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RESPONSE TO AN OUTBREAK OF LOCALLY TRANSMITTED DENGUE IN KEY LARGO, FL, BY THE FLORIDA KEYS MOSQUITO CONTROL DISTRICT

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ABSTRACT. Dengue virus (DENV) is an ever-increasing threat to the residents of South Florida. Seventy-two cases of locally acquired dengue were contracted by residents and visitors of Key Largo, FL, in 2020. The primary vector, *Aedes aegypti*, has been a large focus of the Florida Keys Mosquito Control District's (FKMCD) control measures for over a decade. This paper recounts the 2020 outbreak of DENV in Key Largo, FL, and the FKMCD's *Ae. aegypti* operational response. The overall House Index (13.43%) during the outbreak was considered high (>5%) risk for local transmission. Larval habitat characterized from property inspections was similar to previous larval ($\tau = 0.78$, $P < 0.005$) and pupal ($\tau = 0.745$, $P < 0.005$) habitat studies. Adult surveillance of the active dengue transmission area provided 3 positive pools out of 1,518 mosquitoes tested resulting in a minimum infection rate of 1.976. Increased personnel response with long-term larvicide formulations and increased aerial, truck, and handheld ultra-low-volume adulticide control measures quickly reduced the *Ae. aegypti* surveillance numbers below the action threshold. No active cases of dengue have been reported since October 2020.

KEY WORDS Adulticide, *Aedes aegypti*, arbovirus surveillance, dengue, larvicide

INTRODUCTION

Dengue virus (DENV) (Family *Flaviviridae*, Genus *Flavivirus*) is the most widespread arbovirus in the tropical and subtropical latitudes (Jansen and Beebe 2010). The virus is a group of 4 related serotypes (DENV 1–4) of flavivirus that is primarily transmitted by *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse). Dengue infection is estimated to affect up to 390 million people every year in subtropical and tropical climates (Bhatt et al. 2013). It is estimated that half the global population (~128 countries) is at risk for dengue infection with an endemic cycle risk in ~100 countries (Brady et al. 2012, WHO 2022). Immunity to individual serotypes is achieved through infection, but subsequent infection of a secondary serotype may develop into severe dengue, a medically dire manifestation of dengue fever symptoms. One in 4 infected individuals develop symptoms, 1 in 20 develop severe symptoms, and up to 20% of untreated individuals may die from severe dengue symptoms (WHO 2022, CDC 2023). Although a vaccine is available, adolescents (9–16 years old) are eligible to receive the vaccine only with a lab-confirmed previous infection and a risk of contracting a second serotype of dengue (CDC 2023).

Dengue outbreaks in the USA have been documented periodically since 1780 and continued until 1945, and cases in the late 20th century were predominantly travel related or isolated autochthonous cases near the Texas-Mexico border (Schneider and Droll 2001, Añez and Rios 2013). In the past 14 years, locally acquired dengue outbreaks have been reported in Florida localities including Key West in 2009–2010 (Graham et al. 2011, Radke et al. 2012), Martin County in 2013 (Teets et al. 2014) and most recently in Key Largo in 2020. Though not considered endemic at this time, locally acquired dengue infections have been reported in Miami-Dade County in 10 of the past 12 years (Florida Department of Health 2023).

Aedes aegypti, the primary vector of dengue in South Florida, seeks larval habitats and blood meals near human domiciles and populated urban areas. Gravid females use artificial containers as oviposition sites (Chan et al. 1971; Hribar et al. 2001, 2004). Recent blood meal analysis demonstrated that *Ae. aegypti* collected in the Florida Keys fed predominantly (73.9%) on humans (Pruszynski et al. 2020). These factors contribute to ideal situations for the urban dengue transmission cycle. Secondary vectors such as *Ae. albopictus* are also present in low numbers in the Florida Keys, although they have not been implicated in transmission (Graham et al. 2011, Murray et al. 2018).

