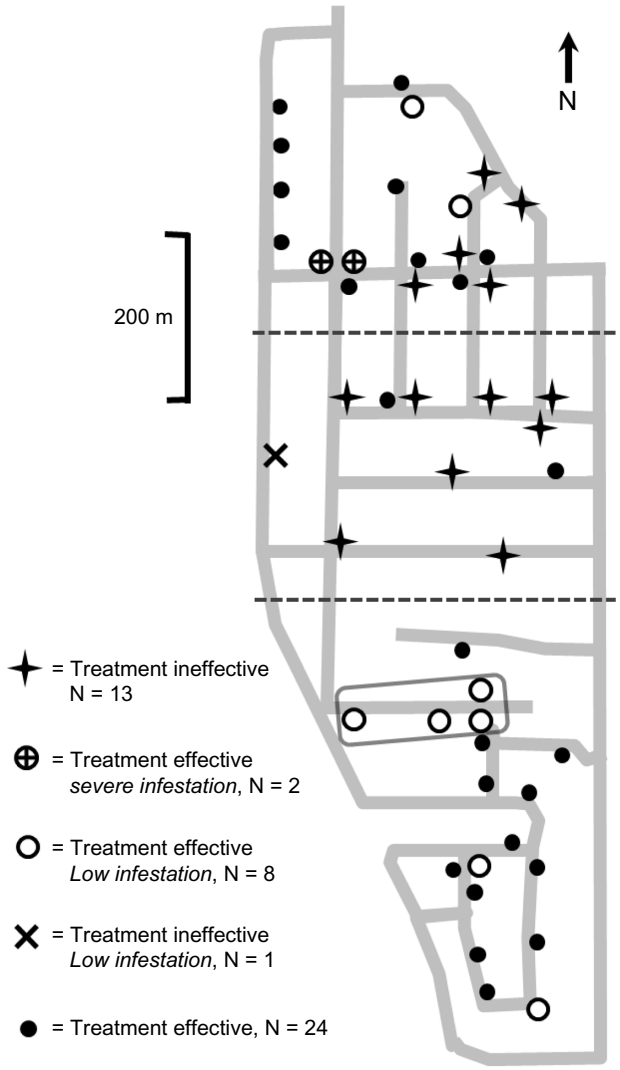


Figure 1. Relative location of 48 curbside catch basins monitored for mosquitoes by weekly dip samples over the course of June–September 2011, 2012, and 2013 for larvicide efficacy trials in a suburb north of the City of Chicago and their assigned categories based on average dip samples with and without larvicide treatment.
Notes: Dotted lines denote the division between the three groups of catch basins utilized in 2011 and 2012. *All catch basins were cleaned once mid-season in 2011 via vacuum-mounted Vactor truck except for four highlighted by rectangle.

numbers of vector species utilizing these structures.[8,26–28] However, what was somewhat unexpected is that from all dip samples from the 60 basins over three seasons, the average number of mosquitoes collected from treated basins was only 4.3 mosquitoes, less than those without treatment. While this difference does appear to be significant statistically, it is smaller than what is desired by the NSMAD as the costs of the larvicide and the expense of labor to apply these larvicides can be substantial. A single Natular XRT tablet, for example, can cost around US$4.

Dip sample counts varied by catch basin, both supporting the idea that unknown individual site characteristics can affect mosquito abundance and allowing for some categorization of structures. There were indeed 13 basins that appeared to be "resistant" to treatments or in which treatments were ineffective. Two more basins had counts in which the larvicides appeared to reduce the mosquitoes but counts in treated samples remained quite high, possibly indicating susceptibility to severe mosquito infestation. The high counts found in the treated samples of these 15 basins could also help explain why such a relatively small difference was observed between total treated and untreated counts. Together, these 15 structures may indicate that, at least during the monitoring period, over a quarter of basins were not receiving adequate control from the single dose of extended-release larvicides. Nine of the basins (categorized as "treatment effective–low infestation" and "treatment ineffective–low infestation") held averages of untreated samples that were low in comparison to all treated samples, suggesting that larvicide application in those specific basins may not have been necessary during the monitoring periods.

