Efficacy of *Aedes aegypti* population control methods in the first two mosquito-borne Zika transmission zones in Miami-Dade County, Florida

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ABSTRACT

In the summer of 2016, Miami-Dade County became the site of the first two mosquito-born Zika transmission zones in the continental United States, first in the Wynwood neighborhood in the City of Miami, and subsequently in the South Beach neighborhood of the City of Miami Beach. Control efforts were carried out by Miami Dade County, guided by the Center for Disease Control, and directed by Florida's Governor. These treatments included sprays of adulticides and larvicides, some of which have caused greater concern among citizens in the affected areas than the Zika virus itself. Trap counts of adult female *Aedes aegypti* mosquitoes were obtained by public record request and analyzed graphically and statistically to determine efficacy of the different insecticides applied. Application of permethrin, a persistent pyrethoid adulticide, had no effect whatsoever on mosquito counts. Naled, a potent organophosphate adulticide applied aerially, produced a transitory suppression in Wynwood but lost efficacy after two or three applications. In Miami Beach, aerial application of naled produced no significant reduction of the *Aedes aegypti* population. Repeated application of Bti, a relatively target-specific toxin cocktail produced by the soil bacterium *Bacillus thurigiensis israelii,* was followed by declines in *Aedes aegypti* populations in both Wynwood and Miami Beach, though potential confounds in the data should not be ignored. Specific recommendations are proposed for future treatments of South Florida's urban mosquitoes, as well as better monitoring procedures, and the creation of approved protocols for sharing information with officials, the press, and the public.

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INTRODUCTION

When Zika reached the New World in 2014, few experts doubted it would take long to reach Florida. Locally transmitted Zika cases were reported in Florida in the summer of 2016. Zika first appeared in the neighborhoods popular with international visitors, the Wynwood neighborhood in the City of Miami, followed a month later by the South Beach neighborhood in the City of Miami Beach.

Governmental response was swift, if somewhat uncoordinated. Miami-Dade County's Mosquito Control Division and Department of Public Health, the state Department of Health, the Florida Governor's office, and the Center for Disease Control (CDC) in Atlanta were involved in the decision process, however local government officials were excluded. Direction came from the CDC to spray the most potent mosquito insecticides available, and the Governor gave orders to carry out the CDC's direction. Pushback from concerned citizens arose, and local officials found themselves caught in the middle, wishing neither to defy the desires of their constituents, nor the advise of experts at the CDC and state and county health departments.

Absent from the mix of inputs was expert statistical guidance. Such advice was needed to design scientifically valid monitoring programs that would enable the collection of data to determine efficacy of treatment regimes, and to analyze the data that were being collected through the tireless efforts of Miami-Dade County staff in the Mosquito Control Division.

Local university scientists were asked time and again by municipal government officials and private citizens for advice and guidance on the wisdom of different mosquito control treatments. Until now local scientists could only give generic advice based on published information, few of which were precisely relevant to fighting urban populations of *Aedes aegypti* in the Cities of Miami and Miami Beach.

Florida's constitution and public records law grant the public access to a broad array of documents. Through public records requests made by local government officials, I have been able to piece together data sufficient to enable a statistical analysis of the efficacy of varied mosquito control efforts. These data were provided only gradually and in response to repeated requests, and with assistance from Miami Herald reporters and CDC staff in guiding me as to what data existed that I had not yet received. The Miami Herald successfully sued to obtain certain data.

METHODS

The data analyzed here were obtained through public records requests to Miami-Dade County pursuant to Chapter 119.07 Florida Statutes. Counts of female *Aedes aegypti* captured in 16 BG-Sentinel traps in the Wynwood neighborhood of Miami, Florida, were obtained from the Miami-Dade County Mosquito Control Division for the period from 27-Jul-2016 to 9-Sep-2016. This Wynwood zone was initially sprayed from "Buffalo" trucks with the adulticide permethrin, then aerially with the organophosphate adulticide naled using an ultra-low volume (ULV) spray, and with the bacterial larvicide Bti (VectoBac WG®). The Wynwood zone also received localized backpack fogging with permethrin, and to door-to-door visits from County personnel searching out and dumping standing water. An additional 16 traps were situated immediately outside Wynwood for

11 days, beginning 9-Aug-2016, two days after the second naled application. These trap sites received the aerial naled application as the Wynwood zone, but not Bti, permethrin, or the same intensity of door-to-door inspections. Comparison of these data with the Wynwood data might give us a clue as to the efficacy of aerial Bti application, qualified by our recognition of the confound of different efforts on the ground.

Counts of female *Aedes aegypti* captured in 19 BG-Sentinel traps in Miami Beach, Florida, were obtained from the Miami-Dade County Mosquito Control Division for the period from 21-Aug-2016 to 15-Sep-2016. The Miami Beach zone was sprayed from the air with the organophosphate adulticide naled and from trucks with the adulticide permethrin and the larvicide Bti.

Statistical comparisons of daily mosquito catch counts were run using the MATLAB® Statistical Toolbox. Daily catch counts did not fit a normal distribution (Kolmogorov-Smirnov tests), and they defied attempts to transform them to normality, so comparisons of daily trap counts were made using the non-parametric Kruskal-Wallis test, applying Tukey's honestly significant difference criterion to correct probabilities for multiple comparisons.

Overall comparison of daily median trap counts inside and outside the Wynwood Bti zone was made with a Wilcoxon signed-rank test (1-tailed). Day-by-day tests of raw trap count differences inside and outside the Wynwood Bti zone were made with separate Kruskal-Wallis tests (1-tailed). For both Wynwood and Miami Beach, I also conducted Spearman rank correlation on median daily counts excluding the two days after a naled application.

Exploratory data analysis has relied on non-parametric boxplots (McGill et al., 1978; Tukey, 1977). Catch data for each date are represented by a box extending from the upper 75% quartile to the lower 25% quartile, with the median (middle) value represented by a horizontal bar. "Whiskers" extend above and below the box by 1.5x the interquartile range, which would include 99.3% of the data if the data were normally distributed, which they are not. Data lying beyond the whisker range are represented by '+' symbols. Triangle symbols within (or sometimes outside) the boxes indicate nonparametric comparison intervals. Two medians are significantly different at the 0.05 alpha level (2-tailed) if their associated comparison intervals do not overlap (Nelson, 1989).

