Managing *Aedes aegypti* populations in the first Zika transmission zones in the continental United States

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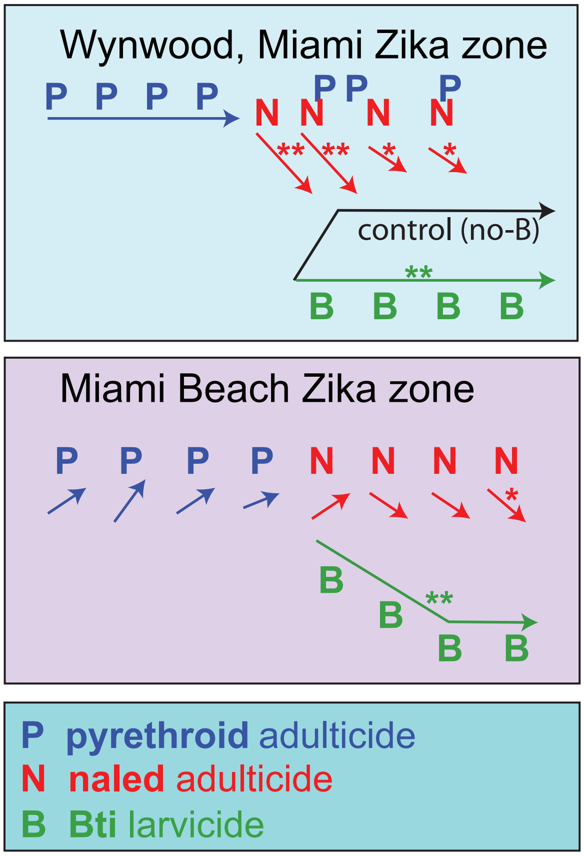
graphical abstract



Two neighborhoods in Miami-Dade County received a mix of adulticide and larvicide treatments during Zika outbreaks in 2016. Arrows signify relative degree of change in trap counts of female *Aedes aegypti.* * $p < 0.05$, ** $p < 0.01$.

ABSTRACT

The African Zika virus swept across the Pacific, reaching the New World in 2014. In July, 2016, Miami-Dade County, Florida became the locus of the first mosquito-borne Zika transmission zones in the continental United States. Control efforts were guided by the Centers for Disease Control and Prevention, including aerial and truck sprays of adulticides and larvicides. To improve our understanding of how best to fight Zika transmission in an urban environment in the developed world, trap counts of adult *Aedes (Stegomyia) aegypti* (L.) mosquitoes from the treatment zones were analyzed to determine efficacy of the different insecticide treatments. Analysis revealed that application of four different ester pyrethroid and one non-ester pyrethroid had no statistically significant effect on mosquito counts. Aerial application of naled, a potent organophosphate adulticide, produced significant but short-lived drops in *Ae. aegypti* counts in the first two applications in the first active transmission zone (Wynwood), then lost some efficacy with subsequent application. In the other active transmission zone (Miami Beach), naled produced no measurable effect in the first three applications, and only a small, transient, and marginally significant reduction in the fourth application. Repeated application of the larvicidal bacterium *Bti* was accompanied by steady declines of *Ae. aegypti* populations in both sites. Zika transmission ceased in the first transmission zone, but expanded in the second transmission zone during this period. Specific recommendations are proposed for future treatments of urban mosquitoes.

Highlights

- Locally acquired Zika occurred in three urban areas of Miami-Dade County, Florida, in the summer of 2016.
- *Bti* larvicide greatly depressed urban *Aedes aegypti* populations when applied weekly.
- The organophosphate naled transiently suppressed *Ae. aegypti* populations in one transmission zone but not another.
- Pyrethroid adulticides did not reduce populations of *Ae. aegypti*.

Keywords: *Bti*; naled; Miami; Miami Beach; vector control

Abbreviations: *Bti*, *Bacillus thuringiensis israelensis*; CDC, Centers for Disease Control and Prevention; MBD, mosquito borne disease

1. Introduction

In the 20th Century, malaria, yellow fever, and dengue fever were eradicated from the continental United States (Gubler, 2004; Zucker, 1996). 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 (Benelli and Mehlhorn, 2016).

The continental United States was invaded by West Nile virus, first detected in New York City in 1999, dengue broke out in Key West in 2009, chikungunya reached the Caribbean in 2013, and Zika did so in 2014 (Fischer and Staples, 2014; Graham et al., 2011; Grubaugh et al., 2017; Nash et al., 2001). Experts were confident that Zika would soon reach the southern United States (Monaghan et al., 2016), and the World Health Organization declared Zika a Public Health Emergency of International Concern (PHEIC) for North America in Feb, 2016. The first locally transmitted Zika cases in the continental United States were confirmed in Miami, Florida in July, 2016. At least four independent introductions occurred, and possibly as many as 40 (Grubaugh et al., 2017),

Mutation of a single amino acid substitution in the Zika virus as it came across the Pacific greatly increased prenatal induction of microcephaly and related neural defects (Yuan et al., 2017). As such, Zika became the first described teratogen to be transmitted by mosquito bites (Rasmussen et al., 2016). For the first time in history, the United States government advised pregnant women to avoid visiting a city within the United States (Health Alert Network, 2016a).

In greater Miami, first Zika appeared in areas popular with international visitors, the Wynwood neighborhood in the City of Miami (Health Alert Network, 2016a) (Fig. 1), followed a month later by the South Beach neighborhood in the City of Miami Beach (Fig. 2) (Health Alert Network, 2016b, c).

Governmental agencies at the federal, state, county, and municipal levels all recognized the need to act decisively (Florida Dept. Health Miami-Dade County, 2016). Miami-Dade County's Mosquito Control Division and Department of Public Health, the state Department of Health, the Florida Governor's office, and the Centers for Disease Control and Prevention (CDC) became involved in the decision process, though local government officials were excluded. Direction came from the CDC to spray the most potent mosquito adulticides available, and the Governor gave orders to carry out the CDC's direction, reversing its initial position on spraying naled in Miami Beach. In September 2016, Zika transmission abated in the Wynwood neighborhood of Miami, while the active Zika infection zone on Miami Beach continued to widen. A third zone of active Zika transmission appeared in the Little River neighborhood of Miami (Health Alert Network, 2016c). Local transmission of Zika ceased in Miami that same year, as predicted from the reduction in numbers of imported cases (Dinh et al., 2016).

Efforts to eliminate Zika in greater Miami were based on the underlying assumptions that that transmission would be halted by reducing bite frequency and by regional suppression of outdoor populations of *Ae. aegypti* in the transmission zones. These assumptions were supported by a transmission model for Zika showing that the transmission rate is most sensitive to the biting rate and to the mortality rate of mosquitoes (Gao et al., 2016). The operational approach centered on promoting personal protection against mosquito bites (e.g., with long clothes and repellants) and neighborhood-scale mosquito control in known transmission zones frequented by tourists, affluent areas of greater Miami where habitats ranged from single-family residential neighborhoods, to forested botanic gardens, to high-rise business, hotel, and condominium districts.

