Small-Scale Trials Suggest Increasing Applications of Natular™ XRT and Natular™ T30 Larvicidal Tablets May Not Improve Mosquito Reduction in Some Catch Basins

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ABSTRACT: Stormwater catch basins are commonly treated with larvicides by mosquito control agencies to reduce local populations of mosquito species capable of transmitting West Nile virus. Recent evidence suggests that extended-release larvicides formulated to last up to 180 days in catch basins may not be effective in some basins due to chronic flushing, rapid dissolution, or burying in storm drain debris. To investigate if increasing the number of applications could improve effectiveness, a small study was performed over 13 weeks in 2015 to evaluate two extended-release larvicides (Natular™ XRT 180–day tablets and Natular™ T30 30-day tablets) and a larvicide oil (CocoBear™). Over the course of 13 weeks, three groups of eight basins were monitored for mosquitoes, each group receiving Natular™ XRT, Natular™ T30, or CocoBear™ larvicides. All basins received a single application at the beginning of the study period. Once mosquitoes in a basin surpassed the treatment threshold during weekly monitoring, an additional application of the associated larvicide was given to that basin. The number of applications during the study period ranged from 1 to 10 for CocoBear™ basins, 1 to 7 for T30 basins, and 2 to 8 for XRT basins. Overall, the average number of applications and the cost of larvicide per basin were 4.4 applications at $0.66 per CocoBear™ basin, 4.4 applications at $6.26 per T30 basin, and 4 applications at $16.56 per XRT basin. Basins treated with XRT and T30 needed reapplications more often than expected, yet were no more effective than CocoBear™, suggesting that increasing the frequency of application of these larvicide formulations may not provide increased mosquito reduction in some basins.

KEYWORDS: larvicide, catch basins, mosquitoes, cost

Introduction
Stormwater catch basins are common sources of permanent or semipermanent standing water that are routinely targeted for mosquito larvicide applications by local mosquito control programs to reduce local mosquito populations and incidence of mosquito-borne diseases.1–14 A common practice is to treat the stagnant water in all or most of the tens of thousands or sometimes hundreds of thousands of basins that can exist in an agency’s operational area with at least one application of an extended-release larvicide formulated to last up to 150–180 days. Since the mid-1990s, extended-release larvicides (eg, Altosid® XR, FourStar®, and Natular™ XRT) have been used by the North Shore Mosquito Abatement District (NSMAD), a publicly funded mosquito control agency serving the northeast Chicago metropolitan area, to reduce mosquitoes in ~50,000 catch basins each season. Extended-release larvicide formulations are appealing logistically as a single treatment could theoretically reduce mosquito larvae and pupae for an entire season (ie, May through September). However, there is growing evidence that these products do not provide control for as long as expected.17–23 The cause of the control failures is not well understood but is likely related to the catch basins’ structure, hydrology, and characteristics of the sediments and debris that they collect. Recently, analyses from three years of small-scale larvicide effectiveness trials conducted by NSMAD20 and a year of district-wide catch basin monitoring21 revealed that extended-release larvicide applications fail to control mosquitoes in a significant portion of basins. Because catch basins capture and retain runoff and debris, extended-release larvicides applied to these structures may be susceptible to being flushed out of basins, dissolving more rapidly than designed, or becoming buried in debris captured in the sump. All of these phenomena can influence the effectiveness of larvicides. The objective of this project was to determine if additional applications of extended-release larvicides could improve the effectiveness of these products in basins in which flushing, rapid dissolution, or burying may occur. To do this, three mosquito larvicides, Natular™ XRT 180–day tablet (Clarke Mosquito Control Products, Inc.), Natular™ T30 30–day tablet (Clarke Mosquito Control Products, Inc.), and CocoBear™ larvicide oil (Clarke Mosquito Control Products, Inc.) were evaluated.
during the 2015 season in catch basins located in the same small residential area used for previous larvicide trials.20

Methods
All trials were conducted between June and September 2015 in catch basins located within a 0.7 km² residential area near the center of the NSMAD operational area. As the study area has been used in previous NSMAD catch basin studies, there were several years of data on the present study’s catch basins to inform the present trials. Prior analyses found that extended-release larvicides failed to exhibit any control of mosquitoes over three consecutive years for 12 individual catch basins in the study area. Therefore, each of the three larvicides, XRT, T30, and CocoBear™, was randomly assigned to eight basins that included four of these failure basins and four in which larvicides appeared to be effective. XRT is formulated with 6.25% spinosad, T30 is formulated with 8.33% spinosad, and CocoBear™ is formulated with 10% mineral oil. CocoBear™ is not considered as an extended-release larvicide but was included for control purposes to compare the extended-release larvicides (one and three applications expected, respectively, for XRT and T30) with a larvicide in which weekly reapplications would be expected. The use of untreated basins as a more stringent control was not incorporated in this study over concerns of that these untreated basins could pose a risk to residents within and near the study area.

