Acta Tropica 227 (2022) 106269

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Proof of concept for eliminating *Aedes aegypti* production by means of integrated control including turtles, copepods, tilapia, larvicides, and community participation in Monte Verde, Honduras

Gerald G. Marten [a,*], Xenia Caballero [b], Arnulfo Larios [b], Hilda Bendaña [b]

[a] *EcoTipping Points Project, Kailua, Hawaii, United States*
[b] *Operación Bendición Honduras, San Pedro Sula, Honduras*

A R T I C L E I N F O

Keywords:
Aedes aegypti
Zika
Dengue fever
Chikungunya
Biological control
Copepods
Turtles
Tilapia
Community participation

A B S T R A C T

Monte Verde, a peri-urban squatter community near San Pedro Sula, virtually eliminated *Aedes aegypti* production in all known larval habitats: wells; water storage containers including *pilas* (open concrete water tanks used for laundry), 200-liter drums, 1000-liter plastic "cisterns," buckets; and objects collecting rainwater. The project began in 2016 when Monte Verde was overrun with dengue, Zika, and chikungunya. During more than a year of experimentation, Monte Verde residents crafted an effective, sustainable, and environmentally friendly toolkit that was inexpensive but required full community participation. Biological control with copepods, turtles, and tilapia was at the core of the toolkit, along with a mix of other methods such as getting rid of unnecessary containers, scrubbing them to remove *Ae. aegypti* eggs, and covering them to exclude mosquitoes or rainwater. Environmentally friendly larvicides also had a limited but crucial role. Key design features: (1) toolkit components known to be nearly 100% effective at preventing *Ae. aegypti* production when fitted to appropriate larval habitats; (2) using *Ae. aegypti* larval habitats as a resource by transforming them into "egg sinks" to drive *Ae. aegypti* population decline; (3) dedicated community volunteers who worked with their neighbors, targeting 100% coverage of all known *Ae. aegypti* larval habitats with an appropriate control method; (4) monthly monitoring in which the volunteers visited every house to assess progress and improve coverage as an ongoing learning experience for both volunteers and residents. Taking pupae as an indicator of *Ae. aegypti* production, from September 2018 to the end of the record in December 2021 (except for a brief lapse during COVID lockdown in 2020), the monthly count of pupae fluctuated between zero and 0.6% of the 22,984 pupae counted in the baseline survey at the beginning of the project. Adult *Ae. aegypti* declined to low numbers but did not disappear completely. There were no recognizable cases of dengue, Zika, or chikungunya after June 2018, though the study design based on a single site did not provide a basis for rigorous confirmation that Monte Verde's *Ae. aegypti* control program was responsible. Nonetheless, Monte Verde's success at eliminating *Ae. aegypti* production can serve as a model for extending this approach to other communities. Key ingredients for success were outside stimulation and facilitation to foster shared community awareness and commitment regarding the problem and its solution, enduring commitment of local leadership, compatibility of the toolkit with the local community, overcoming social obstacles, rapid results with "success breeding success," and building resilience.

* Corresponding author.
E-mail address: gerrymarten@hawaii.rr.com (G.G. Marten).

https://doi.org/10.1016/j.actatropica.2021.106269
Received 1 July 2021; Received in revised form 30 November 2021; Accepted 6 December 2021
Available online 8 December 2021

1. Introduction

Effective control of *Aedes aegypti* and associated diseases has been elusive despite massive effort and a diverse array of control methods that have been in use for years or are now in development (Achee et al., 2015). Meta-analyses of methods have concluded that biological control has been most effective, with a mix of biological control and other methods being more effective than any single method by itself (Horstick and Ranzinger, 2015; Bouzid et al., 2016; Alvarado-Castro et al., 2017). A major advantage of biological control is its low cost, though a heavy dose of community participation (Alvarado-Castro et al., 2019) is required to ensure that every household does what is necessary for it to succeed.

Vietnam's multi-method dengue control program with copepods at the core (Nam et al., 1998, 2005; Marten, 2001; Kay and Nam, 2005) is the only instance we know of completely and sustainably eliminating *Ae. aegypti* and dengue on a large scale since the global DDT program collapsed in the 1970s. Vietnam's program began in the 1990s with community participation from local women's unions that already provided door-to-door health services. Within ten years, *Ae. aegypti* was eliminated from approximately a thousand villages and urban neighborhoods, and eventually northern Vietnam was for all practical purposes free of the mosquito and the disease. *Ae. aegypti* and dengue were also eliminated from much of the rest of Vietnam, though in 2019 there was a serious dengue outbreak in southern Vietnam, where the elimination of *Ae. aegypti* was incomplete.

In 2016, when Honduras was swept by a surge of Zika (Scott et al., 2016), dengue, and chikungunya, Monte Verde (MV), a 30-year-old squatter community of 1400 residents considered by the Ministry of Health's regional office to have the most severe dengue history in the region, embarked on a low-budget project to rid the community of these diseases by adapting Vietnam's approach to ecological and social conditions in Honduras (Marten et al., 2020). MV assembled and deployed a multi-method toolkit with three kinds of biological control at the core: turtles (Marten, 2007), copepods (Marten and Reid, 2007; Lozaro et al., 2015), and tilapia (Han et al., 2015).

The goal was complete local elimination of *Ae. aegypti*, an ambitious but worthy goal if it could be achieved. The feasibility of complete local elimination had already been demonstrated not only in Vietnam but also in a Singapore neighborhood (Chan, 1973) and for *Aedes albopictus* on a small island in Louisiana (Marten, 1990a). No mosquitoes ensure no local disease transmission, and sustaining control is easier when there are no mosquitoes to reseed population rebounds during lapses in a control program.

The overriding research question for MV was "*What are the details that will enable this approach to function effectively*?" The journey to an answer involved several additional major questions:

- What overall strategy and project design are most effective?
- What mix of methods should be in the toolkit?
- Which methods are best for each type of larval habitat?
- How can the community be mobilized to use the toolkit effectively?
- What are the obstacles to success and how can they be overcome?
- Is complete local elimination of *Ae. aegypti* achievable under the conditions at MV?

MV's experience offers a case study with enough detail to show how to develop and successfully put into use a multi-method toolkit built around biological control.

2. Materials and methods

2.1. Project site

MV is located at 15.563 N latitude and 87.969 W longitude in Choloma municipality (Cortés Department) on the outskirts of San Pedro Sula. MV's 350 houses run east-west on the south side of the highway connecting San Pedro Sula and Choloma (Fig. 1). They are bounded to the north by an industrial area and to the south by an area of scrub vegetation and wetland. There is an upscale residential area (Las Colinas) immediately adjacent to MV's eastern end.

MV receives no municipal services such as water supply or sanitation, but it does have a rudimentary community-operated water system that pumps underground water to each house for two hours every eight days. The water is for cleaning and cooking but not drinking. Every household in MV has a variety of containers for storing water during the days that water is not supplied (Fig. 2). Some households also have a small well in their yard (Fig. 3). The water storage containers, wells, and objects that collect rainwater are *Ae. aegypti* larval habitat.

2.2. Project strategy

MV's strategy was to pursue complete elimination of *Ae. aegypti* by:

- offering every household a diverse toolkit, each of whose component methods was known to be highly effective at preventing *Ae. aegypti* production if used properly in appropriate larval habitats;
- targeting "100% container coverage" by managing all known *Ae. aegypti* larval habitats at every house with at least one appropriate method.

2.3. Elements of the toolkit

Three forms of biological control were at the core of the toolkit: turtles, copepods, and tilapia. Additional methods were: environmentally friendly larvicides; *Untadita*, a technique for scrubbing containers with bleach to remove *Ae. aegypti* eggs (Sherman et al., 1998); eliminating unnecessary containers and replacing difficult-to-manage water storage containers with more manageable containers; covering containers tightly to prevent mosquitoes from entering to deposit eggs; covering or positioning containers and other objects to keep them dry instead of collecting rainwater.