The present study evaluates the effectiveness of one piece of this effort, the 2016 campaign to control *Ae. aegypti* with insecticides as evidenced through mosquito trap data. Although the mosquito trap data were not collected as part of a controlled experimental design, sufficient information exists within the data set to inform urban mosquito control decisions moving forward into the coming years when urban *Ae. aegypti* in U.S. cities will be no less nettlesome than in 2016.

2. Methods

2.1 Mosquito control efforts

Following the detection of locally-acquired Zika, 5 km^2 of Wynwood (Fig. 1) were initially fogged from trucks operated by Miami-Dade County Mosquito Control applying the ester pyrethroids permethrin, phenothrin (sumithrin), prallethrin, and deltamethrin (Vasquez, 2016). The Wynwood zone also received localized backpack fogging with ester pyrethroids deployed by certified public health pesticide applicators, and door-to-door visits from County personnel searching out and dumping standing water. Subsequently an area about five times larger, spanning parts of the adjacent neighborhoods of Allapattah, Edgewater, and Overtown received aerial ultra-low volume (ULV) spray with the organophosphate adulticide naled (Dibrom®) and aerial application of bacterial larvicide *Bti* (VectoBac WDG®) droplets. Naled (Dibrom®, AMVAC Chemical Corporation) was applied by ultra-low volume (UVL) aerial spray at a concentration of 73 ml ha^{-1} (1.00 oz/acre) between 05:00 and 07:00. Bti was applied at a concentration of 560 g ha^{-1} (0.5 pound/acre). Flights were conducted by Dynamic Aviation under the direction of Clarke Pest Control, Inc. Winds were 0-10 km/h (Table 1).

The South Beach transmission zone (Fig. 2) was sprayed from the air with naled and from trucks with ester pyrethroids and with *Bti*. Aerial spraying of naled on Miami Beach was conducted from planes that first measured wind velocity, then plotted a back-and-forth flight spray path offshore that would cause the naled mist to drift in strips onto the urban target zone while avoiding the water. Wind speeds were similar, 0-10 km/h. Ground-truthing by smell and fluorescent dye detection indicated that naled did drift into the target zone (Clarke, 2016). Unlike the aerial applications of Bti in Wynwood, Bti (VectoBac WDG) was applied at dawn in Miami Beach from a pickup truck equipped with a Buffalo Turbine Mist Sprayer (CSM2) producing 120 μm droplets.

2.2 Data acquisition.

Staff of the Miami-Dade County Mosquito Control Division captured mosquitoes in BG-Sentinel® traps (Biogents) baited with the BG-lure and enhanced with CO_2 released from dry ice in a small cooler. Daily counts of *Ae. aegypti* captured in 16 traps in the Wynwood neighborhood of Miami, Florida, were analyzed for the period from 27 Jul to 9 Sep, 2016 (Likos et al., 2016). Of the 55-day period, mosquito counts were missing from 26 days. On the days that data were collected, 13% of the traps malfunctioned on any given day for various reasons, e.g., loose or torn catch bags, locked gates, trap invasion by lizards, etc. Sixteen additional traps were situated immediately outside Wynwood for 11 days, beginning 9 Aug, 2016, two days after the second naled application (Fig. 1).

These trap sites received the aerial naled application as the Wynwood zone, but not *Bti*, pyrethroids, or the same intensity of door-to-door inspections. Comparison of trap data inside and outside Wynwood might indicate the efficacy of aerial *Bti* application, qualified by our recognition of different efforts on the ground. Counts of *Ae. aegypti* captured in 19 traps in the South Beach neighborhood of Miami Beach, Florida, were collected by the Miami-Dade County Mosquito Control Division for a 55-day period from 21 Aug to 9 Oct, 2016. These data were of higher quality than those from Wynwood, with no missing days, and far fewer missing data.

2.3 Data analysis

Statistical comparisons of daily mosquito catch counts were run using the MATLAB® Statistical Toolbox and Curve-Fitting Toolbox. Count data are typically overdispersed, and, in the worst case, may be plagued by large numbers of zero counts caused either by disappearance of subjects, sampling error, or some combination of the two (Zuur et al., 2009). The daily trap count data had both of these issues. Zero inflation was present only in the second half of data set, so no single model (e.g., zero-inflated negative binomial) would fit the entire data set. Instead, comparisons of daily trap counts were made using non-parametric tests.

Initially, I determined statistical significance of changes in trap counts following adulticide application using Wilcoxon sign-rank tests to compare mosquito counts for each trap the day before application (N-1) to the day after application (N+1). Whenever possible, I did not use the day of application because the traps were operational those days for a variable mix of hours before, during, and after the spray application, and because some insecticides such as pyrethroids are excito-repellant (Mongkalangoon et al., 2009; Potikasikorn et al., 2005), which reduces host-seeking behavior, and thus taxis to traps such as the BG Sentinel that exploit host-seeking behavior (Kongmee et al., 2004). To detect the frequency of spontaneous declines unrelated to adulticide application, I created sets of control days, comprised of trap counts at least two days before or after days of adulticide application. Statistically significant changes in these control periods were uncommon in the Wynwood data set but very common in the Miami Beach data.

On Miami Beach, a steady, gradual decline in mosquito counts was recorded in days 19-47. To distinguish effects of the adulticide and larvicide applications on trap counts of adult *Ae. aegypti*, I begin with a consideration of their respective modes of action. Adulticides produce adult knockdown within minutes of contact (Mount et al., 1978), and thus their effects on trap counts must be manifest within a day of application. Conversely, the bacterial larvicides in *Bti* are endotoxins which kill larvae upon ingestion, but do not affect pupae or adults which are not feeding on the bacteria (Boisvert and Boisvert, 2000). Thus the effects of *Bti* on adult trap counts will necessarily be delayed, and spread out over many days of recruitment. The two modes of control will necessarily overlap given the treatment regimes used, and acute suppression can lead to chronic changes. However, acute and gradual effects can be separated mathematically. To distinguish acute effects (adulticide) from gradual effects (*Bti* + adulticide or *Bti* alone) I fitted a 2^{nd} order polynomial count medians for this period using least-squares regression. A 2^{nd} order polynomial was the lowest order function that maximized the coefficient of determination (Sokol and Rohlf, 1981), with the added benefit for this analysis of tracking the gradual changes with minimal influence of one-day transient changes. I subtracted the best fit line to obtain trap count residuals. Residuals were normally distributed (Kolmogorov-Smirnov test) and time invariant, indicating that a single normal distribution underlay daily variation in mean mosquito trap counts during this period, irrespective of population density. From these residuals, I determined daily effect sizes by subtracting trap count residuals of the day before (N-1) from those of the day after (N+1). Negative values then represented the acute within-trap declines bracketing a given day, e.g., the acute effect of a naled application on daily trap counts.