For the purpose of this analysis, I have taken the estimated Zika transmission threshold as a 90% reduction of the pre-treatment population, a common, if inaccurate, rule of thumb in mosquito epidemiology. We should recognize that absent modeling based on estimates of human and mosquito density and bite frequency in the affected neighborhoods, that 90% figure could easily be off by a significant amount, as seen in variation in the transmission threshold for dengue in different neighborhoods (Focks et al., 2000).

RESULTS

Permethrin truck sprays

Four successive truck sprays of permethrin over a 5-day period in Wynwood had no measurable effect on trap counts of female *Aedes aegypti* (Figure 1). Despite the complete absence of demonstrable efficacy, the County conducted three more of truck sprays in Wynwood, which likewise had no effect on *Aedes aegypti* counts – the Wynwood mosquito population rose immediately following one such spray application (Figure 2). Following these seven truck applications of permethrin in Wynwood, five such truck sprays of permethrin were carried out in Miami Beach, which likewise had no effect on trap counts of female *Aedes aegypti* (Figure 3).

Naled aerial sprays
Naled (Dibrom®) was applied to Wynwood four times by aerial spray starting at 05:30 - 6:00 between 4-Aug-2016 and 18-Aug-2016 (Figure 2), twice to Miami Beach on 10-Sep-2016 and 12-Sep-2016 (Figure 3), and twice more subsequent to my data set.

Catch counts of female *Aedes aegypti* in Wynwood fell by 60% the day after the first naled application (Table 1, $p = 0.009$), but did not reach the estimated Zika transmission threshold of 90% reduction. Following the second naled application three days later, the mosquito population fell further ($p=0.0001$), to below the estimated Zika transmission threshold, but remained below that line for just one day. Following the third and fourth naled applications, the populations declined less than after the first two applications to levels that were not statistically significant from the day before each application.

Naled's reduction of *Aedes aegypti* counts in Wynwood was transitory. Within three days the female *Aedes aegypti* populations were virtually identical to pre-spray levels (Table 1). Following some of the treatments, the mosquito populations three days later were higher than before the treatment.

The first two naled applications in Miami Beach produced only a modest decline in median trap counts, neither of which was statistically significant (Table 2). Following the first naled application, mean mosquito counts declined slightly, and following second and third naled application, mean mosquito counts rose slightly; none of these changes were significant (following the third application I have mean counts but not individual trap counts). Differences between median and mean mosquito catch numbers (Table 2), which were not seen in the Wynwood data (Table 1), result from upward data skew, indicating that spatially localized pockets of mosquitoes eluded the spray.

Bti (VectoBac WV®) application
The Wynwood zone receiving Bti showed a statistically slower recovery of mosquito populations than the area immediately outside Wynwood that did not receive Bti ($p = 0.002$, Wilcoxon signed-rank test). Daily catch counts started out similar inside and outside the Bti treatment zone, but recovered much faster outside the zone receiving Bti (Figure 4). Excluding the post-naled days, *Aedes aegypti* trap counts declined significantly in Wynwood (Spearman signed-rank correlation, $r = -0.73$, $p > 0.0001$, 1-tailed). Even leaving out the first three counts, which were much higher than the subsequent days, the decline remained statistically significant ($r = -0.41$, $p > 0.05$). Miami Beach trap counts, declined significantly starting two days after the first Bti application (Spearman signed-rank correlation, $r = -0.74$, $p > 0.02$, 1-tailed).

While declines followed Bti application both in Wynwood and Miami Beach, only in Wynwood did the trap counts suggest the population had been depressed below the likely transmission threshold for Zika. At this point the County backed off aerial application of Bti, whereupon the mosquito population began to climb once again (Figure 2). In Miami Beach, the mosquito population did not fall below the estimated transmission threshold following Bti administration, at any time during the period for which data were obtained (Figure 3).

DISCUSSION

Permethrin truck sprays

The first four truck sprays of permethrin in Wynwood had neither a measurable effect nor a statistical effect on trap counts of *Aedes aegypti* females, yet the County proceeded to conduct eight more permethrin sprays. Residents and non-target organisms in Wynwood and Miami Beach were unnecessarily exposed to permethrin, a particularly persistent insecticide. Time, money, and labor spent on permethrin application could have been directed to more effective pursuits. These permethrin applications continued, even after the County Mosquito Control Division, the Public Health Dept., and the CDC had seen data showing clearly that truck sprays of permethrin were not reducing local *Aedes aegypti* populations and could have no effect on Zika transmission. When clear information fails to influence the course of action, something is broken in the decision chain.

Whether local *Aedes aegypti* have evolved chemical resistance to permethrin or whether this mosquito's habits prevent exposure to truck spray cannot be ascertained from these data. Use of positive controls (caged mosquitoes) would have resolved this question early on. CDC scientists have previously commented on the ineffectiveness of truck sprays to kill *Aedes aegypti* in Puerto Rico both because of inaccessibility and evolved chemical resistance. A vast literature has documented the evolution of multiple genes that confer resistance to permethrin and other insecticides (rev. Hemingway and Ranson, 2000). In Florida, the historic use of organophosphate insecticides to control saltmarsh mosquitoes followed the disuse of chlorinated hydrocarbons such as DDT, which had lost effectiveness because mosquitoes had acquired adaptations that conferred resistance. Some of the adaptations to DDT also confer resistance to pyrethroids such as permethrin, which similarly target the voltage-gated sodium channels (Aguirre-Obando et al., 2016; Chuaycharoensuk et al., 2011; Ishak et al., 2015). The County is planning to switch from permethrin to the adulticide etofenprox (Zenivex®), a non-ester pyrethroid that, like permethrin and DDT, targets the voltage-gated sodium channel. The additive insect toxicity of permethrin and etofenprox (Schleier and Peterson, 2012) suggests competitive binding at the sodium channel. If channel mutations are conferring permethrin resistance in *Aedes aegypti* in Miami-Dade County we would not predict improved lethality of etofenprox over permethrin. If resistance stems from mutations of esterases or other detoxification enzymes, a switch to the non-ester etofenprox might improve efficacy.