2.3.1. Turtles

A baby turtle can eat a thousand 3rd/4th instar mosquito larvae or pupae every day. There is no mosquito production when a turtle is in a well or water storage container (Borjas et al., 1993). Turtles become family pets and will survive for years as long as they are fed to keep them healthy.

Baby red-eared slider turtles (*Trachemys scripta elegans*) were obtained from a turtle farm in Louisiana (Fig. 4). The turtles cost approximately one U.S. dollar each and were certified to be salmonella free. Although it is well known that pet turtles can be a source of salmonella infection for humans, extensive study of this issue has established that turtles present no risk of salmonella infection when used for mosquito control in wells or water storage containers like those in MV (Borjas et al., 1993; Marten, 2007). Although the use of an exotic species like *T. scripta* might be questioned because of possible detrimental impacts on native turtles or Honduran ecosystems, we consulted international turtle conservationists and concluded that the use of *T. scripta* was environmentally responsible. Supplement 1 provides details.

After arrival of the turtles in Honduras and before distributing them in MV, pooled fecal samples from the turtles were checked for salmonella at a local medical laboratory and none were positive. Beginning in August 2016, one or two turtles were provided to every household in MV that wanted them. Families were instructed on turtle care. After that, additional turtles were provided as needed.

2.3.2. Copepods

The larger species of cyclopoid copepods (Fig. 5) are highly effective predators of first-instar mosquito larvae (Leonard and Marten, 1994; Marten and Reid, 2007). Although these tiny crustaceans are common in freshwater habitats around the world, they seldom get into *Ae. aegypti* larval habitats on their own. When introduced to a larval habitat, they multiply to large numbers, consuming virtually every mosquito larva that hatches into the water (Marten, 1990b). The copepods take care of themselves, maintaining a large population for as long as there is water. They do not depend on mosquito larvae for survival, because their diet embraces a broad variety of foods, ranging from protozoa and rotifers to aquatic animals twice their size. If an aquatic habitat contains food for mosquito larvae, it also contains food for copepods.

Mesocyclops longisetus is a common copepod species in Latin America. Its large size (1.5 mm in length excluding caudal setae) makes it particularly effective for mosquito control (Marten, 1990b). Its high temperature tolerance, surviving temperatures up to 42 °C (Marten et al., 1994a), enables it to thrive in containers exposed to the tropical sun. The fact that *M. longisetus* spends its time mainly on the sides or at the bottom of a container instead of swimming in the water column gives this species an advantage at surviving in containers with active water turnover (Marten et al., 1994b). Copepod species that swim in the water column are unable to maintain a long-term population because of population losses when water is removed from a container for use.

Mesocyclops longisetus curvatus was collected with a conventional mosquito dipper at a small marsh where it was common only a few hundred meters from MV. Because the collection also contained *Microcyclops dubitabilis*, a copepod too small to be an effective predator of mosquito larvae, it was necessary to establish pure cultures of *M. longisetus* by using a pipette to introduce single copepods to small containers in the laboratory. Copepod cultures could be started with a single copepod because most adult copepods are females that have already been inseminated for life.

A sample of copepods from each single-female culture was examined by an expert on copepod taxonomy to confirm that the culture contained only *M. longisetus*. Cultures confirmed to be pure *M. longisetus* were then combined to form a stock culture for use throughout the project. The copepods could be produced by the thousands at virtually no cost, following procedures described by Suarez et al. (1992) and Marten et al. (1997). The food for the copepods was two kinds of protozoa: *Chilomonas* sp. and *Paramecium caudatum*. The food for the protozoa was wheat seed that decomposed in the culture water. The stock culture was maintained in a small number of 20 L drinking water bottles. Production in several 200 L drums at MV provided copepods for introduction to larval habitats there.

M. longisetus was introduced for the first time to all wells, tires lining wells for structural support (Fig. 3), and many water storage containers in MV (Fig. 2) during December 2016 to February 2017. Copepod introductions continued after that as needed.

2.3.3. Tilapia

The El Carao National Fish Culture Research Center (DIJEPESCA) at Comayagua, Honduras, produces 2.6 million tilapia fingerlings (*Oreochromis niloticus*) each year for aquaculture (Oseguera, 2016), charging approximately two U.S. cents per fingerling. Although *O. niloticus* is mainly herbivorous, feeding on algae covering underwater surfaces, it also eats mosquito larvae. Fingerlings from DIJEPESCA, 4–5 cm in length, were introduced to MV wells during August-September 2016. After that, tilapia were available to any household that wanted them for any larval habitat. MV subsequently set up its own small-scale tilapia production system to supply tilapia fingerlings to the community.

Although the use of *O. niloticus* might be questioned because it is an exotic species, originally from East Africa, *O. niloticus* is already well established in Honduras with commercial fisheries in lakes and aquaculture promoted by the government. Supplement 1 provides further detail about this issue.

A few MV households already had small native poeciliid fish captured from a nearby marsh or river in their water storage containers simply because they liked the fish, and additional native poeciliid fish were collected from ponds at El Carao, introducing them to a selection of wells and containers in MV to assess their potential for larval control. The species involved were the spottail killifish (*Heterandria bimaculata*), dogfish rivulus (*Rivulus tenuis*), mangrove molly (*Poecilia orri*), shortfin molly (*Poecilia mexicana*), largespot livebearer (*Poeciliopsis pleurospilus*), and knife livebearer (*Alfaro huberi*).

2.3.4. Larvicides

Larvicides were not at the core of the toolkit because they could be too expensive for large-scale use in communities such as MV and may also be difficult for untrained community residents to use properly. Nonetheless, environmentally friendly larvicides could be of use for special needs such as stopgap suppression of mosquito production when other methods fell short. The following larvicides were assessed for this role by applying them at label rates to a selection of various kinds of containers under normal conditions of use during the time early in the project when *Ae. aegypti* larvae were common.

Spinosad (Clarke Natular DT). An insect nerve toxin (Darriet et al., 2005) in slow-release tablets that kill all mosquito larvae for as long as

Fig. 1. Monte Verde, highlighted with red dots representing houses in the aerial photograph, is immediately south of Highway CA13 next to a bend in the highway known as "La Vuelta del Cura". The western part of Monte Verde has three rows of houses fronting on dirt roads. The eastern part (narrower and extending to Las Colinas subdivision) has two rows of houses on opposite sides of a dirt road. The large buildings to the north of Monte Verde are factories. The large greenish areas to the north are scrubland, and the large greenish areas to the south are marsh (dark green) and scrubland (light green).

there is spinosad in the water. Because spinosad decomposes within a day when exposed to sunlight, it does not accumulate in natural aquatic ecosystems.

BTI (Valent Biosciences Vectobac WDG and Summit BTI briquettes). A bacterial toxin from *Bacillus thuringiensis israelensis*, highly specific to mosquito larvae and harmless to other animals (Lacey, 2007; Boyce et al., 2013). Vectobac WDG is a granular formulation to be dissolved in water before application to larval habitats and kills larvae only at the time of application. Summit BTI briquettes are slow-release "doughnuts." BTI was known to be useful to introduce at the same time as copepods in order to kill larvae that were already in a container or well and too large to be eaten by copepods (Marten et al., 1993). The copepods could deal with all newly hatched larvae after that.

Pyriproxyfen (Sumitomo Sumilarv 0.5G). An insect growth hormone that persists for months (Invest and Lucas, 2008). Pyriproxyfen may suppress emergence of adult mosquitoes from the pupal stage even when it does not kill the larvae. Pyriproxyfen is harmless to vertebrates, though toxic to some arthropods besides mosquitoes and some mollusks (Devillers, 2020). Two formulations were tested: granular Sumilarv 0.5 G and a custom-made coating on small tiles.

2.4. Egg sinks and local elimination

Because a community's coverage of water storage containers with biological control and other methods will always be less than perfect, it is reasonable to ask whether local elimination is possible under real-world conditions. There is reason for optimism because treated containers can function as "egg sinks," which waste mosquito eggs and if numerous enough, drive down a mosquito population until it disappears (Marten, 1990a; Nam et al., 1998; Marten, 2012a). In fact, the transformation of larval habitat into egg sinks is more effective than getting rid of the habitat. The practical rule is simple: If at all possible, use larval habitat as a resource by transforming it into egg sinks with biological control or some other method. If transformation is not possible, get rid of it or replace it with something that can be transformed into an egg sink.