2.4 Ethical note

The maps in Fig. 1 & Fig. 2 indicate the approximate sites of mosquito traps, but do not indicate which of these traps captured Zika-infected mosquitoes. To further protect the privacy of neighborhood residents, the trap markers are shifted by a small, random distance. Thus, no specific persons exposed to Zika or addresses of specific persons exposed to Zika can be identified or inferred from these maps. Data available to share lists only the street block but not the complete address.

3. Results

3.1. Pyrethroid application

Four successive truck-based fog applications of various ester pyrethroid adulticides, carried out by Miami-Dade Mosquito Control over a 5-day period in Wynwood had no measurable effect on trap counts of female *Ae. aegypti* (Fig. 3). Note, however, that no trap counts were collected for six days after the pyrethroid applications began, and two days elapsed between the fourth application and the next mosquito counts. Thus, a transient decline could have been missed because of gaps in data collection. The County conducted three more truck sprays of ester pyrethroids in Wynwood (Fig. 3). Mosquito counts rose following the 6th pyrethroid application (Fig. 3, day 25). The 5th and 7th pyrethroid applications were coincident with naled applications and declines were seen the following day.

Following the seven pyrethroid applications in Wynwood, five more applications of ester pyrethroids were carried out in Miami Beach (Fig. 4). Three of these five were followed by statistically lower trap counts the day after spray than the day before (sign-rank tests, one-tailed, $P<0.05$), however, in that same period of pyrethroid application (Fig. 4, days 2-17), two of four control comparisons likewise showed statistically significant declines. Thus, *Ae. aegypti* population declines on Miami Beach following ester pyrethroid application were indistinguishable from spontaneous fluctuations unrelated to adulticide application.

The non-ester pyrethroid etofenprox (Zenivex®, Central Life Sciences) was sprayed in the Mid-Beach neighborhood (28 Street to 68 Street, Fig. 2) on two successive days (1-2 Nov, 2016). Trap counts were ~40% higher the day following the etofenprox sprays than the day before ($p = 0.01$, sign-rank test, 2-tailed), presumably due to natural fluctuation unrelated to the spray application.

3.2. Naled aerial sprays

Trap counts of *Ae. aegypti* in Wynwood fell measurably after each naled application (Table 1, sign-rank tests, 1-tailed). The first two applications occurred three days apart and were accompanied by a compound 96% decline in adult mosquito counts. Control comparisons produced no significant declines in this period, so the acute declines following naled application can be safely attributed to the naled application. Statistical significance indicates a discernable pattern, but does not indicate its magnitude. The effect sizes were striking during the first two applications, then diminished with each successive application; the 3^{rd} & 4^{th} naled applications had significantly smaller effects than the first (p = 0.016, p = 0.003; sign-rank test, 1-tailed).

Following naled applications in Wynwood, the reductions in *Ae. aegypti* trap counts were transitory. Within three days the *Ae. aegypti* populations were virtually identical to pre-spray levels (Table 1). In fact, the mean counts were 7% higher three days later than before the treatment.

In Miami Beach, naled applications were alternated with the larvicide *Bti* on a weekly basis for four weeks (Figs. 4, 6). Over that period, the mosquito population declined asymptotically. The first naled application was followed an increase, and the 2^{nd} and 3^{rd} by drops – none of these changes were significantly greater than chance ($P > 0.01$, sign-rank test, 2-tailed). The fourth application was followed by a decrease marginally greater than background (Fig. 6c). Even though the proportional decrease was an impressive-seeming 89% (Table 1), the spontaneous proportional decrease three days later was even larger than following the naled application, and occurred in the complete absence of adulticide application. Loosening the test stringency (increasing alpha from 0.1 to 0.05) triples the number of “significant” changes on control days, but no other naled day becomes significant. Because adulticides produced little or no acute effects in Miami Beach, we can assume they likewise had no chronic effect. Thus, we can attribute any gradual declines in mosquito trap counts to other factors such as *Bti* larvicide application.

3.3. Bti (VectoBac WDG®) application

The Wynwood zone receiving the larvicide *Bti* showed a statistically slower recovery of mosquito populations than the area immediately outside Wynwood that did not receive *Bti* ($p = 0.002$, signed-rank test; Fig. 6). Daily catch counts started out similar inside and outside the *Bti* treatment zone, but recovered much faster outside the zone receiving *Bti*. Excluding the post-naled days, *Ae. 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 following the first *Bti* application ($R^2 = 0.31$, $p < 10^{-44}$).

Both in Wynwood and Miami Beach, sustained use of *Bti* appeared to depress the populations below 90% of their pre-treatment values, which likely contributed to the decline in Zika transmission (Dinh et al., 2016). In Wynwood, the *Ae. aegypti* population began to climb again a week after the last application of *Bti* (Fig. 3). In Miami Beach, *Bti* was applied more systematically at regular 7-day intervals, and the *Ae. aegypti* population fell to less than 90% of its prior level 17 days after the first *Bti* application and remained close to that level (Fig. 4). By that time, 34 days after the initial pyrethroid applications, Zika had appeared outside of the South Beach treatment zone in the Mid-Beach neighborhood (Fig. 2).

4. Discussion

4.1. Did naled application work?

Naled is a potent organophosphate insecticide, which inhibits the action of acetylcholinesterase (Fukuto, 1990). Naled is a very effective adulticide if droplets contact the adult mosquito, although it has significant potential to harm non-target organisms, especially pollinators (Hoang and Rand, 2015a, b) and individuals with pseudocholinesterase deficiency (Purdham and Gutierrez, 1986). Because of the concern over non-target effects, a key question in the minds of residents, scientists, and government officials is whether the controversial application of naled is necessary in conjunction with *Bti* to fight Zika. The CDC noted "although the combination of aerially applied naled and Bti along with source reduction and ground-based applications of larvicide and adulticides reduced *Ae. aegypti* populations to low levels, it cannot be concluded definitively that these reductions were responsible for ending the outbreak" (Likos et al., 2016). While naled did appear to cause transient suppression of *Ae. aegypti* in Wynwood, the data are less than clear in supporting an overall conclusion that naled played an essential role in ending the Zika outbreak because the acute declines in mosquito counts following the different naled applications were so variable and so many other factors were involved. In addition to aerial application of naled and *Bti*, the County was conducting active property-by-property inspections and localized insecticide applications, and public relations outreach to "dump and cover" breeding sites while encouraging personal use of insect repellants.