Because nearly the same percentage (~40%) of late instars' stages was observed from both treated and untreated samples, it does appear that mosquitoes will continue to exist in basins and to survive till final stages of immature development even with existing and/or recent treatments. Whether this suggests that a proportion of larvae emerging from egg rafts could be resistant to or physically able to avoid the active ingredients of evaluated larvicides is unknown. Unfortunately, it is logistically difficult to estimate what percentage of adult mosquitoes successfully emerge from their pupal stage in catch basins. Though samples may be reared out in laboratory settings,[9,28–30] the NSMAD lacks space for rearing facilities, and estimations of emergence were not attempted during these trials.

Though the cleaning of basins is considered to be ameliorative to mosquito management as the process will remove accumulated debris, it was surprising that in 2011, the first year of trials, all basins except four located in this southern portion – where treatment was generally effective – were cleaned once by vacuum by the local village. This involved pumping most water, debris, and (assumed) larvicide from sumps with a Vactor truck (Federal Signal Corporation, Streator, IL).[11] One might then expect these four "uncleaned" basins to have a greater quantity of accumulated debris than the other basins and thus prone to conditions that can hinder larvicide treatments. This, however, was not evident in our study as our multi-year observations appear to show otherwise. An explanation for this could be that these four basins may generally receive much less debris and/or runoff compared to the others and thus require less frequent cleaning. With less accumulated debris in these basin sumps in general, captured water may be less attractive to ovipositing females. It is also worthwhile to note that even though in the first year of trials the other 44 basins were cleaned of debris that could have potentially hindered treatment effectiveness, we still were able to identify a significant number of basins that were "larvicide-resistant" from that and the following two years. This may suggest that the unknown conditions or site characteristics that play a role

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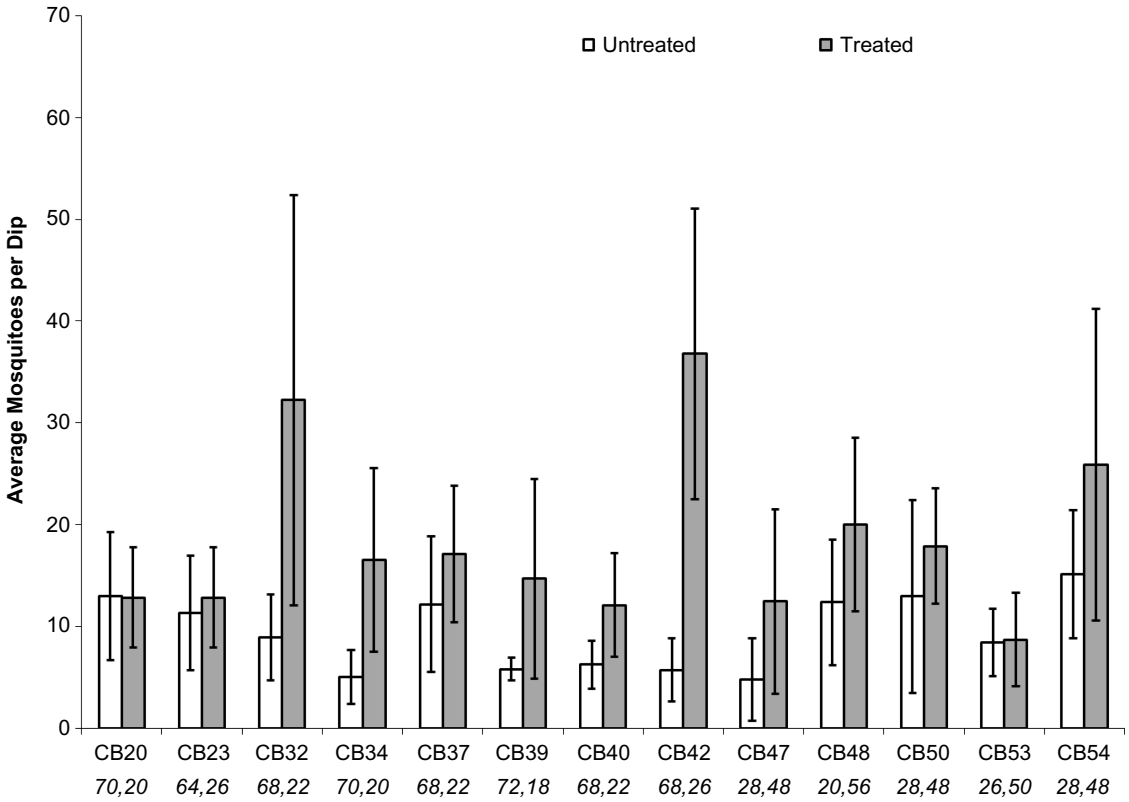