Because an egg sink can be more effective if biological control or a larvicide renders it more attractive for oviposition, and less effective if oviposition is repelled, a field experiment was conducted at MV with ovitraps to measure the attraction or repulsion of oviposition by different kinds of biological control and larvicides. The ovitraps were 473 mL black plastic cups containing water and brown paper around the inside of the cup. Nine different ovitraps, each containing water that was different with regard to biological control or larvicide treatment, were placed in close proximity to one another in the corner of a yard. Similar sets of nine ovitraps were placed at 15 houses. The papers were removed from the ovitraps after one week and the eggs counted.

The nine water treatments were: MV tap water with no chlorine (control); water from a *pila* with no biological control or larvicide (control); water containing copepods; water that had contained a turtle or tilapia; water containing Natular, Sumilarv, or Vectobac, or from a drum containing part of a BTI briquette, all at the label rate. The numbers of eggs from ovitraps with different treatments was compared using the Wilcoxin ranked sum test for paired comparisons.

Fig. 2. Water storage containers (from left to right): Cistern (plastic tank with bleach bottle on top and black cap for the small hole in the top); *pila* (cement tank); white bucket; blue plastic drum; and in the lower right corner a gray plastic tub that was a chemical container at a factory before it was scavenged and the top cut off. Clothes are washed on cement washboards like the one at the left side of the *pila*. The boy is a Monte Verde volunteer using a turkey baster to collect mosquito larvae in the drum.

2.5. Organizing community participation

In early 2016, Operation Blessing Honduras (OBH) proposed an *Ae. aegypti* control project to MV community leaders: the local *patronato* (community council), the priest and pastor of the Catholic and Evangelist churches, teachers at the primary school, and the management committee for MV's water supply system. After a positive response from community leaders and verbal approval from the Ministry of Health's regional office, OBH staff held community meetings to educate residents about the following topics and connections between them: *Ae. aegypti* mosquitoes (recognizing them and understanding their life cycle); *Ae. aegypti* larvae and their habitats; diseases transmitted by the mosquitoes (dengue, Zika, and chikungunya); how biological control and other methods could get rid of the mosquitoes and the diseases. Lessons and field projects about the same topics soon followed at MV's primary school.

A very important outcome of the community meetings was emergence of a core of approximately 20 members of the community who served as unpaid volunteers working with MV residents to develop details of the toolkit and put the toolkit into use throughout the community. Most of the volunteers were local housewives. A few were men or 12–15 year-old boys. The volunteers evolved after several years into a tight-knit team of one man and eight women. After that, several teenage children of volunteers, who started by helping their parents, became full-fledged volunteers.

OBH assigned two facilitators to MV from April 2016 to March 2019. During the last half of that period, one of the facilitators was the MV resident who managed MV's water system. With technical support from an American scientific advisor, the facilitators provided information about biological control and other methods to the volunteers and worked closely with the volunteers and the rest of the community to put the methods into use

The volunteers started by using biological control at their own homes to work out the details of how to do it. Their homes also served as demonstration sites for their neighbors. Then they visited every house in MV, inviting each family to participate in the program by selecting from the toolkit what it needed to eliminate mosquito production on its own premises. The volunteers did their visits in groups of two to four, so they would be taken more seriously than a single person and they could consult one another during a visit. They worked with each household to refine the details of adapting the biological control and other methods to local conditions, including the various ways that people used their water storage containers and the preferences that different families had for copepods, turtles, fish, or some other method. The goal was coverage of all *Ae. aegypti* larval habitat by one or more of the methods. If a family chose to employ more than one method for a particular type of larval habitat, the redundancy made prevention of *Ae. aegypti* production even more effective.

2.6. Baseline survey and monthly monitoring

A baseline survey covering 87% of the houses in MV was conducted in June 2016 to assess the number of water storage containers, wells, and other *Ae. aegypti* larval habitats in MV along with the number of larvae and pupae in those habitats. The survey was performed by approximately 85 people, MV's newly formed volunteers working side-by-side with a group of physicians and other health professionals that had previously participated in OBH "Mobile Brigade" medical clinics in other communities. Participants received two days of training on survey procedures, including practice at visually estimating the number of larvae or pupae without removing them from a container. Then every water storage container, well, or object that could collect rainwater was recorded along with an estimate of the number of larvae and pupae. Information was also collected about family size, pregnancy, and sanitation facilities.

Immediately after the baseline survey, there was an ovitrap survey with the 473 mL black plastic cups placed in the yard at every house to collect eggs for one week. Ovitraps were also placed along a perimeter around MV, several hundred meters outside the edges of the community, to assess the presence of adult *Ae. aegypti* in the surrounding area. This perimeter included the adjacent scrubland and wetland, and the edge of the road bordering the industrial area immediately to the north, but not Las Colinas, which was a gated community to which MV volunteers did not have access at that time.

The volunteers began monthly monitoring in October 2016, visiting approximately 95% of MV houses each month to check whether every well and container was under effective control and assist the families to improve as needed. At each visit, volunteers filled out a data sheet specifying the number of each kind of water storage container on the property, how many were dry, how many contained water, and whether they contained larvae or pupae. The main equipment was a clipboard, a turkey baster to sample larvae from containers or wells for identification, and a flashlight to see larvae in dark wells and containers. Approximately 2100 containers were inspected each month. Beginning

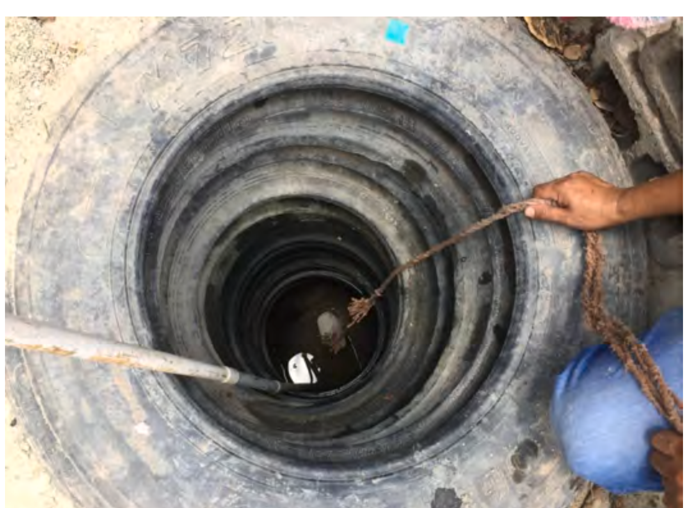


Fig. 3. Household well with tires providing structural support. The white tube on the left leads to a pump that transports water from the well to a storage container in the yard. The rope is attached to a bucket for removing water from the well. The tires above the water level in the well often contain water that provides *Ae. aegypti* larval habitat.

Fig. 4. Red-eared slider turtle (*Trachemys scripta elegans*). Photo by Chris Brown.