4.1.1 Fighting Zika in Wynwood

In Wynwood, unlike in Miami Beach, naled did appear to work in the beginning. The initial two naled applications in Wynwood, conducted three days apart, were followed by a short-term 96% reduction in mosquito counts. If the sole Zika reservoir was a small number of female *Ae. aegypti* and these females were all killed during the initial knockdown when naled appeared to be effective, then indeed the aerial naled applications might have been responsible, since shortening the lifespan of adult female mosquitoes exponentially reduces their vectorial capacity (MacDonald, 1952). If, however, a reservoir of Zika-infected humans remained in Wynwood, a significant possibility given the prevalence of asymptomatic infections, then that reservoir could have reinfected the rebounding mosquito population if infected people were still getting bitten. Female *Ae. aegypti* frequently hide in or on fabric, picture frames, or other dark places for 3-4 days following a blood meal (Clark et al., 1994; Gratz, 1993), making them difficult to kill with aerial insecticide application, which primarily affects flying mosquitoes in outdoor locations. Further, female *Ae. aegypti* double their resistance to pyrethroids following a blood meal (Eliason et al., 1990). In general, among virus-infected females, recently blood-fed females are likely to be overrepresented relative to free-flying females, which may include a majority of nullipars that have not yet fed on humans (Focks et al., 1987). While naled droplets falling from the sky may kill a large fraction of free-flying female *Ae. aegypti*, the most likely females to survive any given aerial spray application are recently blood-fed females who are hiding. For those reasons, the second application of naled in Wynwood three days after the first might have been particularly effective at killing residual infected mosquitoes and breaking the chain of transmission in that neighborhood.

The reason for the apparently reduced efficacy of naled in the third and fourth Wynwood applications is not clear. Wind conditions at nearby Miami International Airport were not different in the first two versus second two applications, but a light rain was falling during the third application (the Dibrom label states: “Do not apply when it is raining in the treatment area.”). Such a rapid evolution of chemical resistance seems unlikely. Another possibility for the loss in effectiveness of naled 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 (Reiter, 2007). 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. A third possibility is upregulation of existing detoxification genes. Increased resistance can follow blood-feeding (Eliason et al., 1990; Moore et al., 1990) or pesticide exposure (Bass and Field, 2011; Guedes et al., 2010). Epigenetic transmission of heightened gene expression following insecticide exposure is documented a variety of insect taxa including mosquitoes (Field and Blackman, 2003; Oppold et al., 2015; Rahman et al., 2010).

4.1.2 Fighting Zika in Miami Beach

Zika suppression in Miami Beach proved more intractable than in Wynwood. Zika became more widely distributed over Miami Beach than in Wynwood, making ground-based control efforts much more difficult

The first three of the four naled applications on Miami Beach were <u>not</u> followed by sharp declines of *Ae. aegypti*. 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. According to J. Conlon, Technical Advisor to the American Mosquito Control Assoc., "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." (Staletovich, 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" (Flechas and Chang, 2016). The first three spray flights were conducted to account for onshore winds. Naled was released over the Atlantic on a flight path that would cause the mist to drift westward onto South Beach. Tall hotels along Collins Avenue on the east side of the island create turbulence that can disrupt UVL drift, and create wind shadows in which weak-flying mosquitoes take refuge (McKenna, 2016). Only the fourth flight was conducted in still air, which allowed the spray to be released directly over the island. Interestingly, that 4th naled application was followed by the only marginally significant reduction detected in the trap counts (Fig. 6).

The persistence of high counts at some trap sites following the first three naled application was consistent with the prediction that the spray would fall unevenly. Comparing trap counts against the trap placement map, no consistent difference could be found between the eastern (windward) and western (leeward) edge of the island, nor any trend that would suggest significant mosquito immigration in the north edge of the treatment zone. However, despite its acute toxicity to *Ae. aegypti,* naled has not been found effective in controlling this species, even in the absence of tall buildings: aerial UVL application of naled over Puerto Rico on four successive mornings killed female *Ae. 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).

A few traps placed in the most affluent single-family neighborhoods in the north end of South Beach and in Mid-Beach produced consistently higher numbers of *Ae. aegypti* than elsewhere, initially in excess of 100 per day. Visual inspection of satellite images shows evidence of residential construction or remodeling. Miami-Dade County has since initiated a program specifically to control *Ae. aegypti* at construction sites.

4.2. Bti application

The contrast in mosquito recovery between the Wynwood zone that received *Bti* plus naled, versus the area just outside Wynwood that received naled alone, indicates a strong effect of *Bti* (Fig. 5). For example, from day 2 to day 3, the median count outside the *Bti* zone increased from 4 to 21, while inside the *Bti* zone, the median count increased from 6 to 7. The population's speed of recovery was about 15 times faster absent use of *Bti*.

The soil bacterium *Bacillus thuringiensis* serovariety *israelensis* (Berliner) or *Bti*, produces a cocktail of four different insect-specific endotoxins, which makes it difficult for mosquitoes to evolve resistance (Tetreau et al., 2012). Further, *Bti* is extremely specific, killing a subset of Diptera when ingested, with non-target toxicity limited to very few other taxa (Boisvert and Boisvert, 2000).

For *Bti* to work on its own, it would have to reduce daily replacement of adult mosquitoes to levels significantly lower than daily mortality. Daily mortality of released female *Ae. aegypti* in Thailand is typically 0.11 to 0.16 (Reiter, 2007), but was lower, 0.28 to 0.39, in an upscale Brazilian neighborhood with lot sizes and landscaping similar to the initial Zika zone on Miami Beach (David et al., 2009). The Wynwood vs. outside Wynwood comparison (Fig. 5) suggests *Bti* was reducing adult daily recruitment more than tenfold. This difference is high enough that, with protracted and systematic use, *Bti* might cause a long-term decline without concurrent use of broadcast adulticides. Aerial application of *Bti* on Key West has suppressed *Ae. aegypti* populations by about two thirds, but cryptic breeding sites such as cisterns and hot tubs are believed to provide larval mosquito sanctuaries impenetrable to aerial spray, thus sustaining *Ae. aegypti* reproduction on this urban island (pers. com. Florida Keys Mosquito Control District staff). Urban Miami-Dade has no cisterns, but hot tubs are popular and often go unused in the hot summer when mosquitoes are abundant. Winter residents may not adequately chlorinate hot tubs while they are away for the summer and fall, mosquitoes can enter through the smallest gap in a cover.

In Miami Beach, *Bti* was applied every seven days. However, field studies in subtropical Australia found that *Bti* completely lost its efficacy after seven days and did so sooner in water with high organic content (Russell et al., 2003). Given the cost of *Bti* application, while recognizing its specificity and the promise it affords as a larvicide that resists acquired resistance, we need to know how well *Bti* reaches different breeding sites as applied Buffalo turbine truck in Miami's urban habitats. In Miami's summer rainy season, *Bti* may need to be reapplied more frequently than once a week to maintain a high level of potency against larval *Ae. aegypti,* with no gaps in coverage. At temperatures 27°C (81°f) or above, *Ae. aegypti* develop from egg to pupa as little as 4.5 days (Rueda et al., 1990), a particular problem if rain washes out or dilutes *Bti* in between applications. If *Bti* were scheduled to be applied weekly, and significant rain falls within the 60-hour period following an application, larval development could resume in in rain gutters and other structures prone to rain washout.

Controlled experiments with *Bti* should be conducted in structurally comparable neighborhoods that are not believed to be infected with Zika, to determine the best application methods and regimes and the degree of mosquito population suppression that can be achieved with optimized methods. Such experiments will pay dividends for the control of ongoing and future outbreaks of Zika and other arboviruses.