Naled

After the initial naled applications in Wynwood, the mosquito population never rebounded to its pre-naled levels, possibly because of subsequent Bti application. One is tempted to attribute the suppression to the combined effects of the adulticide and larvicide. In Miami Beach, however, the same decline in *Aedes aegypti* trap counts was seen over the first 13 days of the data set before either naled or Bti were applied, so we cannot be sure about the Wynwood effect in the absence of broader spatial controls.

Naled did appear to have short-lived success during its two (possibly three) initial applications in Wynwood, after which its efficacy diminished. Naled produced little effect in Miami Beach. Aerial UVL application of naled over Puerto Rico on four successive mornings killed female *Aedes aegypti* in outdoor cages, but produced only a temporary decrease in the wild population of ovipositing females, the short-term reduction being of a small magnitude that would not halt transmission of Dengue (Clark et al., 1989). In Puerto Rico, as in Miami, the mosquito population rebounded quickly.

The reason for naled's loss of efficacy in Wynwood is not certain. Such rapid evolution of chemical resistance is not expected, but not impossible given historic annual exposure of Miami's *Aedes aegypti* population to naled and malathion during aerial sprays to control the non-disease carrying "nuisance mosquito" *Aedes taeniorhynchus*. Another possibility for the loss of naled's effectiveness in Wynwood is behavioral resistance. Population-wide variation is seen in many behavioral traits, and has been documented in the clock genes that regulate circadian rhythms for activity and hiding (Costa and Kyriacou, 1998; Kyriacou et al., 2008). *Aedes aegypti* is a diurnal species, most active around the edges of the day and midday in shady areas, but hiding in inaccessible locations at night. The first spray applications between 05:30 and 06:00 might have killed the early risers, leaving the remaining population of later-rising mosquitoes untouched and free to reproduce. Rapid directional selection on natural clock gene variation could make this mosquito population rapidly resistant to early morning adulticide treatments.

When Zika was first identified in Miami Beach, mosquito control experts stated that aerial naled application was unfeasible in that neighborhood because buildings would interfere with even dispersal of the spray. *"These tiny droplets are very much impacted by wind currents…. In cities with tall buildings, you've got wind currents that won't keep the pesticide on the ground where it will do any good."* (J. Conlon, Technical Advisor to the American Mosquito Control Assoc., Miami Herald, 23 Aug 2016). Subsequently, the CDC and County decided to carry out aerial sprays on Miami Beach anyway. *"The announcement* [to spray naled] *represented a reversal by state officials and the Centers for Disease Control and Prevention, who previously said Miami Beach's dense urban environment and high-rise buildings made aerial spraying infeasible"* (Miami Herald, 6 Sep 2016). The mosquito catch data from Miami Beach indicate that the experts' skepticism was well-founded; aerial spray on Miami Beach had no positive effect and apparently missed most of the mosquitoes on the ground, though non-target effects were reported.

Bti

Declines in adult female *Aedes aegypti* counts following Bti application in Wynwood and Miami Beach could have resulted from the Bti, from localized ground efforts to reduce standing water, or from some combination of the two. Controlled experiments should be conducted as soon as possible in structurally comparable neighborhoods that are not believed to be infected with Zika, to determine the best methods and regimes for Bti application, and the degree of mosquito population suppression that can be achieved.

Such experiments will pay dividends for the control of ongoing and future outbreaks of Zika and other arboviruses.

Bti is a unique insecticide because it is naturally composed of four different insecticidal compounds with different actions, which makes it difficult for mosquitoes to evolve resistance against (Tetreau et al., 2012). Further, Bti is extremely specific, killing only a subset of Diptera (the order of two-winged flies), which includes mosquitoes. Given the high monetary cost of Bti application, while recognizing its specificity and the promise it affords as an agent that retains efficacy, we need to know how well is working as applied by helicopter and truck in our urban habitats.

Intepreting trap count statistics

The trap placement layout did not include systematic negative controls for naled, permethrin, or door-to-door water dumping. Thus we cannot account for routine, area-wide fluctuation in mosquito populations that could have confounded the effects of pesticide application. Our inability to factor out natural variation limits our confidence in the causality of insecticide treatment inferred from the Wynwood and Miami Beach datasets. Failure of particular statistical tests to detect a significant difference means that any observed difference might reasonably have resulted from chance effects. Absence of statistical significance for a particular test does not mean that the pesticide application had no effect, just that we have low confidence in any possible effect observed. Median and mean effect sizes should be emphasized, however, because we are seeking reductions in mosquito populations of a magnitude sufficient to suppress transmission.

Experimental design

Given the importance of data collected on efficacy of mosquito control during a Zika outbreak, greater attention should have been given to experimental controls. Positive controls typically take the form of caged mosquitoes situated in the sorts of places wild mosquitoes would be expected to reside. Negative controls would include traps situated in areas not receiving particular treatments. The situation of traps in the zone that received naled but not Bti was helpful, but it was one of several controls that were needed. The absence of stronger experimental controls begs for an expertly guided experimental design created before future treatments are needed. The CDC has the expertise to design well-controlled experiments, as do local universities and health departments. The absence of such guidance speaks both to a lack of coordination with available experts and insufficient internal agency staffing. Unlike Florida's other mosquito control districts, Miami-Dade County's mosquito control division employs no trained scientists with advanced degrees.

How much suppression is enough to stop Zika?

A rule of thumb in mosquito epidemiology holds that to stop a mosquito-borne disease, the adult mosquito population must be reduced by 90%. The transmission threshold is determined not by a generic reduction percentage but by the ratio of adult female mosquitoes to humans at which transmission fails. Thus the proportional depression of the mosquito population necessary to stop an epidemic of mosquito-transmitted Zika in a neighborhood depends on the initial density of both humans and mosquitoes in that area and the frequency of bites (Focks et al., 2000). The modeling can be done for Zika in Miami, but, to my knowledge, has not been done. As a consequence, County and CDC staff have been forced to shoot in the dark, knowing their target threshold only by whether new Zika cases are diagnosed that can be traced back to mosquito-transmission in particular neighborhoods.