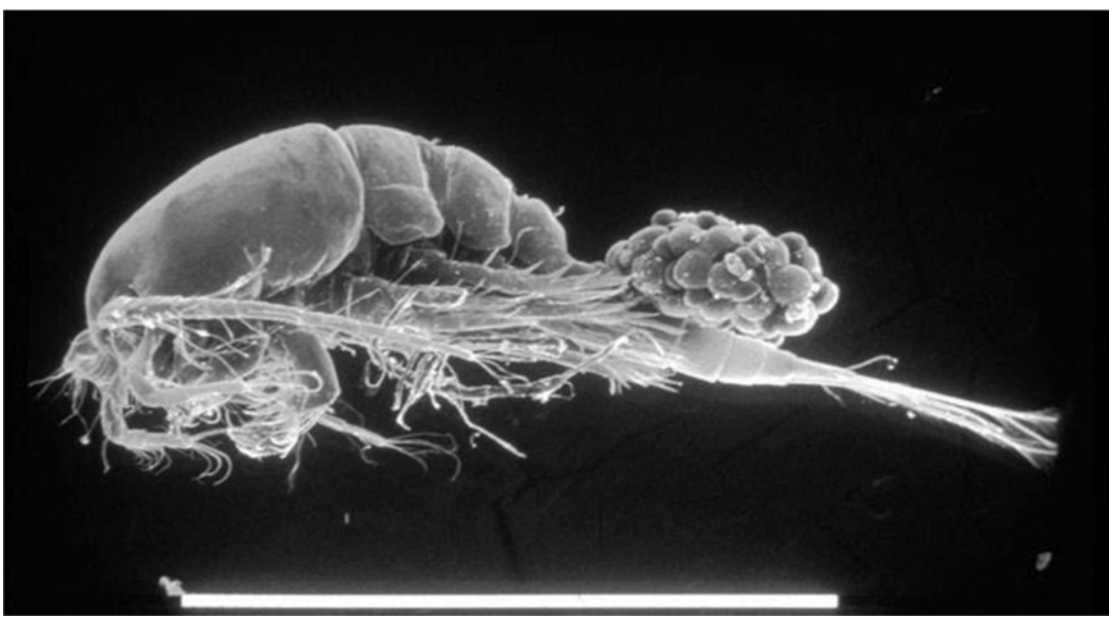


Fig. 5. Copepods are voracious predators of newly hatched mosquito larvae and other aquatic animals up to twice their size. The bar at the bottom of the photo represents one millimeter. An egg sac is visible above the tail. Photo by Michael Brown.

in June 2018, counts of mosquito larvae and pupae seen in water storage containers were added to the monthly monitoring record. The monthly monitoring also included questions to every family about febrile illness during the previous month and symptoms including headache, body pain, joint pain, and rash, which are known to be characteristic of dengue, Zika, and chikungunya (Elson et al., 2020).

Each month, a Breteau Index (the number of containers with larvae or pupae per 100 houses) was calculated from the monthly monitoring data for MV as a whole, and a Container Index (the percentage of containers with water that also had larvae or pupae) was calculated for each major type of larval habitat. While these indices do not have a reliable relationship with disease transmission (Bowman et al., 2014), they were useful for assessing the progress of container coverage. It was not necessary to calculate confidence limits for these indices because the confidence limits were very small when sampling a large percentage of a finite population. OBH continued to assist with data processing and technical support after the intensive facilitation ended in 2019.

Beginning in March 2020, the government imposed a national COVID lockdown that allowed people to leave their homes only one day a week and prohibited visits to other households. The volunteers discontinued monthly monitoring. Although the government might have allowed them to continue as essential workers, the volunteers feared MV residents would not welcome their visits, and they did not want to risk carrying COVID back to elderly people in their own households. The lockdown relaxed slightly in July, by which time people had adapted to the extreme circumstances. Monthly monitoring resumed, and the volunteers found that most households welcomed them. The record of illnesses was not resumed because families did not want to divulge information about household illnesses for fear of COVID stigma among neighbors or possible government action such as removal to a COVID isolation camp.

Ovitrap surveys were conducted several times a year with an ovitrap at nearly every house for a week. Adult mosquitoes were raised from samples of ovitrap eggs, as well as samples of larvae collected from wells and water storage containers during the monthly monitoring, to ascertain whether they were *Ae. aegypti, Ae. albopictus*, or other species. Ten BG sentinel traps collected adult mosquitoes at a random selection of houses during June-September 2017, rotating to different houses each week. BG traps were not used during 2018–2020, but five returned to continuous use beginning in March 2021. Mosquito counts were based on mosquito identification information in Clark-Gil and Darsie (1983), Pecor et al. (2002), Rueda (2004), and Brown et al. (2009).

Although ovitraps and BG traps were used to assess the impact of the program on the adult *Ae. aegypti* population, caution was necessary when interpreting changes in trap captures from one time to another. The number of eggs in ovitraps can be a consequence not only of mosquito numbers but also changes from one time to another in weather, the attractiveness of the ovitrap paper, and competition for oviposition from larval habitat in the area around the trap. The effectiveness of a BG trap declines continuously after the lure cartridge has been replaced, and the mosquito catch at different times can depend upon how the traps are operated. A field study comparing BG traps and ovitraps found no correlation of results from the two methods (Lourenço-de-Oliveira et al., 2008).

In August 2019, the project conducted a baseline survey in San Antonio, a community similar to MV and located 13 km north of MV at 15.647 N latitude and 87.949 W longitude in Choloma municipality. The survey included the same information as MV's baseline survey in June 2016. As San Antonio did not have an *Ae. aegypti* control program, a plan to assist San Antonio to establish a program similar to MV's was begun in 2019, delayed by COVID lockdown in 2020, and abandoned in 2021 because of numerous gang murders there.

In March 2020, a baseline survey of larval habitats was conducted in Las Colinas, the residential area immediately adjacent to MV. The survey covered 18% of the houses and included the same information as the other baseline surveys, except no information about the families or illnesses. Although Las Colinas' continuous water supply and screened houses made it very different from MV, Las Colinas was of particular interest because it could be a source of mosquitoes entering MV.

3. Results

3.1. Baseline survey

Table 1 lists the types of *Ae. aegypti* larval habitats and numbers of larvae and pupae seen in MV's baseline survey at the beginning of the project. A total of 57,148 mosquito larvae and 22,984 pupae were counted in all larval habitats. More than 95% of the larvae in all larval habitats except wells were *Ae. aegypti*, the rest being *Ae. albopictus, Culex quinquefasciatus*, or other *Culex species*.

Wells. 57% of the 333 households had wells. More than half of all larvae and pupae observed in Monte Verde were in the wells, which contained large numbers of *Ae. aegypti* and *Cx. quinquefasciatus*. Hundreds of adult mosquitoes typically surged out of a well whenever the cover was lifted.

Pilas. 77% of households had one or more *pilas*, which are cement tanks with a faucet and drain and a typical water storage capacity of 800–1000 L. *Pilas* store water for a variety of uses, including washing clothes on a concrete washboard immediately adjacent to the *pila* (Fig. 2). In total, more *Ae. aegypti* larvae were recorded in *pilas* than in any other kind of water storage container in the baseline survey.

Drums. 67% of households had one or more 200 L plastic drums for water storage. Although the total number of larvae in drums was less than half as much as the number of larvae in *pilas*, the total number of pupae, which more directly reflects mosquito production, was nearly the same in drums and *pilas*.

Cisterns. 12% of households had 1000 L plastic tanks called *cisternas*. Most cisterns were enclosed except for a small opening for a screw cap at the top (Fig. 2). The top side of a few cisterns was cut away, making them completely open at the top. There were only 36 cisterns in MV, but there were more pupae per cistern than any other type of water storage container.

Buckets. 90% of households had 20 L plastic buckets for water storage. The number of buckets was greater than the number of all other water storage containers combined. Buckets were particularly numerous at households that could not afford a *pila* or drum, some families having as many as 20 buckets. Buckets were smaller than *pilas* or drums, and not a stable habitat for mosquito larvae, so each bucket contained far fewer larvae and pupae than other water storage containers. The mosquito production from such a large number of buckets was nonetheless significant.

Tires. 17% of the wells were lined with used truck tires for structural stability, about eight tires in each well (Fig. 3). Tires above the surface of the well water usually provided ideal *Ae. aegypti* larval habitat because they contained water from when the tires were submerged by a higher water level.

Bottles. 72% of households had bottles lying around as trash or piled up for recycling, and the bottles could collect rainwater. Only 0.5% of the 6487 bottles contained *Ae. aegypti* larvae, but the 422 pupae in those bottles reflected noteworthy mosquito production.

Other. Animal dishes and trash that collected rainwater had a total of 0.4% of the larvae and 0.1% of the pupae observed in the survey.