Beginning the first week of June and ending the first week of September, each of the 24 study basins was monitored for the presence of mosquito larvae and pupae by removing the circular grate of each structure with a manhole hook and taking two dip samples using a standard 350-mL dipper. Mosquitoes collected in a dip sample were counted, with the number of pupae and fourth instar larvae noted. All 24 basins received their respective larvicide treatments in the second week of monitoring. Each subsequent week, a basin was found to have reached or surpassed an average of 12 mosquito larvae and/or pupae per dip, and that basin received an additional treatment of its respective larvicide. The 12-mosquito threshold was found by dip samples taken from untreated basins in the study area from 2011 to 2014 that showed that the average number of mosquitoes from a total of 1,752 dip samples in untreated basins was 13.7 (95% confidence interval [CI] = 12.5–14.9). The total number (sum of two dips) of mosquitoes in basins across weeks was compared among the treatments using a repeated measures analysis of variance. The number and the total cost of larvicide applications were also recorded each week. In 2015 a single XRT tablet cost NSMAD $4.14, $1.43 for a T30 tablet, and estimated $0.15 per single basin for CocoBear™. Precipitation data were downloaded from a nearby weather station of the National Oceanic and Atmospheric Administration’s National Weather Service Forecast Office located at the Chicago O’Hare Airport (http://www.nws.noaa.gov/climate/index.php?wfo=lot).

Results
All basins except two (one treated with CocoBear™ [CBA] and one with T30 [T30h]) surpassed the threshold for retreatment at least once (Table 1). The number of applications to a single basin during the study period ranged from 1 to 10 for CocoBear™ basins, 1 to 7 for T30 basins, and 2 to 8 for XRT basins (Table 1). Generally, there did not appear to be a large difference among the effectiveness of larvicide treatments each week (Table 2). The mean total mosquitoes (sum of two dips) and 95% CI were 17.13 (11.58–22.67) for CocoBear™ basins, 21.38 (15.05–27.70) for T30 basins, and 21.70 (14.97–28.43) for XRT basins over 12 weeks with active treatment. There was no statistical significance found between treatments: $F(2, 21) = 0.32, P = 0.73$. The percentages of the fourth instars and pupae observed from the total mosquitoes found among the treatments each week ranged from 6.9% to 87.9% for CocoBear™, 9.0% to 78.2% for T30, and 9.2% to 75.3% for XRT. The total number of applications to the eight basins was 35 for CocoBear™ and T30 basins and 32 for XRT basins. Overall, the average number of applications and the cost of larvicide per basin were 4.4 applications at $0.66 per CocoBear™ basin, 4.4 applications at $6.26 per T30 basin, and 4 applications at $16.56 per XRT basin.

Discussion
Over the monitoring period, basins appeared to hold similar numbers of mosquitoes across the three larvicide treatments, suggesting, at least in the study basins, approximately equal effectiveness for the amount and type of larvicides used. Certainly, it appears that the use of the extended-release tablets (both XRT and T30) may not be the most cost-effective way to reduce mosquitoes in the study basins. Most extended-release basins needed more than the expected applications throughout the monitoring period, yet their effectiveness was no better than that of the oil. The use of the active ingredient (spinosad) of two extended-release pesticides for mosquito control, including the effect on nontarget species, has been described at length elsewhere.24–30 Although larvicide oils are not formulated to be effective for longer durations, it did appear to be more cost effective than tablets in the study basins. The cost of labor for larvicide applications appears to be about the same for the three products as the total applications were similar. However, the cost of product is significantly lower for CocoBear™ per application, making it the most economical treatment.

Larvicide oils are designed to leave a thin film on the surface of treated water potentially killing larvae and pupae via contact or suffocation. However, because this film tends to break down quickly when the water surface is disturbed (eg, from an influx of rain and runoff), this type of larvicide is considered to be effective for a much shorter duration than the extended-release larvicides. Alternatively, the XRT and T30 tablets are formulated to slowly release their active ingredients over time, killing mosquito larvae and
Increasing applications of Natular™ XRT and Natular™ T30 larvicide tablets

Table 1. A comparison of the number of larvicide applications made to eight CocoBear™ oil-treated, eight Natular™ T30-treated, and eight Natular™ XRT-treated catch basins from June to September 2015. T indicates that the threshold for treatment (≥12 mosquitoes per dip) was surpassed and an application of the associated larvicide to the basin was made.