Non-target effects

Most humans in residential zones receiving ULV spray of naled normally show low exposure residues and experience no health complications (Duprey et al., 2008). Some vulnerable individuals, however, may experience problems following limited exposure to organophosphate insecticides such as naled. One in 2800 people is homozygous for a gene that results in pseudocholinesterase deficiency, making them particularly sensitive to organophosphates (Purdham and Gutierrez, 1986). Fetuses and children below the age of 7, individual with paraoxonase 1 enzyme gene variant $PON1_{-108TT}$ may be particularly susceptible (Huen et al., 2010; Roberts et al., 2007). Other vulnerable groups may include individuals with multiple chemical sensitivity, chronic fatigue, asthma, or those coming off chemotherapy. The CDC and Florida Dept. of Health have distributed literature stating that naled, as applied in ULV spray, is safe for birds and fish. The application of naled over Miami Beach coincided with a mass influx of migratory barn swallows on 12 Sept. Following the naled applications on Miami Beach, residents reported finding dead mosquitofish, koi, swallows, and honeybees (pers. com.). Mosquitofish and koi skim dead insects off the water's surface. Swallows take to the air at first light when the naled was sprayed from planes, capturing small insects from the air or picking them off the water's surface. Naled has been shown in controlled studies to be highly toxic to honebees and butterflies (Hoang and Rand, 2015a, b; Zhong et al., 2003).

Recommendations going forward

1. Stop spraying permethrin and naled to suppress *Aedes aegypti* in urban areas. Permethrin had no effect. Naled failed to depress mosquitoes on Miami Beach and lost its efficacy in Wynwood. Both insecticides inflict documented harm on wildlife, and cause health problems for a small but vulnerable subset of the human population, agency flyers to the contrary not withstanding.
2. Continue using Bti until new guidance is provided concerning its efficacy.
3. Engage statisticians or quantitative ecologists to produce experimental designs for monitoring mosquito populations and treatment effects.
4. Test the efficacy of etofenprox (Zenivex®) in a small-scale, experimentally controlled, pilot study on *Aedes aegypti* in the Wynwood neighborhood where these mosquitoes were unaffected by permethrin.
5. Conduct carefully designed studies of Bti efficacy in potential Zika outbreak zones such as Coconut Grove, South Miami, or Coral Gables.
6. Initiate carefully designed (i.e., with the aid of quantitative professionals) pilot studies with emerging mosquito-suppression technologies, including improved ovotraps, transgenic mosquitoes, and *Wolbachia*-infected mosquitoes.
7. Engage mathematical modelers to determine accurate transmission thresholds for Zika in urban and suburban neighborhoods.
8. Analyze and release field data promptly and share all available information up and down the chains of command, and with the press and elected officials, without forcing them to resort to court orders.

In the 20th Century, the United States eliminated malaria, yellow fever, and dengue fever from the continent. Subsequently, both state and federal government lost interest in maintaining institutional capabilities for fighting mosquito-borne pathogens, even as these pathogens rebounded in the tropics and subtropics of the New World. As demonstrated by our response to the recent Zika outbreaks in Miami Dade County, we need to improve our knowledge and our coordination, and rebuild our institutional capabilities.

Specific policies and protocols for sharing information should be set by the bodies politic that answer directly to the public, in coordination with the agencies. Secrecy erodes confidence in decision-makers at all levels. When it came to the advisability of naled application, the citizen skeptics proved more right than the experts. For agencies to restore their credibility, they will need to analyze data more quickly and share new information more readily with the public and the press in the future.

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Literature Cited

Aguirre-Obando, O.A., Pietrobon, A.J., Dalla Bona, A.C., Navarro-Silva, M.A., 2016. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in *Aedes aegypti* populations from Jacarezinho (Brazil) after a Dengue Outbreak. Rev Bras Entomol 60, 94-100.

Chuaycharoensuk, T., Juntarajumnong, W., Boonyuan, W., Bangs, M.J., Akratanakul, P., Thammapalo, S., Jirakanjanakit, N., Tanasinchayakul, S., Chareonviriyaphap, T., 2011. Frequency of pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Thailand. J. Vector Ecol. 36, 204-212.

Clark, G.G., Reiter, P., Gubler, D.J., 1989. *Aedes aegypti* control trials using aerial ULV applications, in: Uren, M.F., Siok, J., Manderson, L. (Eds.), Proceedings Fifth Symposium on Arbovirus Research in Australia. CSIRO Tropical Animal Science, Brisbane, pp. 192-193.

Costa, R., Kyriacou, C.P., 1998. Functional and evolutionary implications of natural variation in clock genes. Curr. Opin. Neurobiol. 8, 659-664.

Duprey, Z., Rivers, S., Luber, G., Becker, A., Blackmore, C., Barr, D., Weerasekera, G., Kieszak, S., Flanders, W.D., Rubin, C., 2008. Community aerial mosquito control and naled exposure. J. Am. Mosq. Control Assoc. 24, 42-46.

Focks, D.A., Brenner, R.J., Hayes, J., Daniels, E., 2000. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. Am. J. Trop. Med. Hyg. 62, 11-18.

Hemingway, J., Ranson, H., 2000. Insecticide resistance in insect vectors of human disease. Annu. Rev. Entomol. 45, 371-391.

Hoang, T.C., Rand, G.M., 2015a. Acute toxicity and risk assessment of permethrin, naled, and dichlorvos to larval butterflies via ingestion of contaminated foliage. Chemosphere 120, 714-721.

Hoang, T.C., Rand, G.M., 2015b. Mosquito control insecticides: A probabilistic ecological risk assessment on drift exposures of naled, dichlorvos (naled metabolite) and permethrin to adult butterflies. Sci. Total Environ. 502, 252-265.

Huen, K., Harley, K., Bradman, A., Eskenazi, B., Holland, N., 2010. Longitudinal changes in PON1 enzymatic activities in Mexican-American mothers and children with different genotypes and haplotypes. Toxicol. Appl. Pharmacol. 244, 181-189.

Ishak, I.H., Jaal, Z., Ranson, H., Wondji, C.S., 2015. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. Parasit Vectors 8, 181.

Kyriacou, C.P., Peixoto, A.A., Sandrelli, F., Costa, R., Tauber, E., 2008. Clines in clock genes: fine-tuning circadian rhythms to the environment. Trends Genet. 24, 124-132.