3.2. Surveys at other locations

Results from the August 2019 baseline survey in San Antonio were similar to MV's baseline survey in June 2016 (Table 2). The Breteaux Index, the total number of larvae and pupae, and the number of eggs in ovitraps were all very high in both surveys. Most wells at San Antonio were dry at that time, and wells with water contained no larvae. The bulk of *Ae. aegypti* production in San Antonio was from *pilas*, and the rest from drums. About 700 bottles and pieces of trash that collected rainwater contained one or two larvae, but judging from pupae, *Ae. aegypti* production was nil.

The March 2020 baseline survey in upscale Las Colinas revealed far fewer open water storage containers than MV and correspondingly few *Ae. aegypti* production sites compared to MV's 2016 baseline survey (Table 2). Las Colinas' Breteaux Index was less than MV's 2016 baseline Breteaux Index, but more than four times MV's 2020 Breteaux Index at the time of the Las Colinas survey. Although many houses in Las Colinas had washing machines, nearly all houses had a *pila* for laundry, and the *pilas* accounted for virtually all *Ae. aegypti* larvae and pupae. There were no wells in Las Colinas, all cisterns were sealed, most drums were covered, and buckets, bottles, and trash contained no larvae.

3.3. Attraction or repulsion of oviposition by biological control and larvicides

Table 3 compares the number of eggs deposited in ovitraps containing water under different treatments with respect to turtles, copepods, tilapia, and larvicides. While a more thorough study would be necessary for precise comparison, comparison of the treatments with the tap water control shows unequivocally that biological control and larvicides did not repel oviposition. In fact, if any of the methods had an effect, it was attraction. Tilapia attracted more than twice as many eggs as tap water (one-sided Wilcoxin test, $P = 0.001$), as did copepods ($P = 0.025$). Turtles attracted 72% more eggs than tap water ($P = 0.046$). Two of the larvicides, Sumilarv ($P = 0.07$) and BTI briquettes ($P = 0.02$), also attracted more eggs than tap water, though the Wilcoxin test found no significant difference between these two larvicides and "*pila* water" that had no biological control or larvicide. The Wilcoxin test showed no significant difference for the other two larvicides (Vectobac and

Table 1
Results of the Monte Verde baseline survey in June 2016.[1]

Larval habitat	Number of houses[2]	Number of containers[3]	Number of positive containers[4]	Percent of containers positive	Number of larvae[5]	Percent of all larvae	Number of pupae[5]	Percent of all pupae	Number of larvae and pupae per container[6]
Wells[7]	167	170	48	28%	32,050	56%	16,460	71%	285
Pilas	223	245	100	41%	13,011	23%	2214	10%	62.1
Drums	196	409	85	21%	5133	9%	2342	10%	18.3
Cisterns	32	36	8	22%	2313	4%	575	3%	80.2
Buckets	263	1986	101	5%	2137	4%	741	3%	1.5
Tires[7]	28	29	9	31%	2070	3%	200	1%	78.3
Bottles	209	6847	34	0.5%	258	0.5%	422	2%	0.1
Other[8]	ND	4348	16	0.3%	176	0.4%	30	0.1%	0.05
TOTAL		13,710	381		57,148	100%	22,984	100%	

[1]The number of houses sampled was 291 out of Monte Verde's 333 houses.
[2]Number of houses with the specified type of larval habitat.
[3]"Container" in this table refers to water storage containers, wells, and objects that collected rainwater.
[4]Number of containers with larvae or pupae in the specified type of larval habitat.
[5]Total number of larvae or pupae in all containers of the specified type of larval habitat.
[6]Average number of larvae and pupae for all containers of the specified type of larval habitat (including containers without larvae or pupae to calculate the average).
[7]Based on an August 2016 survey of wells and tires, instead of the June 2016 survey which recorded 12,125 larvae and 1418 pupae in wells and 266 larvae and 57 pupae in tires. Counts in wells included *Culex* as well as *Aedes*.
[8]Flower pots, animal dishes, garden implements, and trash that collected rainwater.

Table 2
Comparison of survey results in San Antonio and Las Colinas with Monte Verde.

	Monte Verde (baseline) June 2016	San Antonio[1] (baseline) August 2019	Monte Verde August 2019	Las Colinas[2] (baseline) March 2020	Monte Verde March 2020
Total houses	333	401	341	650	363
Houses sampled	291	323	283	115	330
Positive containers[3]	381	1519	16	25	17
Breteau Index	84	81[4]	4	22	5
Wells (% positive)	28%	0%	0%	—	0%
Pilas (% positive)	41%	46%	3%	12%	3%
Drums (% positive)	21%	33%	1%	0%	2%
Cisterns (% positive)	22%	9%	0%	0%	0%
Buckets (% positive)	5%	9%	0%	0%	0%
Other (% positive)	0.3%	10%	0%	0%	0%
Total larvae	57,148	52,057	376	1363	405
Total pupae	22,984	4908	15	80	17
Ovitraps	244	259	ND	68	ND
Percent positive[5]	94±2%	92±2%	ND	83±4%	ND
Eggs/ovitrap[6]	73±5	47±3	ND	15±2	ND
Dengue cases[7]	39	37	0	ND	0

[1]San Antonio: 418 *pilas*, 193 drums, 69 cisterns, 478 buckets, 12,202 "other" (157 tires, 4747 bottles, 7176 pieces of trash that collected rainwater, 122 animal dishes).
[2]Las Colinas: 112 *pilas*, 45 drums (mostly covered), 70 cisterns (sealed or covered), 122 buckets, 23 tires (not associated with wells).
[3]Number of containers with larvae or pupae.
[4]The Breteau Index for San Antonio was 81 when only *pilas*, drums, and cisterns (which accounted for nearly all larvae and pupae) are included in the tabulation. However, if bottles and trash (which were very numerous but with a large number containing only one larva) are also included, the Breteaux Index for all containers was 470.
[5]Percentage of ovitraps containing *Ae. aegypti* eggs (±standard error).
[6]Average number of eggs/ovitrap including ovitraps with no eggs (±standard error).
[7]Febrile illness during the previous month with distinctive dengue symptoms.

Natular) compared to tap water.

3.4. Refining and implementing the toolkit

It took about a year for the MV volunteers and residents to work out the details of how to use the biological control and other methods. The following is a summary of what happened with the various methods in each kind of larval habitat (Table 4):

Wells. While all three forms of biological control – copepods, turtles, and tilapia – were virtually 100% effective at eliminating *Aedes* and *Culex* larvae in wells, copepods emerged as the method of choice because it took no effort to maintain them. Copepods were abundant in every well in MV within a few months of the copepod introductions during December 2016 to February 2017, and there was no known *Ae. aegypti* production from wells after that, though *Culex* larvae continued sporadically in wells for another year. If a well dried out, the copepods could survive in the sediment at the bottom as long as it contained some moisture. The copepod population usually rebounded as soon as a well had water again. BTI briquettes, which killed larvae reliably for two or three weeks, became the method of choice for immediately eliminating larvae if they appeared in a well for any reason.

Several wells were severely polluted by use as urinals, and elimination of mosquito production by copepods broke down in those wells. *M. longisetus* and *Culex* larvae were very abundant because the water contained a high density of microbial food for both mosquito larvae and copepods, and the copepods were apparently eating protozoa instead of

Table 3
Average number of *Ae. aegypti* eggs laid in ovitraps (± standard error) to test oviposition attraction or repulsion by turtles, copepods, tilapia, and larvicides.

Treatment	Number of eggs
Tap water[1]	7.1±3.2
Pila water[1]	11.1±2.9
Turtles	12.1±4.0
Copepods	15.1±4.4
Tilapia	19.5±7.1
Natular	8.6±2.5
Sumilarv	12.9±4.0
BTI briquettes	19.1±7.2
Vectobac	5.1±1.8

[1]Controls.

mosquito larvae. As these wells were no longer providing water for household use, they were put out of action for mosquito production by sealing them over with concrete.

When native poeciilid fish were introduced to wells, they did not reliably establish the populations necessary to prevent mosquito production. Consequently, native fish did not become part of the toolkit. Some households chose to have turtles or tilapia in their well, and they were completely compatible with copepods. Copepods were too small for turtles to prey upon them, and although tilapia fingerlings would eat all the copepods in a small container, tilapia had no noticeable impact on copepods in a well. It should be noted, however, that tilapia and turtles could not be together in the same well because tilapia harassed turtles by nipping at them.