Figure 2. Average mosquito counts with 95% confidence intervals from 13 of 48 study catch basins in which larvicide treatments appeared ineffective from weekly dip sample monitoring during June–September 2011, 2012, and 2013 larvicide efficacy trials. Larvicides evaluated were Natular XRT tablets, FourStar™ briquettes, and the combined use of FourStar and CocoBear larvicide oil.
Note: Italicized numbers below catch basins represent the total number of dip samples during untreated and treated weeks.

in reducing the larvicide effectiveness in these basins occur chronically and/or are part of the basins' physical structures.

Further investigation into site characteristics of the study catch basins and their surrounding landscape could help identify specific factors that may help explain the observed variations in mosquito counts. All basins in the southern portion of the study area were found to be "treatment effective," while the middle and the northern portions held more of a mix of categories. However, even though a number of abiotic and biotic factors have been associated with mosquito abundance in catch basins, a determination of the cause or causes may be of limited practical use to vector control programs with ongoing constraints of time and resources. This would certainly be the case if the factors relate to the site-specific locations where basins were installed (ie, hydrology) and their physical structure and design. These would be variables that local governmental agencies other than those primarily involved with vector control would have control over (eg, municipal public works or engineering departments). Additionally the NSMAD has only 8 full time staff members and approximately 12–15 seasonal workers. Attempting to identify and monitor the potential factors that could contribute to "larvicide resistance" in all or even a significant portion of the approximately 60,000 basins, it services would be beyond this agency's capacity and severely reduce the other important services it provides.

Our study indicated that during any given season, a proportion of all catch basins may not be protected effectively by a single dose of larvicides as commonly administered in this climate zone. Furthermore, our results suggest that up to 30% of monitored basins continued to have high counts of mosquitoes despite the use of larvicides used by NSMAD. Extrapolation from our findings should be taken with caution because the monitoring area in this study was 3/1000 of the size of the District operational area. Similar evaluations elsewhere with other larvicide formulations would help to inform if larvicide-resistant basins are a more widespread and common phenomena. If larvicide-resistant basins are indeed found elsewhere in significant numbers, the prospect of collecting mosquito data from thousands of basins to identify resistant basins may present more of a strain on resources than the application of two or more doses of larvicides per basin, an increase in the number of applications, and/or using larvicides with more toxic active ingredients. At the same time, these solutions have weaknesses for a number of reasons. If catch basins are prone to conditions that reduce efficacy and hinder dispersal of larvicides, then use of a larger quantity and/or more toxic active ingredients may still not achieve the level of desired control. Using more toxic active ingredients may additionally create a concern of harmful effects to non-target species. It is also unknown if or how commonly flushing of