4.3. Why were pyrethroids ineffective?

Pyrethroids have been effective as mosquito adulticides through their binding of voltage-gated sodium channels (Davies et al., 2007). In late October, 2016, as the mosquito season was winding down, the University of Florida's Medical Entomology Laboratory, under contract with the Florida Dept. of Health, conducted CDC bottle bioassays of susceptibility of *Ae. aegypti* to various pyrethroid and organophosphate adulticides (Connelly and Rey, 2016). *Ae. aegypti* local to Miami-Dade County were found to be “resistant” by the CDC standard to a variety of pyrethroids, both ester and non-ester types, and showed “developing resistance” to the organophosphate malathion. Susceptibility to naled was not tested in these mosquitoes but has proven high in other populations (Connelly and Rey, 2016).

Applications of pyrethroids in Wynwood and Miami Beach had no measurable effect on trap counts of *Ae. aegypti*. Data on local *Ae. aegypti* from Wynwood and Miami-Beach indicate these populations have evolved significant chemical resistance to pyrethroids (Connelly and Rey, 2016). After ester-pyrethroids failed to suppress trap counts of *Ae. aegypti* populations in Wynwood and South Beach, the County applied the non-ester pyrethroid etofenprox (Zenivex®) in the Mid-Beach neighborhood. Like the ester pyrethroids, etofenprox had no effect on trap counts of *Ae. aegypti*, consistent with the discovery of resistance to efentoprox in lab tests of *Ae. aegypti* from Miami-Beach (Connelly and Rey, 2016), and in field tests conducted by the CDC (J. McAllister unpubl.). If resistance had stemmed from mutations of esterases, a switch to the non-ester pyrethroid might have improved efficacy. However, if resistance is caused by changes in the other detoxification enzymes (glutathione *S*-transferases and cytochrome P450 monooxygenases), then the resistance may readily transfer to non-ester pyrethroids. In California, *Ae. aegypti* are homozygous for the V1016I mutation in the voltage-gated sodium channel gene, which confers some resistance to pyrethroids of all classes (Cornel et al., 2016).

4.4. Integrated vector management

Ae. aegypti are hard to control with adulticides and getting harder. The traditional role for adulticides might be to reduce an infected standing crop of adults as a way to break an infection cycle, but *Ae. aegypti* poses many problems to this approach (Fernandes et al., 2018). Urban *Ae. aegypti* are increasingly resistant to many adulticides (revs. Chareonviriyaphap et al., 2013; Hemingway and Ranson, 2000; Kasai et al., 2014; Moyes et al., 2017), and females double their capacity for detoxification following a blood meal (Eliason et al., 1990). Multiple factors outside of resistance can interfere with adulticide efficacy, such as wind, and the tendency of females to rest where sprays do not reach them (Dzul-Manzanilla et al., 2017; Perich et al., 2000). The rapid life cycle of *Ae. aegypti* in warm weather recruits new adults quickly, but even faster following adulticide application (Focks et al., 1987). Data from the 2016 control efforts show that *Bti* can serve as the backbone of an urban suppression program for *Ae. aegypti*, while modern variants on sterile male techniques might provide an effective compliment. Directed applications of the right adulticides under the optimal atmospheric, structural, and temporal conditions still might be a useful supplement to larvicides if a viral outbreak can be localized, as was the case in Wynwood. For wide applications of adulticides, promotion of resistance and non-target effect remain areas of concern.

4.5. Experimental design

The first priority during the 2016 Zika outbreak was maximal suppression of *Aedes aegypti*, rather than crafting the ideal experimental design. If greater effort is given to incorporating strong experimental design features going forward, especially when the region is not in the middle of a viral outbreak, statistical analysis will become easier and more definitive in determining efficacy of single treatment applications. Negative controls should include traps situated in areas not receiving particular treatments. For example, the placement of traps in the zone outside Wynwood that received naled but not

Bti was essential to showing the efficacy of *Bti* (Figs. 1, 5). Positive controls can take the form of caged mosquitoes, some in the open and others situated in the sorts of places wild mosquitoes would be expected to reside.

4.6. How much suppression is enough to stop Zika?

Local transmission of Zika ceased in Miami that same year, as predicted from the reduction in numbers of imported cases combined with the low local transmission rate (Dinh et al., 2016). Increased personal protection from bites and government-sponsored vector control may have reduced the transmission rate, though by an unknown degree. Extensive control efforts were applied in Wynwood and Miami Beach, with differing results: Zika was quickly contained in Wynwood but spread in Miami Beach. Differing introduction rates may have been responsible, however multiple Zika strains were identified in both Wynwood and Miami Beach (Grubaugh et al., 2017). In the less affluent Little River neighborhood, a small Zika transmission area, with a single Zika strain (Grubaugh et al., 2017), was treated from the ground, not aerially, and transmission abated quickly.

Absent accurate models of transmission dynamics, mosquito control staff are reduced to guesswork, knowing their target population threshold only by whether new Zika cases are diagnosed that can be traced back to mosquito-transmission in particular neighborhoods, a process that can take weeks or months. Daily survival rate of adult mosquitoes, the most critical factor in disease transmission (MacDonald, 1952; Smith et al., 2012) is unknown for female *Ae. aegypti* in the affected neighborhoods of Miami-Dade County; survival estimates vary widely from sites around the world (Buonaccorsi et al., 2003; Focks et al., 1987; Fouque et al., 2006), and are likely age-dependent (Harrington et al., 2001).

4.7. Recommendations going forward

As demonstrated by our response to the recent Zika outbreaks in Miami-Dade County, we need to rebuild our institutional capabilities and improve our knowledge and our coordination. Despite an early success at containing the localized outbreak in Wynwood, Zika successfully exploited our institutional and social vulnerabilities and created new outbreaks in Miami-Dade County. The risk of mosquito-borne disease in the coming years is projected to increase rather than decrease (Fernandes et al., 2018; Patz et al., 2005). Accordingly, Miami-Dade County and local universities are cooperating to apply lessons learned from the first season to provide a more effective response. Some of the improvements include:

1. Emphasize the use of the larvicide *Bti*, but begin using it prophylactically before a virus outbreak, apply it at night after people are indoors, and apply it more frequently if heavy rains are expected.
2. Conduct carefully designed studies to improve *Bti* efficacy in urban and suburban mosquito habitat (e.g., Jacups et al., 2013; Pruszynski et al., 2017; Williams et al., 2014).
3. Rethink the spraying of adulticides to suppress *Ae. aegypti* in urban areas. If an effective adulticide is identified, it should be used as a compliment to an effective larval control program. Adulticide should be repeated 3-4 days later to hit blood-fed females that were hiding and/or resistant during the previous application.