The Florida Keys Mosquito Control District (FKMCD) is an independent taxing district charged with the control of both nuisance and vector mosquitoes in the 120-mile (192-km) -long populated archipelago of Monroe County, FL, that separates the Gulf of Mexico from the Atlantic Ocean and borders Miami-Dade County to the north. The unique linear geography necessitates 3 separate FKMCD offices to operate efficiently: Big Coppitt Key in the Lower Keys (Key West to the Seven Mile Bridge), Marathon in the Middle Keys (the Seven Mile Bridge through Lower Matecumbe Key), and Key Largo in the Upper Keys (Upper Matecumbe Key through Angelfish Key). While the majority of District funds are spent on controlling for nuisance salt marsh mosquitoes, *Ae. taeniorhynchus* (Wiedemann), the 2009–2010 dengue outbreak in Key West prompted an updated integrated pest management program (IPM) for including the adoption of new treatment techniques, new pesticide formulations, and an increase

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in personnel to reduce the population of the dengue vector, *Ae. aegypti*. Since then, locally acquired dengue cases had not been reported in the Florida Keys for over a decade.

On March 3, 2020, the first suspected case of dengue was reported in Key Largo and serologically confirmed by the Florida Department of Health (FDOH). The infected individual had not traveled abroad or out of the Florida Keys prior to infection. No more suspect cases were reported until June 17, but later FDOH patient interviews and antibody tests provided evidence that transmission was occurring in late May into early June 2020. Reported infections did not continue past early October 2020. In late 2021 the FDOH notified the FKMCD that 2 cases were confirmed to have occurred in early January 2020, though these cases were unreported at the time. In total, 72 locally acquired dengue cases were reported in the Upper Keys. Lessons learned from the 2009–2010 Key West dengue outbreak and methods developed in the decade since were applied to the 2020 dengue outbreak in Key Largo. This paper details the *Ae. aegypti* surveillance and response to the 2020 dengue outbreak in Key Largo.

MATERIAL AND METHODS

Aedes aegypti *surveillance:* Normal daily operations at FKMCD are discussed in detail by Hribar et al. (2022). After FKMCD received word from the FDOH of the initial suspected locally acquired dengue case in March, between 12 and 16 FKMCD inspectors performed property inspections within the area, moving outward from the suspect case property. These rigorous inspection events are referred to as "sweeps." Teams of 2 inspectors "swept" entire neighborhoods by visiting every accessible domestic, commercial, and public property and treated or dumped larval habitats. When live mosquito immature stages were observed at domestic sites, samples were collected and brought to the Key Largo FKMCD laboratory in 6 oz straight-walled polystyrene jars (US Plastics, Lima, OH, 70/400 lid) to identify species, quantity, and stage of development under magnification by trained staff using mosquito identification keys. No attempt was made to collect every immature mosquito in sampled habitats. Sampling and habitat information was entered in FieldSeeker® (Frontier Precision, Bismarck, ND), a geographic information systems vector control mapping and data management system used by FKMCD. In addition to location and samples, habitat type (plastic container, boats, hot tub, garbage cans, flower pots, plant trivets, etc.) was recorded for each inspection and for each sample taken. Plastic containers are defined as any plasticized item capable of holding water that is not recognized as any other recordable larval habitat. To discern if any historical changes occurred in *Ae. aegypti* oviposition habitat, Kendall's tau statistic (Ghent 1963) is used to investigate correlation between rank order containers positive for larvae and pupae between historical data (July numbers only) and the sample collections from the 2020 sweep (Hribar and Whiteside 2010). The House Index (HI) was calculated as (no. of infested houses) × 100/houses inspected (Connor and Monroe 1923).