It was necessary to remove water gently from wells containing tilapia, because throwing a bucket roughly into a well appeared to harm the fish. Some households raised tilapia for food at the same time they used them for mosquito control, but other families did not eat their tilapia because they considered them to be family pets.

Pilas and drums. Some families used *Untadita* to scrub *Ae. aegypti* eggs off their *pilas* or drums at least once a week. Many covered their plastic drums with a tight-fitting lid to keep mosquitoes out. It was not practical to keep copepods or fish in most *pilas* and drums that were in active use, because many housewives cleaned their *pilas* and drums as frequently as once a week and *pilas* were used for laundry. It was too much trouble to remove copepods before cleaning so they could be returned to the *pila* or drum afterwards, and laundry chemicals could harm copepods and fish. Turtles emerged as the most common method for *pilas* and also drums lacking lids. Turtles thrived in *pilas*, apparently unperturbed by laundry activities, and they were easily removed and held on the side whenever a *pila* or drum was cleaned. It was typical for a family to keep its turtle in a *pila* and move it for a short time to any other container where larvae were discovered. The impact of turtles and their feces on water quality was not an issue for the great majority of households. There was no visible effect on the water. The few households that had reservations about turtles could choose another method.

When turtles were first introduced to MV, there was a problem of theft by boys who wanted to collect turtles, but the problem faded away within a few months as the novelty wore off. Another problem at the beginning was turtles escaping because people were not accustomed to keeping track of them. Sometimes turtles went "over the top" of a *pila* when the water overflowed after a housewife left the faucet running unattended. Some households dealt with this problem by drilling a small hole in the *pila* a few inches below the top, so water could not reach the top. Other households simply developed the habit of watching more carefully. A family that lost its turtle was offered a replacement turtle after pledging to care for the turtle correctly.

Another problem during the first year was turtle mortality, which was particularly high during the first winter. Some turtles with signs of malnutrition such as a thin carapace died when there was cold rainwater during storms from the north. Survival improved dramatically when families established a routine of feeding a variety of kitchen scraps such as tortilla, rice, cabbage, lettuce, cucumber, oats, and banana to their turtles. A few families chose commercial turtle food. Turtle losses due to escape or death were near zero after the first year. Because *T. scripta elegans* is known to be more carnivorous when small and herbivorous when large, it was uncertain how well the turtles would function as predators of mosquito larvae when the turtles grew larger. By 2020, the turtles ranged up to 18 cm ventral carapace length, and they continued to eat all available larvae. There was never a salmonella problem with the turtles.

Although copepods were not suitable for *pilas* or drums in active use, they were ideal for these containers when not in active use and collecting rainwater. Without copepods, unused *pilas* or drums could produce large numbers of mosquitoes, particularly if they contained fallen leaves that decomposed to provide food for mosquito larvae. A few families kept tilapia or local fish in their *pilas* or drums, and the fish were generally effective at preventing mosquito production.

During the testing of larvicides, Natular was popular among residents because an unobtrusive tablet attached to the side of a *pila* or drum in a simple holder provided 100% larval mortality for about three weeks. However, Natular was not suitable for containers with copepods because it killed them. BTI was useful for those containers because it provided 100% larval mortality and inflicted no harm on copepods or fish.

Cisterns. Some households were able to seal the opening at the top of their cistern so mosquitoes could not enter to lay eggs. However, many households did not do this well enough to prevent mosquito production, so copepods were maintained in all cisterns. It was not practical to use turtles in cisterns with limited access through a small hole at the top, but a small hole was a benefit for copepods because it meant those cisterns were seldom cleaned. They often contained algae, which made them ideal habitat for mosquito larvae and copepods, and there was no mosquito production as long as copepods were there. It was necessary to restock cisterns with copepods whenever the cisterns were cleaned. One family had local fish in a cistern with the top removed.

Buckets. Buckets were not practical for long-term management with biological control, because copepods and fish could be lost when water was poured out of buckets, and turtles could escape. However, people could get rid of mosquito larvae in a bucket by simply pouring the bucket water (containing larvae) into a *pila* that contained a turtle. When not in use, buckets were stored upside down or under cover where they would not collect rainwater.

Bucket management also included a community campaign to get rid of unnecessary buckets, replacing them with cisterns, *pilas*, or drums, all of which could be managed with biological control or some other method. The total number of buckets in MV was reduced from

Table 4
Applicability of each method to each kind of larval habitat.[1]

	Pilas	Drums	Buckets	Cisterns	Wells	Tires
Turtles	++	+	–	–	+	–
Copepods	+	+	–	++	++	++
Tilapia	+	+	–	–	+	–
Larvicides	+	+	–	+	+	+
Untadita[2]	+	+	–	–	–	–
Sealing containers[3]	–	++	–	+	–	–
Elimination/ replacement[4]	–	–	+	–	–	–
Nets[5]	+	+	–	–	–	–

[1]Subjective assessment based on Monte Verde's experience with each method.
[2]Scrubbing mosquito eggs from sides of containers.
[3]Covering a container to exclude mosquito access.
[4]Getting rid of unnecessary containers or replacing them with more manageable containers.
[5]Removing mosquito larvae with an aquarium net.
+ Effective method for that kind of container.
++ Preferred method for that kind of container.
– Not a suitable method for that kind of container.

approximately 2000 buckets when the project began to 1147 buckets a year later. This was achieved in part by a community subsidy to build a *pila* or purchase a cistern if a household got rid of unnecessary buckets.

Tires. Copepods proved to be the most effective way to prevent mosquito production in the tires lining wells for structural support. If a well contained copepods, some of the copepods were usually in tires exposed by a drop in the well's water level. However, copepods could be lost from tires if the tires dried out completely, requiring restocking if the tires subsequently contained water but not copepods. It proved practical to keep copepods in tires by routinely introducing them every few months to all tires above the well's water level.

Pyriproxyfen-coated tiles in tires and pyriproxyfen with Sumilarv 0.5 G in water storage containers did not reliably prevent mosquito production even though they worked well in simple laboratory containers. As larvae could be present even when mosquito production was suppressed (or not suppressed) by pyriproxyfen, it was difficult to know whether or not the pyriproxyfen was actually working. Because larvae and pupae were often seen in water storage containers treated with Sumilarv, MV residents did not trust it, and Sumilarv was not adopted as part of the toolkit.

3.5. Achieving full container coverage (problem houses)

By the beginning of 2018, the great majority of households were on board with the goal to manage every container so it would not produce *Ae. aegypti*. However, there were some "problem houses" that continued to have *Ae. aegypti* larvae month after month, frequently in several containers at the same house. Eliminating the mosquito production at these houses became a priority during 2018.

Rooming houses. There were 18 rooming houses in MV, single-story buildings with a corridor running from the front of the house to behind it, and single rooms on each side of the corridor. Although an entire family might occupy one of the rooms, the occupants were generally transient, and there was no coherent family structure for a rooming house as a whole to provide proper management of larval habitats, including caring for turtles or fish in *pilas* and drums. The most effective management for rooming houses was routine larvicide application by a volunteer.

Closed and abandoned houses. When the project began, the occupants of 32 houses were never or seldom seen. Many of these houses had locked gates and walls that made it difficult to see the yard. Because volunteers considered it inappropriate, and possibly dangerous, to enter a property without permission, these houses not only did not participate in the program, but there was no information about whether water storage containers or wells on the property were producing mosquitoes. After considerable effort, the volunteers established contact with all the owners, and these people were generally cooperative.

Chose not to participate. Three households declined to participate at the beginning of the program. That number eventually increased to eight households. While these households were not included in the monthly monitoring or provided turtles, copepods, or tilapia, they pledged to manage their water storage containers with *Untadita* or other appropriate measures, and they generally did so.