4. Initiate carefully designed pilot studies with emerging mosquito-suppression technologies, including improved biocidal ovitraps, and *Wolbachia*-infected mosquitoes.
5. Engage statisticians or quantitative ecologists to produce experimental designs for monitoring mosquito populations and treatment effects.
6. Engage mathematical modelers to determine accurate transmission thresholds for Zika in urban and suburban neighborhoods.
7. Release field data promptly over a data portal and share all available information with research partners to improve the speed of modeling and get the quickest feedback on program efficacy. Redacting patient names and addresses is sufficient to comply with patient confidentiality requirements of federal and state law.

Fortunately, most of these improvements are under consideration or have already been implemented by Miami-Dade County. Unfortunately, mosquito-borne diseases (MBDs) are not on the wane. Air travel and incursion into wild areas has only increased the capacity of MBDs to emerge from tropical forests and move into developed areas (Fernandes et al., 2018). Lessons learned from fighting urban mosquitoes in the 2016 Zika epidemic will remain relevant for the foreseeable future.

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Supplemental materials

Raw data are available in spreadsheet form.

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TABLES

naled #1	Wynwood					Miami Beach				
	day -1	day 0	day 1	day 2	day 3	day -1	day 0	day 1	day 2	day 3
median counts	n/a	20.0	n/a	7.0	4.5	12.0	15.0	7.0	10.0	7.0
mean counts	n/a	22.9	n/a	8.5	5.7	22.6	16.4	9.2	13.5	11.9
relative median	n/a	1.00	n/a	0.35	0.23	1.00	1.25	0.58	0.83	0.58
relative mean	n/a	1.00	n/a	0.37	0.25	1.00	0.73	0.40	0.60	0.53
Sign-Rank test P-value			*0.0001*							
Wind		*SSE 11*					*NNE 8*			

naled #2	day -1	day 0	day 1	day 2	day 3	day -1	day 0	day 1	day 2	day 3
median counts	7.0	4.5	0.0	3.0	6.0	7.0	10.0	7.0	5.0	5.0
mean counts	8.5	5.7	0.9	4.3	8.0	9.2	13.5	11.9	9.5	9.4
relative median	1.00	0.64	0.00	0.43	0.86	1.00	1.43	1.00	0.71	0.71
relative mean	1.00	0.67	0.11	0.50	0.94	1.00	1.47	1.30	1.03	1.03
Sign-Rank test P-value			*0.0002*							
Wind		*N 0*					*N 0*			

naled #3	day -1	day 0	day 1	day 2	day 3	day -1	day 0	day 1	day 2	day 3
median counts	7.0	4.0	2.0	5.0	9.0	7.0	4.0	3.0	3.0	2.0
mean counts	10.4	8.1	4.2	6.2	9.9	5.9	4.8	5.7	5.6	3.7
relative median	1.00	0.57	0.29	0.71	1.29	1.00	0.57	0.43	0.43	0.29
relative mean	1.00	0.78	0.41	0.60	0.96	1.00	0.81	0.97	0.95	0.64
Sign-Rank test P-value			*0.013*							
Wind		*N 0 (light rain)*					*S 10*			

naled #4	day -1	day 0	day 1	day 2	day 3	day -1	day 0	day 1	day 2	day 3
median counts	11.0	7.0	5.0	5.0	n/a	3.0	1.0	0.0	1.5	2.0
mean counts	11.2	12.5	6.5	5.8	n/a	4.9	1.9	0.5	3.4	2.5
relative median	1.00	0.64	0.45	0.45	n/a	1.00	0.33	0.00	0.50	0.67
relative mean	1.00	1.11	0.58	0.52	n/a	1.00	0.39	0.11	0.70	0.50
Sign-Rank test P-value			*0.017*							
wind		*ENE 10*					*N 5*			

	day -1	day 0	day 1	day 2	day 3	day -1	day 0	day 1	day 2	day 3
mean of relative means	1.00	0.62	0.25	0.53	1.07	1.00	0.78	0.48	0.55	0.56
mean of relative medians	1.00	0.85	0.37	0.54	0.95	1.00	0.89	0.79	0.89	0.72

Table 1. Median and mean trap counts for *Ae. aegypti* on the day before naled application, the day of application, and the three days following. Relative medians and means are adjusted to counts the day before the application. For Wynwood data, sign-rank tests compared trap counts the day before to the day after, except the first application, where missing data required a comparison of the spray day with two days after. All naled applications produced statistically significant declines however the effect size (proportion alive on day 1) diminished across the applications, indicating the emergence of some sort of acquired resistance (see Discussion). *Ae. aegypti* populations in Wynwood recovered to pre-spray levels in three days in the second and third application, evident in the lower sub-table. In the Miami Beach data declines in mosquito populations following naled application were smaller, and combined with strong ongoing declines from *Bti* application. Statistical analysis of Miami Beach data is presented in Fig. 6. Wind directions and speeds (km h^{-1}) at the hour of spray application were recorded by the National Weather Service at nearby Miami International Airport.

FIGURES

(Note: all figures require color. Figures 1 & 2 can fit a single column; the others are two columns wide.)

Figure 1. East-central Miami showing the 5 km^2 Wynwood area where both naled and *Bti* were applied, and the 25 km^2 outer area where only naled was applied. Red markers are locations where BG Sentinel traps were placed inside Wynwood. Eighteen additional traps were deployed for 11 days in the naled-only zone but locations could not be obtained.

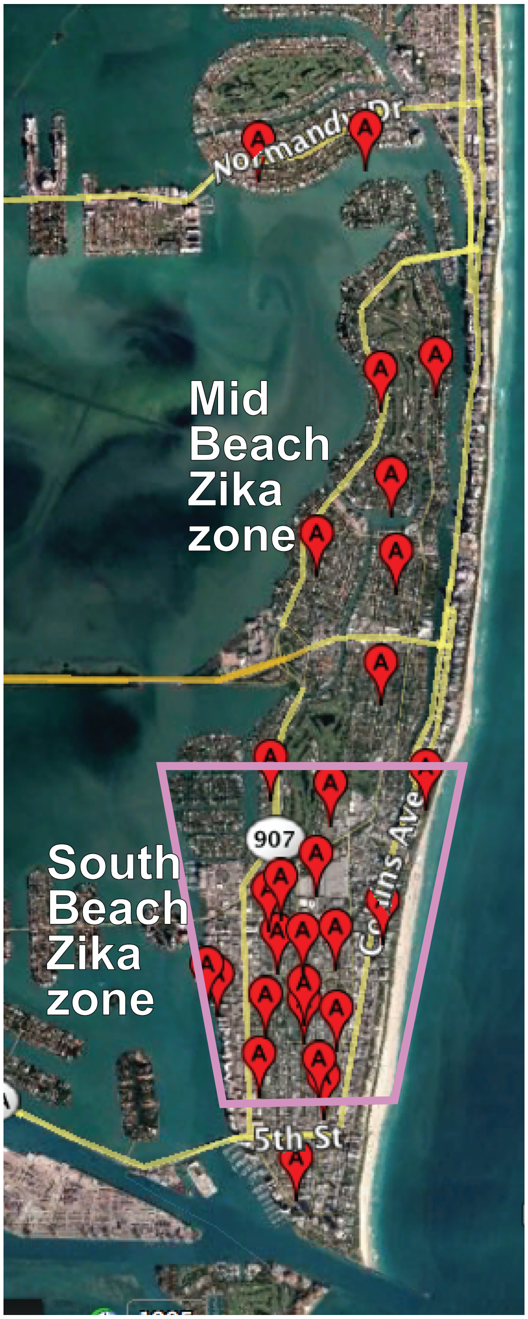

Figure 2. Miami Beach neighborhoods of South Beach where Zika appeared first and Mid-Beach into which Zika expanded. Ester pyrethroids, naled, and *Bti* were applied to the South Beach Zika zone (pink line). Subsequently *Bti* and the non-ester pyrethroid etofenprox were applied in Mid-Beach after active Zika transmission spread into that area. Red markers indicate locations of BG Sentinel traps.