Since the 2009–2010 Key West dengue outbreak, FKMCD has consistently set BG-Sentinel® (BG) traps (Biogents GmbH, Regensburg, Germany) weekly to monitor adult container breeding *Aedes* species populations throughout the urbanized Florida Keys. The BG traps are set at 1400 h and retrieved at 1000 h the following day, and each trap is baited with 3 lb (1.6 kg) of dry ice as CO_2 and a BG-Lure (Biogents GmbH, Regensburg, Germany) (Hribar et al. 2022). In the Upper Keys, 4 BG traps were initially set at densely populated *Ae. aegypti* areas. Over the next decade, *Ae. aegypti* monitoring increased to 9 BG traps set weekly in the Upper Keys. When staff were notified of a suspected DENV case, a BG trap was set near the suspected or confirmed infection case residence. In total, an additional 18 BG traps were set weekly from June 14, 2020, through December 15, 2021. Not all traps were set for the entire duration of the monitoring period, and no traps were placed within 100 ft (33 m) of another set on the same day. Two of these dengue surveillance BG traps have remained on the weekly trapping schedule for operational *Ae. aegypti* surveillance since 2020. *Aedes aegypti* adult females collected from active transmission area BG traps were pooled (max. 20 females/per pooled sample) and sent to the Centers for Disease Control (CDC) Dengue Laboratory in Puerto Rico for reverse transcription polymerase chain reaction (RT-PCR) testing until March 2021. Further testing was completed in house using a RAMP® Dengue machine (Response Biomedical, Vancouver, BC, Canada) at the Key Largo FKMCD facility.

Aedes aegypti *control measures:* Methodologies used to reduce larval infestations were site dependent. Containers that could not be dumped or covered were treated with larvicides (Table 3 below). Multibrood or residual larvicide products (e.g., Altosid pellets®, Vectobac DT®, Natular DT®) were preferentially used over single-brood larvicides (e.g., Vectobac G®, monomolecular films) to provide a longer reinspection window. Handheld ultra-low-volume (ULV) foggers using Duet® (active ingredient [A.I.] sumithrin and prallethrin, Clarke, St. Charles, IL) were used to knock down any existing adult mosquitoes seen during inspections.

From June 29, 2020, to October 8, 2020, aerial (helicopter) larvicide treatments with Vectobac® WDG (A.I. *Bacillus thuringiensis israeliensis* de Barjac [*Bti*], Valent Biosciences, Libertyville, IL) were used to treat large swathes of Key Largo based on study results that showed weekly applications of aerial *Bti* treatments in Key West provided a >50% reduction of *Ae. aegypti* populations under various canopy covers (Pruszynski et al. 2017). Truck-based *Bti* larvicide treatments using an A1 Super Duty Mister® (A1 Mist Sprayers, Ponca, NE), capable of providing up to >90% larval mortality at 24 h past treatment (Murray et al. 2021), were used for targeting smaller neighborhoods or junk yards where

Table 1. Total containers sampled positive for larvae in Key Largo, FL, in March and June to October 2020. Ranked by total *Aedes aegypti* larvae.

Container type	Total containers (%)	Total *Aedes aegypti* larvae (%)
Plastic container	316 (23.20)	3,780 (26.83)
Flower pot	192 (14.1)	2,200 (15.61)
5 Gallon bucket	148 (10.87)	1,567 (11.12)
Garbage can	121 (8.88)	1,365 (9.69)
Metal container	77 (5.65)	1,202 (8.53)
Boat	65 (4.77)	756 (5.36)
Tire	62 (4.55)	700 (4.97)
Bird bath	43 (3.16)	581 (4.12)
Plant trivet	55 (4.04)	491 (3.48)
Bromeliads	69 (5.07)	403 (2.86)
Standing water	30 (2.2)	197 (1.39)
Cooler	33 (2.42)	182 (1.29)
Fountain	26 (1.91)	165 (1.17)
Glass container	41 (3.01)	148 (1.05)
Pool	26 (1.91)	104 (0.74)
Ornamental pond	16 (1.17)	84 (0.59)
Toilet	11 (0.81)	58 (0.41)
Rain barrel	17 (1.25)	54 (0.38)
Concrete unit	4 (0.29)	36 (0.26)
Drain	7 (0.51)	17 (0.12)
Cistern	1 (0.07)	2 (0.01)
Pipewell	2 (0.15)	1 (0.01)

safety, time, and efficacy of a human inspection are considered impractical.