McGill, R., Tukey, J.W., Larsen, W.A., 1978. Variations of boxplots. The American Statistician 32, 12-16.

Nelson, L.S., 1989. Evaluating overlapping confidence intervals. Journal of Quality Technology 21, 140-141.

Purdham, R.S., Gutierrez, D.S., 1986. Pseudocholinesterase deficiency and organophosphorus insecticide use. Journal of the Association of Nurse Anesthetists 53, 240-244.

Roberts, E.M., English, P.B., Grether, J.K., Windharn, G.C., Somberg, L., Wolff, C., 2007. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environ. Health Perspect. 115, 1482-1489.

Schleier, J.J., Peterson, R.K., 2012. The joint toxicity of type I, II, and nonester pyrethroid insecticides. J. Econ. Entomol. 105, 85-91.

Tetreau, G., Bayyareddy, K., Jones, C.M., Stalinski, R., Riaz, M.A., Paris, M., David, J.P., Adang, M.J., Despres, L., 2012. Larval midgut modifications associated with Bti resistance in the yellow fever mosquito using proteomic and transcriptomic approaches. BMC Genomics 13, 248.

Tukey, J.W., 1977. Exploratory Data Analysis. Addison-Wesley, Reading, Massachusetts.

Zhong, H., Latham, M., Hester, P.G., Frommer, R.L., Brock, C., 2003. Impact of naled on honey bee *Apis mellifera* L. survival and productivity: Aerial ULV application using a flat-fan nozzle system. Arch. Environ. Contam. Toxicol. 45, 216-220.

trap site	Wynwood naled application #1			
	8/4/16	8/5/16	8/6/16	8/7/16
1	22			1
2	12		2	4
3	11			3
4	20		9	10
5	11			1
6	9		2	6
7	38		18	8
8	13		1	0
9	11		3	1
10	21		6	5
11	10		1	1
12	36		7	5
13	55		23	4
14	57		10	13
15	20		16	13
16	20		13	16
mean	**22.9**	**N/A**	**8.5**	**5.7**
sd	15.5	N/A	7.2	5.0
median	**20.0**	**N/A**	**7.0**	**4.5**

trap site	Wynwood naled application #2			
	8/7/16	8/8/16	8/9/16	8/10/16
1	1		1	
2	4	0	2	2
3	3	0	5	
4	10	1	2	3
5	1		11	
6	6	0	1	
7	8	2	1	22
8	0	0	2	
9	1	0	3	
10	5	3	11	10
11	1	0	0	2
12	5		5	
13	4	0	3	
14	13	4	10	11
15	13	1	7	
16	16	1	4	6
mean	**5.7**	**0.9**	**4.3**	**8.0**
sd	5.0	1.3	3.7	7.2
median	**4.5**	**0.0**	**3.0**	**6.0**

trap site	Wynwood naled application #3			
	8/12/16	8/13/16	8/14/16	8/15/16
1	36	N/A	15	16
2	7	1	5	13
3	1	2	3	4
4		2	5	11
5		4	6	1
6	2	2	2	0
7	2	4	1	2
8	1	0		1
9	4	0	5	11
10	10	3		
11	1	0	2	0
12	7		5	9
13	17	14	5	11
14	3	20	19	54
15	18	0	8	9
16	4	7		7
mean	**8.1**	**4.2**	**6.2**	**9.9**
sd	9.8	5.9	5.2	13.2
median	**4.0**	**2.0**	**5.0**	**9.0**

trap site	Wynwood naled application #4			
	8/18/16	8/19/16	8/20/16	8/21/16
1	42	0		
2	14	3	18	
3		3		
4	7	10	8	
5	5	3	8	
6		5	3	
7	1	9	0	
8	3	3	9	
9	7	7	5	
10	9	16	5	
11	2	0	0	
12	25	6	0	
13	13	0	14	
14	34	23	1	
15		10	5	
16	0			
mean	**12.5**	**6.5**	**5.8**	**N/A**
sd	13.3	6.4	5.6	N/A
median	**7.0**	**5.0**	**5.0**	**N/A**

Naled	**Spray 1**	1 day	2 days	3 days	**Spray 2**	day 1	day 2	3 days
reduction post Naled(mean)		N/A	**63%**	**75%**		**84%**	25%	-41%
reduction post Naled(median)		N/A	**65%**	**68%**		**100%**	26%	-44%
Kruskall-Wallis P-value		N/A	**0.0090**	**0.0001**		**0.0005**	0.15	0.48

Naled	**Spray 3**	1 day	2 days	3 days	**Spray 4**	1 day	2 days	3 days
reduction post Naled(mean)		48%	23%	-23%		48%	53%	N/A
reduction post Naled(median)		50%	47%	-35%		29%	58%	N/A
Kruskall-Wallis P-value		0.47	1.00	0.98		0.54	0.43	

Note: negative reduction values indicate a population increase.

MEDIAN REDUCTION		
1 day	**2 days**	**3 days**
60%	**49%**	**-4%**

MEAN REDUCTION		
1 day	**2 days**	**3 days**
60%	**41%**	**4%**

Table 1. Summary of Wynwood trap data on the days of naled application and the three days following. Percent population reductions in the lower part of the table indicate the change in mean or median trap counts on days after the spray relative to the counts immediately before the spray. Kruskal-Wallis p-values larger than 0.05 are not considered statistically significant. The first two naled applications, conducted three days apart, produced significant declines. The second two naled applications produced only marginal declines that were not statistically significant. Mosquito populations in Wynwood recovered to pre-spray levels in three days except when sprayed again.

trap site	Miami Beach naled application #1 9/10/16	9/11/16	9/12/16	9/13/16	Miami Beach naled application #2 9/12/16	9/13/16	9/14/16	9/15/16
40	11	9	12	7	12	7	10	5
41	13	12	10	6	10	6	9	11
42	8	9	14	5	14	5	2	3
43	19	32	44	27	44	27	27	21
44	15	16	13	7	13	7	4	3
45	28	24	26	14	26	14	16	11
46	21	6	10	4	10	4	4	7
47		4	2	2	2	2	2	4
48a	6	1	14	7	14	7	3	5
48b	17	10	2	5	2	5	10	5
49	5	6	9	6	9	6	5	5
50	13	3	15	3	15	3	5	5
51	15	7	8	18	8	18	12	7
52	15	7	25	8	25	8	0	16
53	22	3	4	10	4	10	11	11
54	40	11	38	45	38	45	28	20
55a	2	0	2	1	2	1	2	5
55b	4	1	1	4	1	4	4	4
56	42	13	7	48	7	48	26	31
mean	**16.4**	**9.2**	**13.5**	**11.9**	**13.5**	**11.9**	**9.5**	**9.4**
sd	11.2	8.0	12.0	13.6	12.0	13.6	8.8	7.6
median	**15.0**	**7.0**	**10.0**	**7.0**	**10.0**	**7.0**	**5.0**	**5.0**