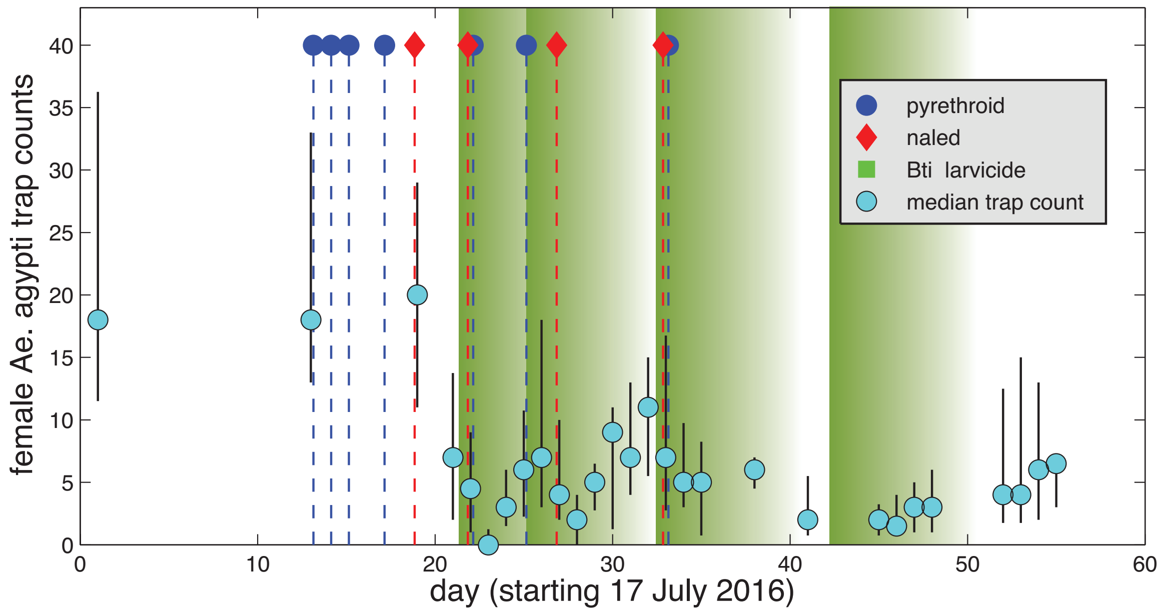

Figure 3. *Ae. aegypti* trap counts in Wynwood starting on 17-July-2016 (day 1). Trap counts did not change following four truck applications of ester pyrethroid adulticides, but declined significantly when the adulticide naled was applied concurrently with the larvicide *Bti*. Trap counts continued to fall under the Bti regimen after naled application ceased, but began to rise again a week after Bti application ceased. Error bars represent 25% and 75% data quartiles.

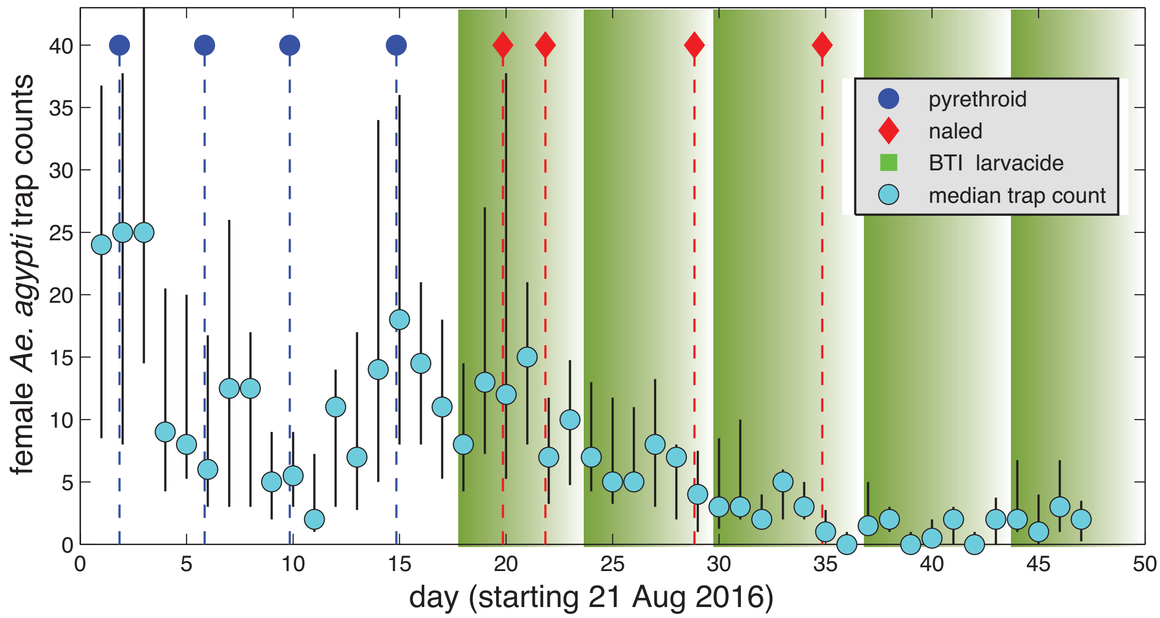

Figure 4. *Ae. aegypti* trap counts in the South Beach neighborhood of the City of Miami Beach, starting on 21 Aug, 2016 (day 1), were unaffected by truck applications of ester pyrethroid adulticides. Naled had no significant effect on trap counts in the first three applications, and a small effect in the fourth. Counts declined steadily under the weekly *Bti* larvicide regime. Note that trap counts took sharp spontaneous declines on days 4 and 9, with no pesticide treatment, emphasizing the need for caution when attributing the cause of declines following pesticide application. Error bars are 25% and 75% quartiles.