Pyrethroid adulticide products (e.g., Permanone 30–30®, Bayer, Whippany, NJ) are used to control the pestiferous *Ae. taeniorhynchus* species in normal operations (3 times the average daily landing rate count), but *Ae. aegypti* has shown some resistance to permethrin products in Florida (Estep et al. 2018, Scott et al. 2021). When *Ae. aegypti* numbers reached the action threshold of >10 *Ae. aegypti* per trap/night, ULV trucks with Fyfanon EW® (FMC, Philadelphia, PA) or aerial adulticiding with Dibrom® (AMVAC, Los Angeles, CA) was used to lower the adult mosquito numbers.

RESULTS

Inspectors collected 826 water samples from 6,156 inspections throughout the Upper Keys (March and June 3 to October 31, 2020), containing 14,091 *Ae. aegypti* larvae and 1,419 *Ae. aegypti* pupae. A total of 1,362 positive containers were recorded, of which the most reported larval habitats were plastic containers ($n = 316$, 26.83%), flower pots ($n = 192$, 14.1%), and 5 gallon buckets ($n = 148$, 10.87%) (Table 1). Plastic containers ($n = 3{,}780$, 26.83%), flower pots ($n = 2{,}200$, 15.61%), and 5 gallon buckets ($n = 1{,}567$, 11.12%) were also the most productive when comparing the total larvae sampled (Table 2).

The HI (Connor and Monroe 1923) for the total inspection period was 13.43%, though it varied from week to week (Fig. 1). Species collected during inspections included *Ae. albopictus*, *Ae. taeniorhynchus*, *Ae. triseriatus* (Say), *Culex nigripalpus* (Theobald), *Cx. quinquefasciatus* (Say), and *Wyeomyia vanduzeei* (Say). Except for *Ae. taeniorhynchus*, the listed mosquito species were found cohabitating with *Ae. aegypti*. *Culex quinquefasciatus* was the secondmost collected species in 208 samples, totaling 4,448 larvae and 258 pupae. *Aedes albopictus* was collected in 14 samples, cohabitating with *Ae. aegypti* in 71.43% ($n = 10$) of the identified samples. The bromeliad-associated mosquito, *Wy. vanduzeei*, was found cohabitating with *Ae. aegypti* in 78% ($n = 14$) of the 18 samples that contained *Wy. vanduzeei* larvae. *Aedes triseriatus* was only found in 3 samples, cohabitating with *Ae. aegypti* in 2 (66.67%) of the 3 recorded samples.

Kendall's tau coefficient for *Ae. aegypti* positive containers for larvae ($\tau = 0.78$, $P < 0.005$) and

Table 2. Rank order of containers most frequently positive for all Florida Keys 2010 wet season and 2020 dengue virus (DENV) area *Aedes aegypti* larvae; $\tau = 0.78$, $P = 0.00005$. Rank order of containers most frequently positive for all Florida Keys 2010 wet season and 2020 DENV area *Aedes aegypti* pupae; $\tau = 0.745$, $P = 0.00071$.

Container type	2010 (larvae)		2020 (larvae)		2010 (pupae)		2020 (pupae)	
	Containers (*n*)	Rank	Containers (*n*)	Rank	Containers (*n*)	Rank	Containers (*n*)	Rank
Plastic containers	62	1	316	1	42	1	144	1
Flower pot	37	2	192	2	22	3	86	2
Plant trivet	30	3	55	9	12	5	27	6
5 Gallon bucket	28	4	148	3	17	4	72	3
Garbage can	27	5	121	4	27	2	55	4
Bromeliad	22	6	69	6	9	6	26	7
Tires	19	7	62	8	5	10	26	8
Boat/jet ski	14	8	65	7	7	8	25	10
Metal container	13	9	77	5	8	7	37	5
Bird bath	9	10	43	10	6	9	25	9
Cooler	8	11	33	11	N/A	N/A	N/A	N/A
Fountain	8	12	26	12	1	11	9	11
Pool/hot tub	7	13	26	13	N/A	N/A	N/A	N/A
Pond	2	14	16	14	N/A	N/A	N/A	N/A