3.6. Decline of Aedes aegypti production

Supplement 2 presents the original data for the more than 100,000 container inspections during the monthly monitoring from 2016 to 2021. The number of water storage containers and wells positive for *Ae. aegypti* larvae declined substantially during 2016–2017, though there were two major lapses in the decline during 2017 (Figs. 6-7,Table 5). While there were numerous challenges and even some serious setbacks during the first 18 months of the project, the entire system was working smoothly enough by February 2018 for the number of containers with mosquito larvae to decline month after month to only 3 positive containers and 25 larvae in all of the monitored containers in MV by December 2018 (Figs. 6-8). After that, and except for several months during the lapse in monthly monitoring due to COVID lockdown in 2020, the total number of positive containers ranged between 5 and 35 (Fig. 6), and the total number of larvae ranged between 0.1% and 2.4% of the number in the baseline survey at the beginning of the project (Fig. 8). The great majority of larvae after December 2018 were in *pilas* with a lesser number in drums and occasionally cisterns. A count of mosquito species in positive water storage containers during 2021 revealed that the larvae were exclusively *Ae. aegypti* in 67% of the positive containers, only *Cx. quinquefasciatus* or another *Culex* species in 11% of the containers, and a mixture of *Ae. aegypti* and *Culex* in 22%.

The total number of mosquito pupae observed in all known *Ae. aegypti* larval habitats in MV declined to zero by September 2018 and continued to be zero during the approximately 20,000 container inspections over the 10 months until June 2019 (Fig. 9). No pupae were encountered during 30% of the months from July 2019 to December 2021, and when pupae were observed, they were almost always in *pilas*, ranging from 0.02% to 0.6% of the number in the baseline survey. It was possible to have no pupae in *pilas* and drums despite the occasional presence of larvae, because turtles and tilapia ignored I/II instar larvae but always consumed the larvae when they were larger.

In ovitraps (Table 6), there was a progressive decline from 73 eggs/trap in the June 2016 baseline survey to 6.5 in March 2018. Eggs/trap fluctuated between 6.7 and 14.3 during the three years after that (except for an increase to 32 eggs/trap during the COVID lockdown). *Ae. aegypti* captures in BG traps averaged 3.99 mosquitoes/trap-week during June-September 2017 and 0.74 mosquitoes/trap-week during the same months in 2021 (Table 7). *Ae. albopictus* was not seen in BG traps in 2017, but two adult *Ae. albopictus* were captured in 2021. *Cx. quinquefasciatus* and other *Culex* were common in the BG traps. *Coquillettidia* was also common, and along with *Mansonia, Uranotaenia*, and *Anopheles* captured in smaller numbers, probably came from the nearby wetland. From 2017 to 2021, there was a 91% reduction in the ratio of *Ae. aegypti* to other kinds of mosquitoes in BG traps (Table 7).

3.7. Results from different phases of the project

Ae. aegypti production in the different larval habitats passed through distinct phases that were instructive with regard to developing the toolkit, putting it fully into use, and sustaining the program. The depiction of each phase presented below shows how Breteaux Indices (BI) in Fig. 6, Container Indices (CI) in Fig. 7 and Table 5, and larval and pupal counts in Figs. 8-9 behaved over the course of the study as toolkit details were worked out and social and climatic disturbances disrupted the monthly monitoring routine and other elements of the control program from time to time.

August–December 2016. Initial deployment of turtles, copepods, and tilapia. The BI dropped from 173 in September 2016 to 35 by December. Initial use of turtles in *pilas* and drums, tilapia in cisterns and wells, and bucket management reduced CI in all of these habitats from about 45% to 10%−15%. Larvae increased in cisterns and wells at the end of this period as tilapia disappeared due to inadequate care. The CI for tires lining wells stayed at 40%−50% because pyriproxyfen-coated tiles failed to eliminate mosquito production reliably.

January–June 2017. Maintaining the gains while reorganizing and refining the use of the toolkit. The BI did not improve, fluctuating in a range of 22–42 while the volunteers and their neighbors were working out the details of maintaining turtles, copepods and tilapia in the containers. CI for *pilas*, drums, and cisterns stayed about the same during this period (20%−25%), buckets were less (9%−15%), and mosquito larvae in wells and tires declined to 5%.

July 2017–January 2018. Lapse, recovery, and lapse again. The BI increased during June-August, the rainy season period that *Ae. aegypti* usually shows a surge, and the surge was exacerbated by a slackening of project effort during that time. The CI for *pilas*, drums, cisterns, wells, and tires increased to almost what they were at the beginning of the

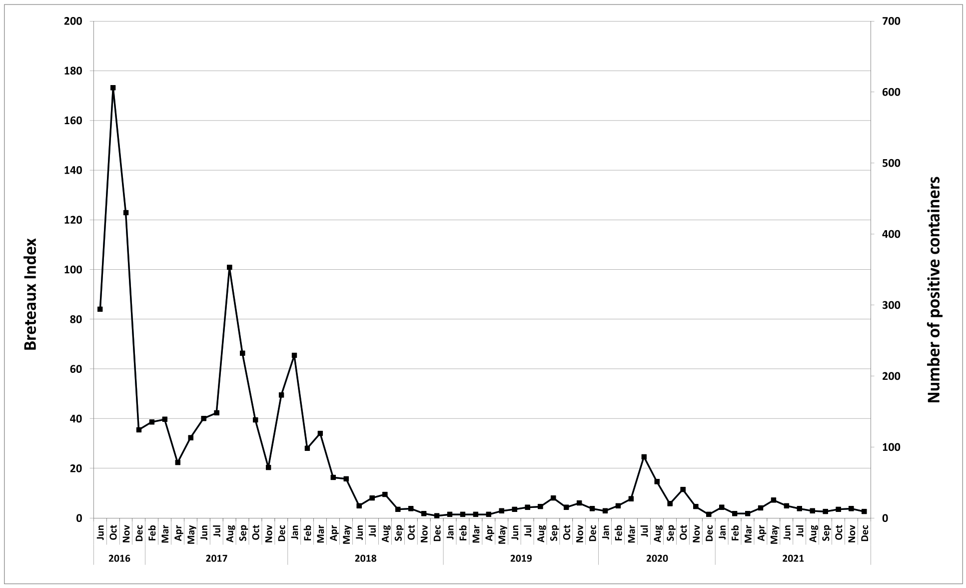


Fig. 6. Breteau Index (total number of water storage containers and wells positive for mosquito larvae or pupae per 100 houses) during each month's monitoring from the beginning of the project to December 2021.