Naled	**Spray 1**	1 day	2 days	3 days	**Spray 2**	day 1	day 2	3 days
reduction post Naled(mean)		44%	18%	27%		11%	30%	30%
reduction post Naled(median)		53%	33%	53%		30%	50%	50%
Kruskall-Wallis P-value		0.22	0.68	0.29		0.95	0.69	0.99

MEDIAN REDUCTION		
1 day	**2 days**	**3 days**
42%	**42%**	**52%**

MEAN REDUCTION		
1 day	**2 days**	**3 days**
28%	**24%**	**29%**

Table 2. Summary of Miami Beach trap data on the days of naled application and the three days following. Declines in mosquito populations following naled application were small and neither naled application produced a statistically significant decline. Differences between medians and means (not seen in the Wynwood data) indicate data skew to the high end: pockets of high density eluded the spray.

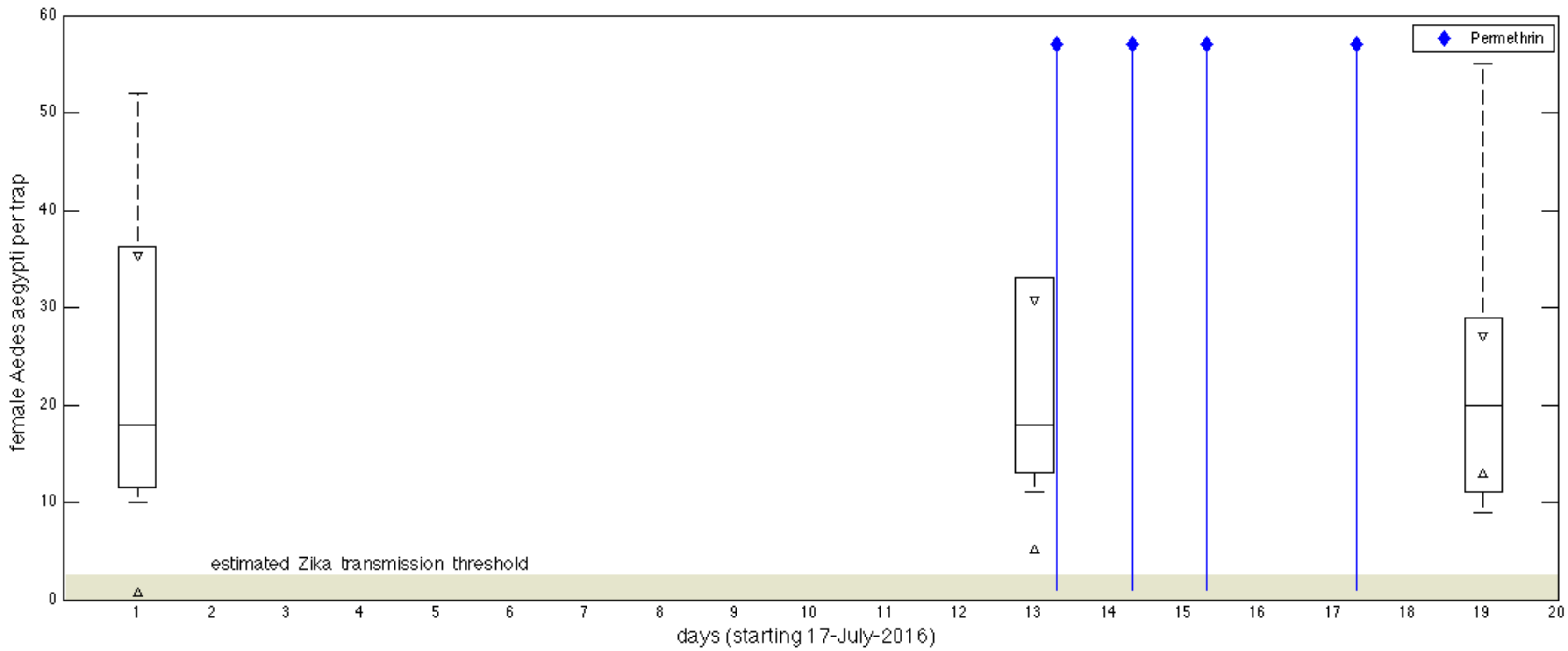

Figure 1. Mosquito trap counts in Wynwood preceding and following four applications of permethrin from trucks across five days. The mosquito population was unaffected. In this figure, and those that follow, two mosquito count medians are significantly different at the 5% alpha level (Kruskal-Wallis test) if their comparison intervals (triangle markers) do not overlap vertically. The shaded strip at the bottom represents the estimated transmission threshold (>90% reduction from pre-spray population level); if the mosquito population is suppressed into this zone and held there, mosquito transmission of Zika should cease.