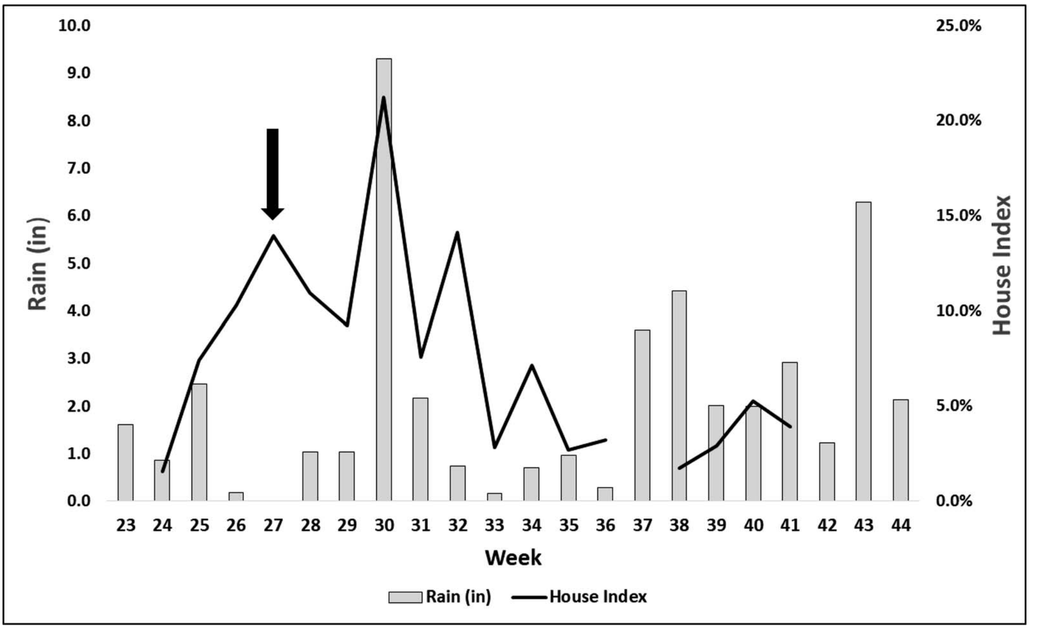

Fig. 1. House Index (%) and weekly rain (in.) in Key Largo, FL, June 1 to October 31, 2020 (weeks 23–44). Arrow indicates start date of weekly (June 29 to October 9, 2020) aerial Vectobac WDG missions for the Key Largo area.

pupae ($\tau = 0.745$, $P < 0.005$) between the 2010 wet season and the 2020 dengue sweep showed a strong correlation of larval habitat preference (Table 2). A significant correlation was seen between containers positive for larvae and pupae ($\tau = 0.833$, $P < 0.005$) during the 2020 sweep.

Inspectors used a variety of larvicides to control *Ae. aegypti* larvae when source reduction techniques were not applicable (Table 3). The most preferred products used by inspectors were Natular G30® and Natular DTs (A.I. spinosad, Clarke, St. Charles, IL), treating 40.99 and 5.39 acres of water at domestic sites, respectively. Pupacides were the least used treatment options, with 1.12 acres of treated surface area recorded.

Between March 16, 2020, and April 28, 2021, 1,518 *Ae. aegypti* females were collected and pooled for DENV testing. A total of 189 pools containing 1,330 female *Ae. aegypti* were sent to the CDC Dengue Lab for RT-PCR testing. An additional 48 pools containing 188 female *Ae. aegypti* were tested by FKMCD personnel using a RAMP Dengue machine. Three pools returned positive results with DENV serotype 1 from pools tested by the CDC Dengue Lab. Two positive pools were collected on week 26 (June 23 and June 27, 2020) and a third on July 29, 2020 (collection area not shown) resulting in a total minimum infection rate of 1.976. No positive mosquito pools were found through RAMP testing at FKMCD facilities.

The maximum mean BG trap collection of adult *Ae. aegypti* was 30.8 (SE = ±21.78) female mosquitoes/trap on week 25 (June 14–20) (Fig. 2). Adult collections were reduced to <10 females/trap by week 27. After collections increased on week 30 (12.5, SE = ±17.48 females/trap) mean *Ae. aegypti* numbers declined to <5 females/trap for the remainder of the 2020 calendar year. Over 48,376 acres were treated with Fyfanon EW (A.I. malathion) from a truck-mounted ULV sprayer, making it the preferred method of adult control by acreage. A total of 126,536.9 acres were treated with various adulticide products and methods (Table 3).