| BASIN     | WEEK NUMBER | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | TOTAL |
|-----------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|-------|
| CocoBear Oil |             |    |    |    |    |    |    |    |    |    |    |    |    |    |       |
| CBa       |             | T  |    |    |    |    |    |    |    |    |    |    |    |    | 1     |
| CBb       |             | T  | T  |    |    |    |    |    |    |    |    |    |    |    | 6     |
| CBc       |             | T  |    |    |    |    |    |    |    |    |    |    |    |    | 2     |
| CBd       |             | T  |    |    |    |    |    |    |    |    |    |    |    |    | 3     |
| CBc       |             | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | 10    |
| CBF       |             | T  |    |    |    |    |    |    |    |    |    |    |    |    | 2     |
| CBe       |             | T  | T  |    |    |    |    |    |    |    |    |    |    |    | 5     |
| CBf       |             | T  | T  |    |    |    |    |    |    |    |    |    |    |    | 6     |
| T30 30-day Tablets* | |    |    |    |    |    |    |    |    |    |    |    |    |    |       |
| T30a      |             | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | 7     |
| T30b      |             | T  |    |    |    |    |    |    |    |    |    |    |    |    | 3     |
| T30c      |             | T  | T  |    |    |    |    |    |    |    |    |    |    |    | 3     |
| T30d      |             | T  | T  | T  | T  | T  |    |    |    |    |    |    |    |    | 5     |
| T30e      |             | T  | T  | T  | T  | T  |    |    |    |    |    |    |    |    | 4     |
| T30f      |             | T  | T  |    |    |    |    |    |    |    |    |    |    |    | 6     |
| T30g      |             | T  | T  | T  |    |    |    |    |    |    |    |    |    |    | 6     |
| T30h      |             | T  |    |    |    |    |    |    |    |    |    |    |    |    | 1     |
| XRT 180-day Tablets | |    |    |    |    |    |    |    |    |    |    |    |    |    |       |
| XRTa      |             | T  |    |    |    |    |    |    |    |    |    |    |    |    | 2     |
| XRTb      |             | T  | T  |    |    |    |    |    |    |    |    |    |    |    | 3     |
| XRTC      |             | T  | T  | T  |    |    |    |    |    |    |    |    |    |    | 5     |
| XRTd      |             | T  | T  |    |    |    |    |    |    |    |    |    |    |    | 4     |
| XRTe      |             | T  | T  | T  |    |    |    |    |    |    |    |    |    |    | 5     |
| XRTf      |             | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | T  | 8     |
| XRTg      |             | T  |    |    |    |    |    |    |    |    |    |    |    |    | 2     |
| XRTt      |             | T  | T  |    |    |    |    |    |    |    |    |    |    |    | 3     |

Note: *The duration of T30 tablets is formulated to last four weeks, and three treatments would be expected for 13 weeks on weeks 23, 28, and 33.

pupae through contact or ingestion. Since the NSMAD was established in 1927 and until the mid-1990s, the larvicide oils were predominantly applied to the district’s catch basins. Oils are somewhat easier to apply than the extended-release larvicides because often the applicator does not need to leave his or her vehicle to treat basins using a long nozzle to spray the larvicide into basins. As such, all NSMAD basins could usually receive three to four oil treatments in a season. However, our results suggest that three to four applications of oil, as well as T30 and XRT, may not be enough for some basins. Currently, it takes approximately two months or more for NSMAD technicians to treat all basins with extended-release larvicides, and often most basins do not receive an additional treatment before the seasonal technicians become unavailable. Looking for longer lasting alternatives to extended-release tablets and more clearly identifying areas with a significant number of basins in which larvicides fail will be important next steps.

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Table 2. A comparison of average mosquitoes per two dips ± SE taken weekly from eight CocoBear™ oil-treated, eight Natular™ T30-treated, and eight Natular™ XRT-treated catch basins from June to September 2015 and weekly rainfall. All basins received a single application of their associated larvicide at week 23.

| WEEK | COCOBEAR™ | T30 | XRT | RAINFOLL (cm) |
|------|-----------|-----|-----|--------------|
| 24   | 0.0 ± 0.0 | 0.06 ± 0.06 | 0.0 ± 0.0 | 8.33 |
| 25   | 4.88 ± 1.95 | 5.56 ± 1.81 | 4.5 ± 1.99 | 3.18 |
| 26   | 7.25 ± 4.17 | 20.3 ± 5.54 | 12.06 ± 3.93 | 2.01 |
| 27   | 3.06 ± 1.64 | 7.87 ± 2.63 | 9.94 ± 3.78 | 2.18 |
| 28   | 5.37 ± 2.27 | 7.31 ± 1.46 | 11.19 ± 3.18 | 5.05 |
| 29   | 6.13 ± 2.25 | 1.12 ± 0.45 | 11.5 ± 3.45 | 0.00 |
| 30   | 17.88 ± 4.96 | 18.88 ± 4.59 | 12.69 ± 3.03 | 1.35 |
| 31   | 4.44 ± 1.61 | 2.69 ± 0.96 | 1.19 ± 0.67 | 0.00 |
| 32   | 11.44 ± 3.93 | 18.63 ± 3.89 | 21.06 ± 7.47 | 0.94 |
| 33   | 3.63 ± 2.03 | 1.13 ± 0.64 | 1.31 ± 0.52 | 1.32 |
| 34   | 24.5 ± 6.07 | 27.81 ± 6.98 | 31.0 ± 5.1 | 1.88 |
| 35   | 14.19 ± 3.67 | 16.88 ± 4.6 | 13.75 ± 5.88 | 0.00 |

Author Contributions
Conceived, designed, and performed the experiments: JEH, MH. Analyzed the data: JEH, MH. Wrote the first draft of the article: JEH, CZ, CX, PCC, MH. All authors reviewed and approved the final article.

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