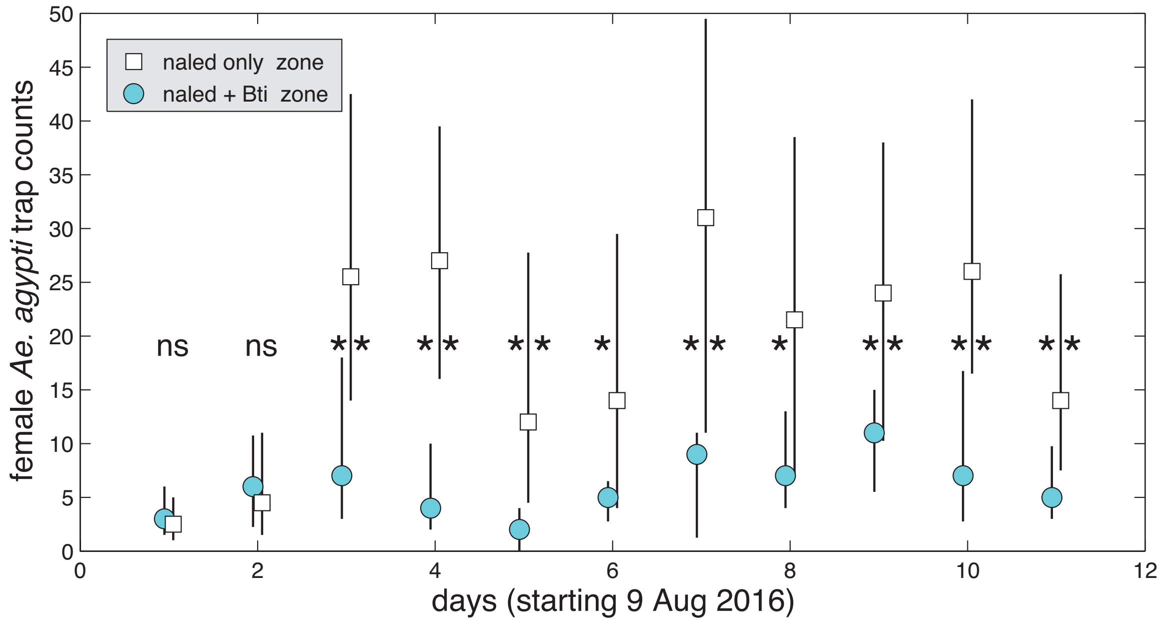

Figure 5. Effect of *Bti* can be seen by comparing median trap counts in the Wynwood [naled+*Bti*] application zone and the surrounding [naled only] zone that did not get *Bti*. Counts suppressed by a pair of naled applications in rapid succession rose again in the absence of weekly *Bti* application, but stayed low in the area where *Bti* was applied (see Fig. 1). Naled was applied to both zones on 7 Aug, 2016 and again on 12 Aug, 2016. From day 2 to day 3, the median count outside the *Bti* zone increased from 4 to 26, showing the population's capacity for rapid increase absent *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. Error bars are 25% and 75% quartiles.

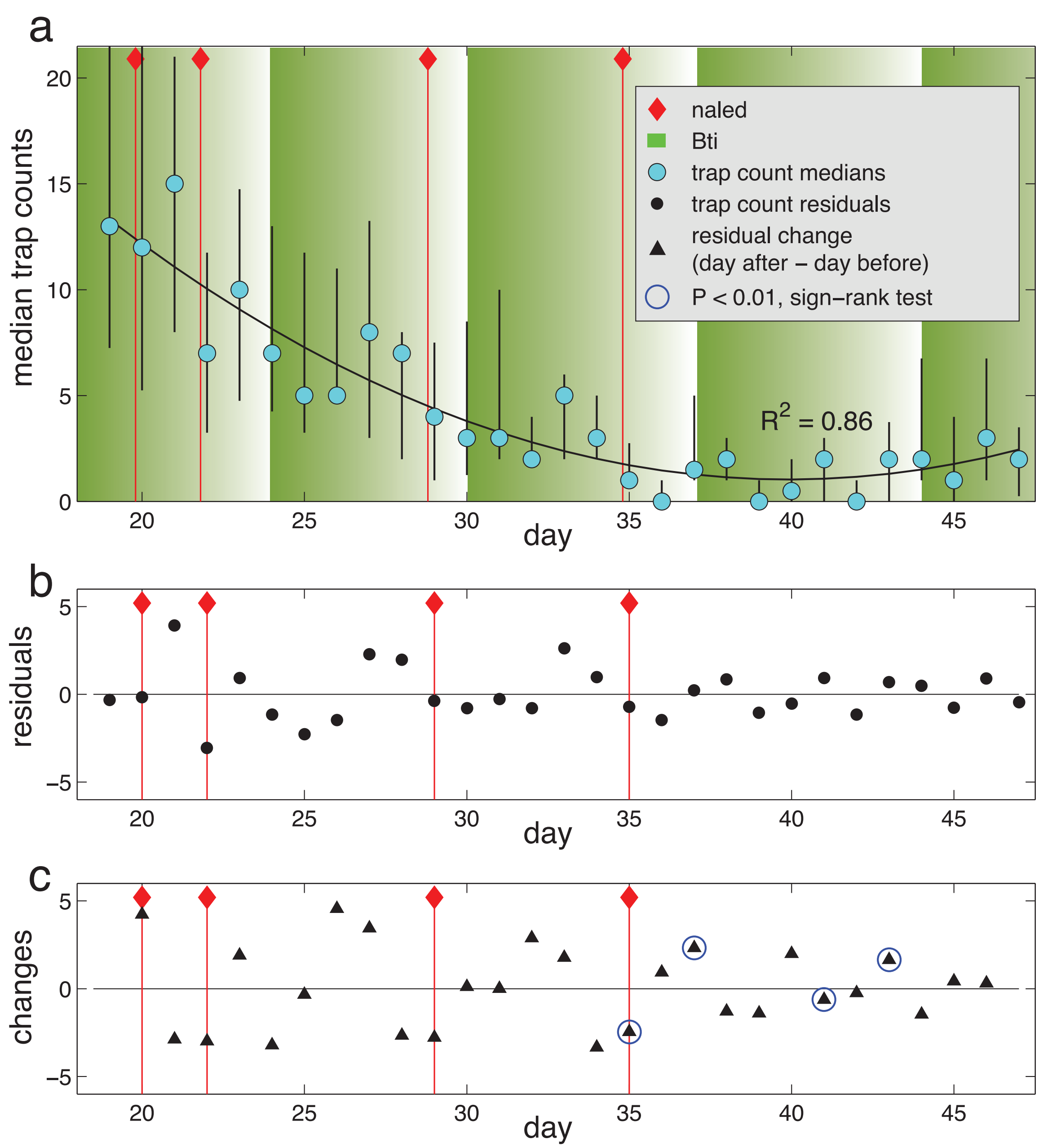

Figure 6.a. Median trap counts on Miami Beach during the naled-*Bti* treatment period. The medians are best fit by a 2nd order polynomial. **(b)** Median residuals remove the gradual declines attributed to the larvicide *Bti*. **(c)** Median effect sizes obtained by subtracting each trap's count on day N+1 from the count on day N-1. Four count changes were significantly different from zero ($p < 0.01$; sign-rank tests, 2-tailed), but only one associated with a naled application, by which time counts were greatly depressed.