DISCUSSION

Lessons learned from the 2009–2010 dengue outbreak in Key West informed the FKMCD response to the outbreak in Key Largo. Container types used by gravid *Ae. aegypti* have not changed much in the decade between dengue outbreaks. Although the mean HI for Key Largo was considered high risk for dengue transmission, the adult populations of *Ae. aegypti* were reduced over the course of the outbreak. In this report, we recount the techniques used to reduce both the larval and adult populations and surveillance methods used to monitor the change in population.

The mean HI (13.43%) for Key Largo from June to October 2020 was considered well above the high-risk limit (>5%) for arbovirus transmission potential in an endemic area, but less information is available for areas where dengue occurs only sporadically (Patz et al. 1998, Rivera et al. 2020). In our report, larval infestation indices such as the Container index and the Breteau index could not be accurately calculated based on gaps and reporting errors in the data records.

Table 3. Larvicides and adulticides used by FKMCD inspectors between June 1, 2020, and October 31, 2020. Total acreage of Key Largo, FL, dengue virus response included.

Product name	Active ingredient	Mode of action	Formulation longevity	Application	Total acres treated
Larvicide					
Altosid pellets	Methoprene	Juvenile hormone analog	30 days	Hand	1.2802
Altosid XR			150 days	Hand	0.4798
Altosid XR-G			21 days	Hand	1.4015
Natular DT	Spinosad	Nicotinic acetylcholine receptor allosteric modulator	60 days	Hand	5.3904
Natular G30			30 days	Hand/backpack sprayer	35.1221
Natular XRT			180 days	Hand	0.4798
Vectobac DT	*Bacillus thuringiensis israelensis*	Microbial disruptors of midgut membranes	30 days	Hand	3.6685
Vectobac G			7 days	Hand/backpack sprayer	4.82287
Vectobac WDG			24–48 hours	Aerial/truck/backpack	32,581.5/250/1
Coco Bear	Monomolecular film	Physical barrier (suffocation)	N/A	Spray bottle	0.0135
BVA Oil					0.0019
Kontrol					1.1066
Adulticide					
Permanone 30-30	Permethrin	Sodium ion channel inhibitor	N/A	Truck ULV	36,540
Duet/Duet Mix	Sumithrin/prallethrin	Sodium ion channel inhibitor	N/A	Handheld ULV	23.2417
Fyfanon EW ULV	Malathion	Acetylcholinesterase inhibitor	N/A	Truck ULV	48,376.3636
Dibrom	Naled	Acetylcholinesterase inhibitor	N/A	Aerial	41,597.3

Aedes aegypti in the Florida Keys have overall consistent container utilization rates. Hribar et al. (2001) reported that rates for the most used containers by *Ae. aegypti* in Key West were equal (trash cans and plastic containers), whereas in Key Largo *Ae. aegypti* preferred plastic containers (23.20%) (Table 1), and garbage cans ranked fourth behind flower pots (14.1%) and 5 gallon buckets (10.87%). A later study found no significant differences between container-type usage in 10 US Census tracts throughout the Florida Keys (Hribar and Whiteside 2010). The strong correlation (Table 2) between positive container 2010 wet season data and the 2020 collections suggests *Ae. aegypti* container use had remained the same. To some degree, this correlation indicates that community sanitation practices have not changed in the preceding decade.

An unofficial action threshold was created for adult *Ae. aegypti* numbers during the 2009–2010 Key West outbreak when FKMCD researchers observed no dengue cases during months when weekly BG trap collections averaged <10 *Ae. aegypti* females/trap/night (FKMCD, internal communication). This action threshold guided FKMCD treatment plans for Key Largo in 2020, using aerial adulticide missions for large areas, ULV truck missions for neighborhoods, or ULV hand-held sprayers at localized sites when BG trap collections exceeded the action criterion. The ability to quickly schedule and use ULV spray trucks for smaller neighborhoods made them the primary adulticiding methodology over aerial methods, which required more stringent meteorological conditions to complete. Adult monitoring with BG traps allowed FKMCD to keep the *Ae. aegypti* numbers below a mean of 5 females per night/trap from the beginning of August (week 25) to the end of 2020 (Fig. 2). Adult *Ae. aegypti* monitoring continued through December 2021 in case of resurgence.