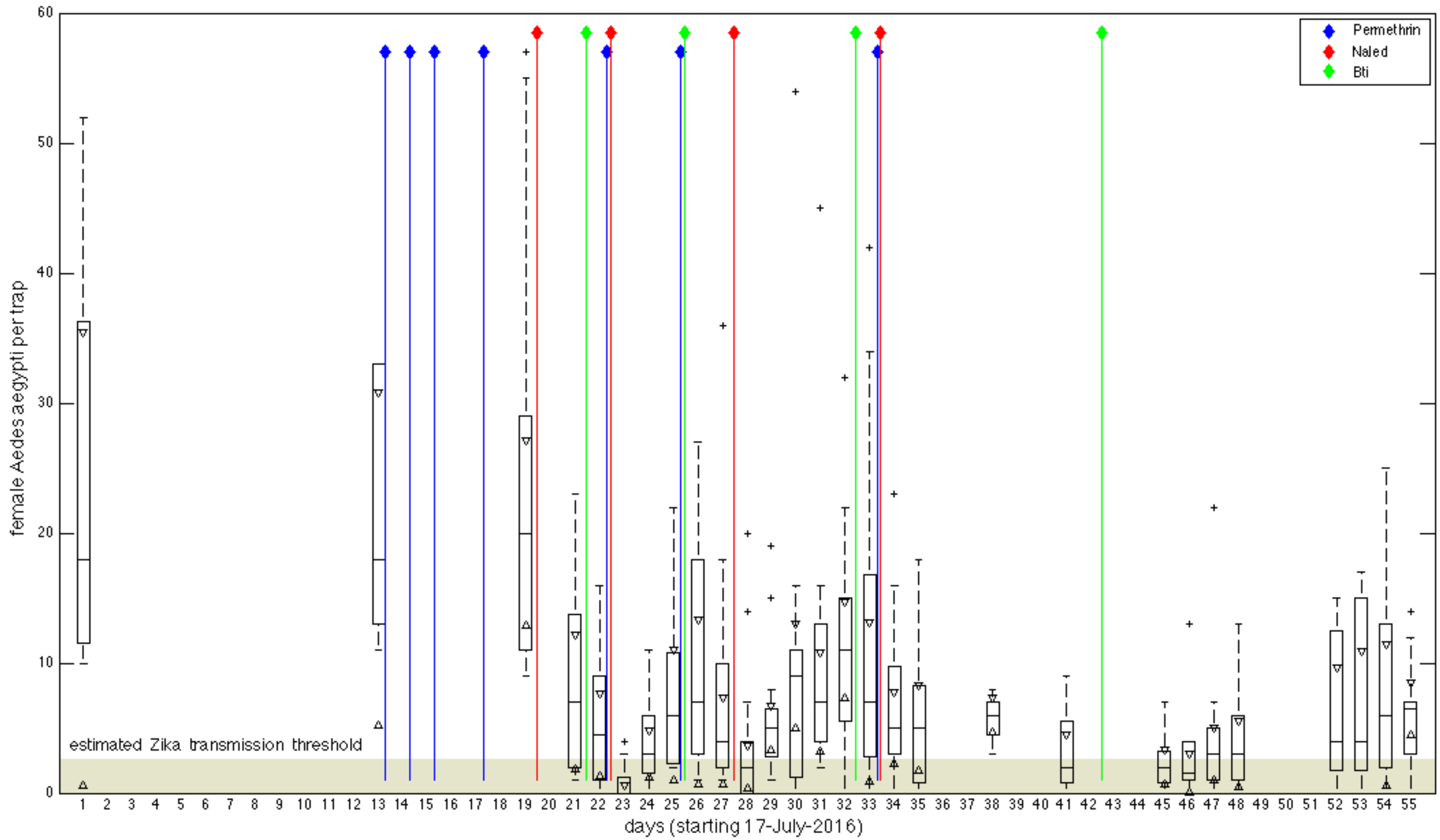

Figure 2. Box plot of trap counts and insecticide treatments in Wynwood over 55 days starting on 17 July 2016. Following the second and third naled treatments, the *Aedes aegypti* population dropped below the estimated transmission threshold (shaded area), but it recovered quickly. The subsequent naled application produced an insignificant drop that did not reach the estimated transmission threshold. Meanwhile, Bti applications had begun, which were accompanied by a gradual, statistically significant drop with sustained suppression. Mosquito populations began to recover a week after the last Bti application.