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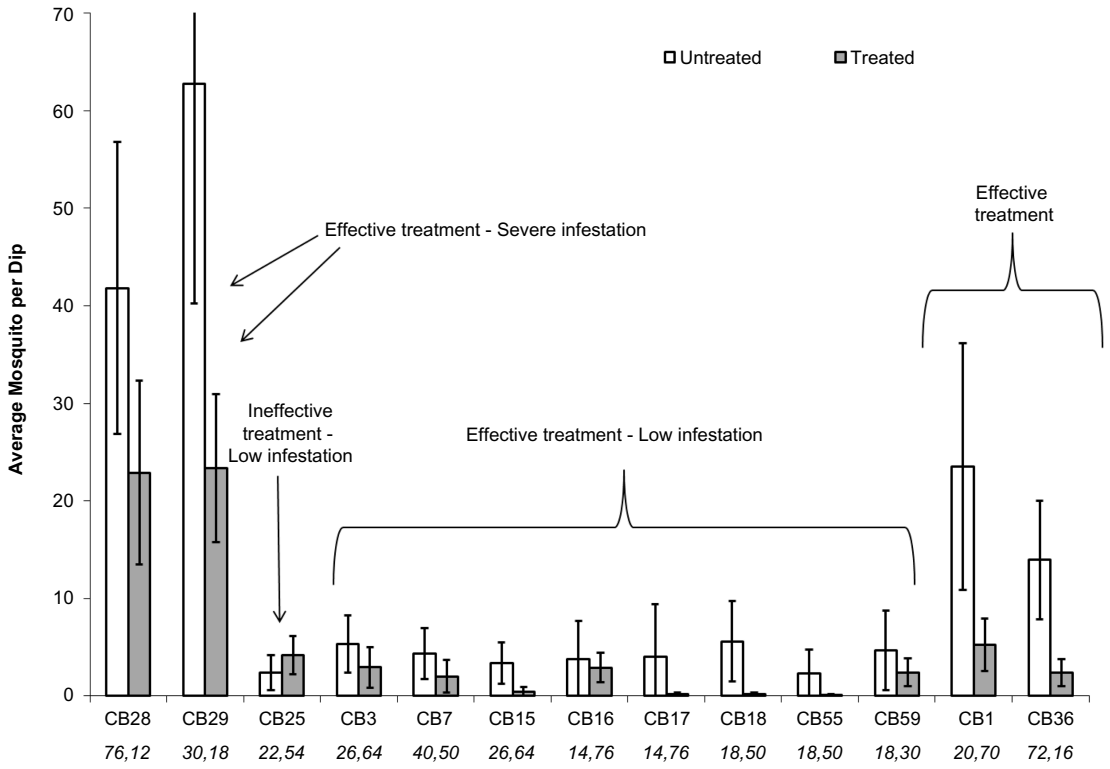


Figure 3. Average mosquito counts with 95% confidence intervals from weekly dip sample monitoring during June—September 2011, 2012, and 2013 larvicide efficacy trials in 13 of 48 study catch basins. Larvicides evaluated were Natular XRT tablets, FourStar™ briquettes, and the combined use of FourStar and CocoBear larvicide oil.

Note: Italicized numbers below catch basins represent the total number of dip samples taken from those specific structures during untreated and treated weeks.

basins caused from heavy runoff may in fact remove larvicide entirely from basins. Finally, in the United States the process of applying a larvicide to standing water is considered a point source of water pollution that requires permitting through National Pollutant Discharge System,[31] and therefore increasing the dose and/or applications of larvicide treatments may be associated with a decrease in water quality. While there may be no simple answer, agencies such as local sanitation departments responsible for the planning, installation, and maintenance of structures, may make different decisions about these structures after better communication and collaboration with vector control programs. This has been suggested over the past century at varying intensities, and is one strong recommendation based on the results from our study.[32–37]

Author Contributions

Conceived, designed, and performed the experiments: JEH, MH, CX. Analyzed the data: JEH, JMS, LRD, MOR. Wrote the first draft of the manuscript: JEH, LRD, MOR. All authors reviewed and approved of the final manuscript.

Supplementary Data

Supplemental Table 1. Average mosquito counts with 95% confidence intervals from 60 study catch basins from weekly dip sample monitoring during June to September 2011, 2012, and 2013 larvicide efficacy trials and their assigned study category.

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