This report would be remiss not to mention the COVID-19 pandemic concurrent with the dengue outbreak in Key Largo. Symptoms of dengue and COVID-19 both include fever, headache, muscle aches, nausea, vomiting, rash, and even conjunctivitis as both diseases display varying severity from case to case (Mayo Clinic 2018, 2020). Patients' symptom confusion between dengue and COVID-19 may have delayed an accurate and timely response from the FDOH and FKMCD. Some reported dengue cases were confirmed later by antibody tests as the patient delayed seeking medical assistance, affecting the accurate timeline of active dengue infection. Social distancing at home but outdoors, which increased vector interaction, may have played a small role in the Key Largo dengue outbreak, but to what degree is unknown. Personal contact with the public by FKMCD personnel was dampened due to fear of COVID as well, removing community education and involvement—a key component in an effective dengue response (Pilger et al. 2010). Adult and larval collection data were interrupted in the weeks prior to the 2020 dengue outbreak due to FKMCD social distancing

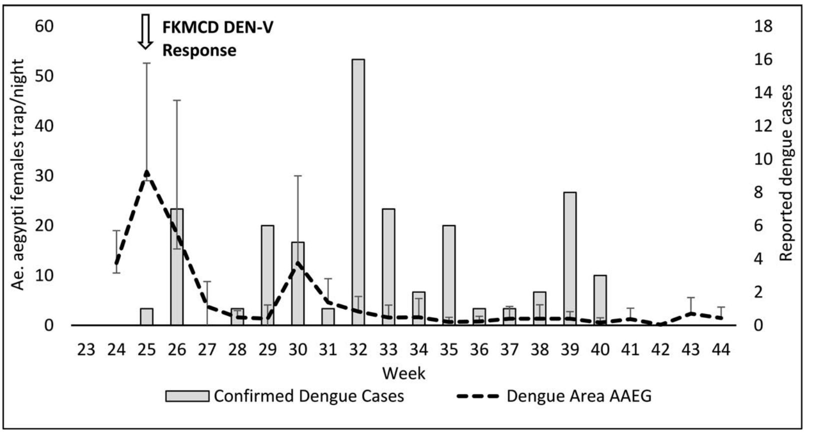

Fig. 2. Confirmed dengue cases in Key Largo between June 17 and October 4, 2020 (weeks 25–40) and the mean *Aedes aegypti* collections from BG Sentinel traps in the areas of dengue transmission. Cases are shown when they were reported to FKMCD, not when the patient was purportedly infected.

efforts as well, limiting FKMCD's ability to gauge mosquito population trends prior to the outbreak.

Globalization, rapid transit, effects of climate change, and increase of endemic dengue transmission will likely facilitate outbreaks in areas where dengue is not endemic (Domingo et al. 2011, Brathwaite Dick et al. 2012, Rivera et al. 2020). In Florida, where *Ae. aegypti* populations already thrive, the CDC (2023) has reported travel-related dengue cases every year since 2010 and locally transmitted cases in 11 of the last 13 years. This trend is likely to continue and provide South Florida and the Florida Keys with an ever-increasing threat of arbovirus introduction. Increased temperatures may allow *Ae. aegypti* to expand its range north into more temperate regions as well and provide longer periods of propagation (Iwamura et al. 2020).

Vector control methods used to reduce dengue incidence differ across the world to varying effect (Ballenger-Browning and Elder 2009). Most studies of intervention strategies are focused in areas with endemic dengue or where vector response is limited by resources, testing 1 or 2 control methods. In the Florida Keys, FKMCD attempted to stop dengue transmission by using all available vector control strategies as part of an IPM program. Lacking proper controls to determine operational control effects, successful interruption of dengue transmission in Key Largo can only be inferred from the brevity of the outbreak, reduction in adult collections, and lack of autochthonous dengue resurgence since 2020.

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