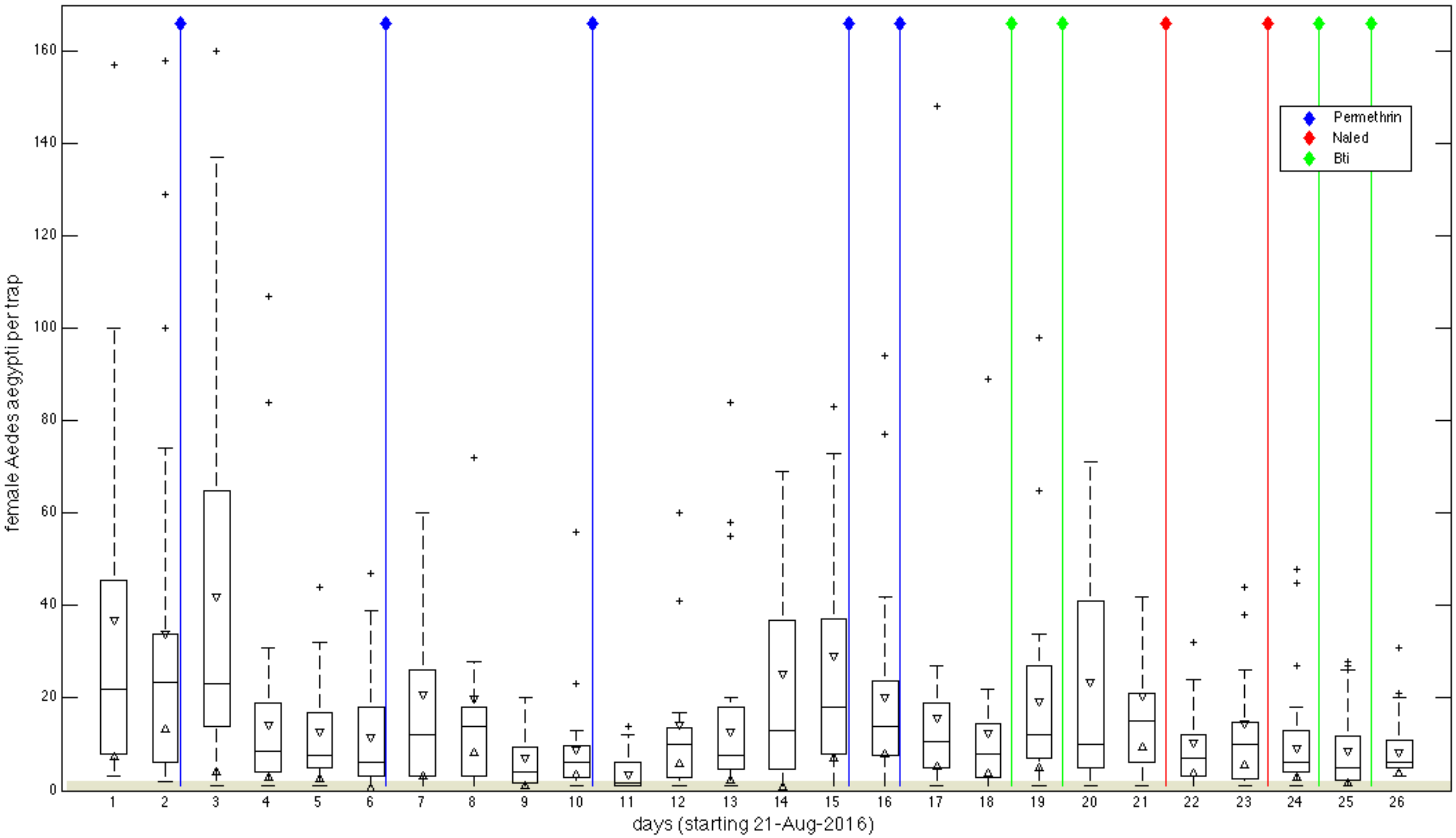

Figure 3. Box plot of trap counts and insecticide treatments in Miami Beach over 26 days starting on 21 August 2016. Permethrin produced no noticeable or statistically significant declines in the local mosquito population. Naled may have produced small declines, but these were not statistically significant and naled was sprayed after Bti, so the modest declines following naled application may have resulted from prior application of Bti, or from chance. The population did start to decline around day 21 following application of the larvicide Bti beginning on day 18, and that decline was statistically significant. Note, however, that natural variation in the mosquito population is extreme, as exemplified in the variation over the first 16 days of trap data, so, absent a controlled experiment, we cannot be sure that the decline following Bti was caused by the Bti treatments.

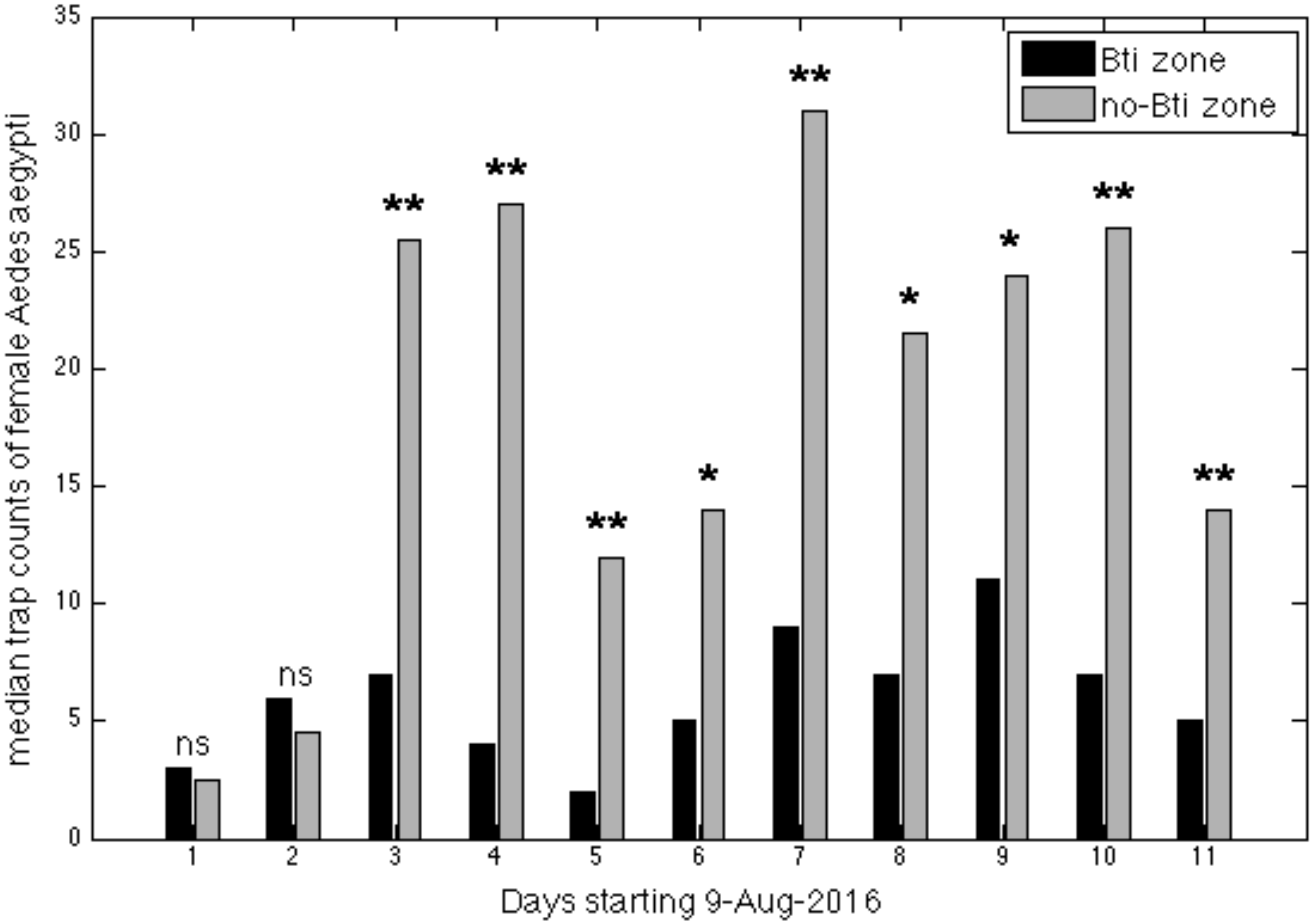

Figure 4. Bars indicate median trap counts in the Wynwood Bti application zone and the surrounding area that did not get Bti. Asterisks indicate significant 1-tailed differences (* <0.05, ** <0.01, ns = not significant) for Kruskal-Wallis tests of catch differences in the two zones on a particular day. Note that naled was applied to both zones two days before, on 7-Aug-2016, and again on 12-Aug-2016 (day #4), as reflected in the reduced counts on day #5.