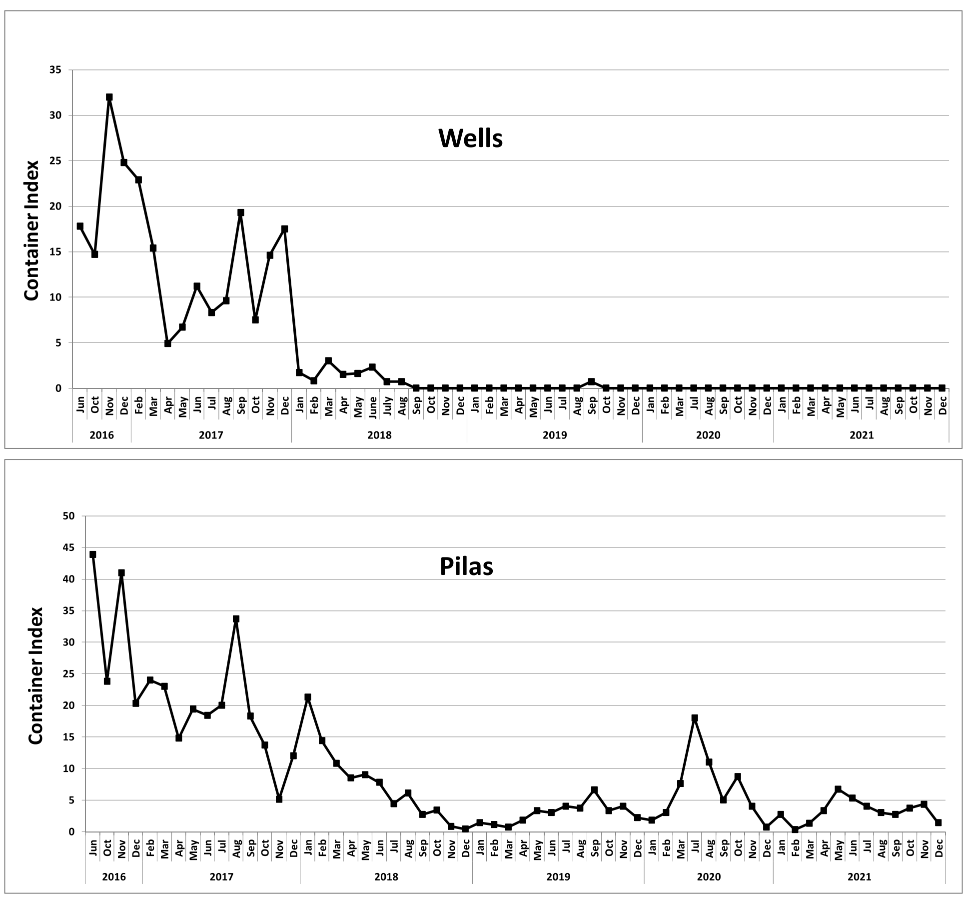


Fig. 7. Container Index (percentage of each kind of container with water that also had mosquito larvae or pupae) for *pilas*, drums, buckets, cisterns, wells, and tires lining wells during monthly monitoring. The Container Index for tires was the percentage of wells with any larvae or pupae in tires above the water line.

project, but buckets increased only slightly. The increases revealed how quickly the mosquito population could rebound with a drop in container coverage. The project quickly reestablished coverage in September, and suppression of mosquito production was rapid because the details of "how to do it" and responsibilities of volunteers for household monitoring had been largely worked out by that time. *Ae. aegypti* disappeared from wells and tires, and after that only *Culex* larvae were seen in wells, though *Ae. aegypti* larvae were sometimes in tires. By November, the BI declined to 20, and the CI for *pilas*, drums, cisterns, and tires dropped to 5% or less. Then there was a second lapse due to

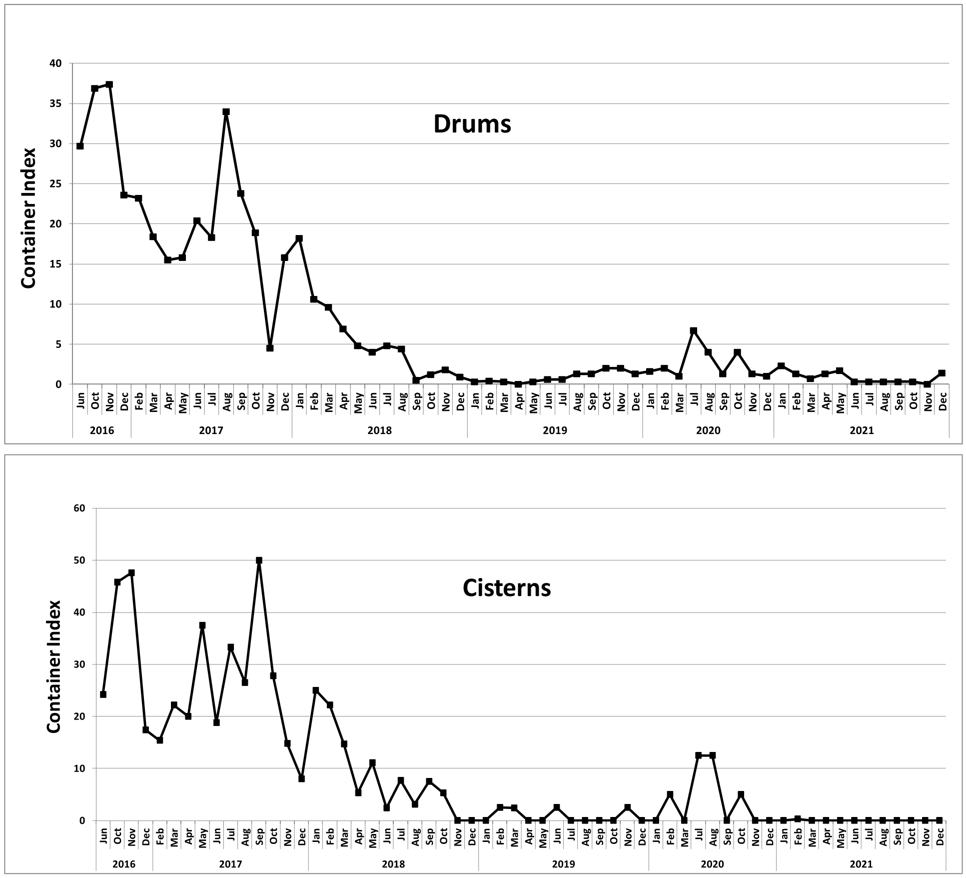


Fig. 7. (*continued*).

protests following the national presidential election in November 2017. Chaos throughout the region crippled transport and commerce, increased risks to personal safety, prevented the project facilitator from going to MV, and generally disrupted activities within the community. The BI increased to 65 in January, and the CI for *pilas*, drums, buckets, and tires increased to more than 30%.

February–August 2018. Steady decline in Ae. aegypti production. Life returned to normal by February 2018, and MV returned quickly to the high level of container coverage it had before the political turmoil. "Problem houses" received intensive though patient attention from the volunteers during this period, gradually reducing the few remaining shortfalls in container coverage. By June the BI was only 5, followed by a slight rainy season increase in August. CI for *pilas*, drums, and cisterns declined to about 5%, buckets and wells (only *Culex*) to 1%, and tires to zero.

September 2018–April 2019. No* known *Ae. aegypti production. The few problem houses that remained were finally brought under effective coverage. The BI declined to 0.9 in December and continued almost that low until April. Less than 200 *Ae. aegypti* larvae, all of them first or second instars, were observed in all of MV each month from November to April. The CI for *pilas* was less than 1% after October, and drums declined to zero by April. There were no larvae in cisterns, wells, and tires, and no pupae were seen in any container during the entire eight months of this period.

May 2019–March 2020. Maintaining container coverage despite more disruptions. There was a slight increase in larvae compared to the previous period, fluctuating between 200 and 400 larvae in all of MV each month. BI increased to 4 by August, the usual time of a seasonal increase, but BI did not decline to its previous level afterwards. Instead, it ranged between 3 and 8, in large part due to two disruptions: first, the project was without larvicides for problem houses for many months, due to delays in shipping a replacement supply from abroad and clearing Honduran customs; second, many household wells went dry due to drought, and the delivery interval from MV's water system increased to 12–15 days because of equipment failures. From May to December, families often used every drop of water in their storage containers before the next water delivery, a change that required adjustments in container use and management procedures for turtles, copepods, and tilapia in containers that were at times completely dry. Most of the larvae were in *pilas* (CI fluctuating between 3% and 7%) and drums (CI = 0.3%−2%). There were almost no instances of larvae in buckets or cisterns, and no larvae in wells or tires during this period. No *Ae. aegypti* pupae were observed in MV during May-November 2019, but an average of 22 pupae/month was seen from December 2019 to March 2020.

July–December 2020. Suspending and resuming monthly monitoring during COVID. Monthly monitoring was suspended during April-June 2020. By July, container management had deteriorated at many households, particularly with regard to moving turtles among containers where they were needed, and the rooming houses had been completely neglected for four months. BI had increased to 25, CI to 18% for *pilas*, 7% for drums, 13% for cisterns, and 1% for buckets (a total of 2610 larvae). Monthly monitoring resumed in July. By December, BI decreased to 1.4, CI in *pilas* and drums to 1% (80 larvae), and there were no larvae in all other containers. Despite heavy flood damage from two hurricanes (Eta and Iota) in November, MV maintained monthly monitoring without interruption.

January–December 2021. Continuing the routine with a challenge from resident turnover. After December 2020, BI and all the CI remained at the same low level that had prevailed during the months before COVID lockdown. Wells and tires, which contained copepods unaffected by COVID disruption, continued without larvae during 2021. However, a new challenge came in 2021 with an unusually high

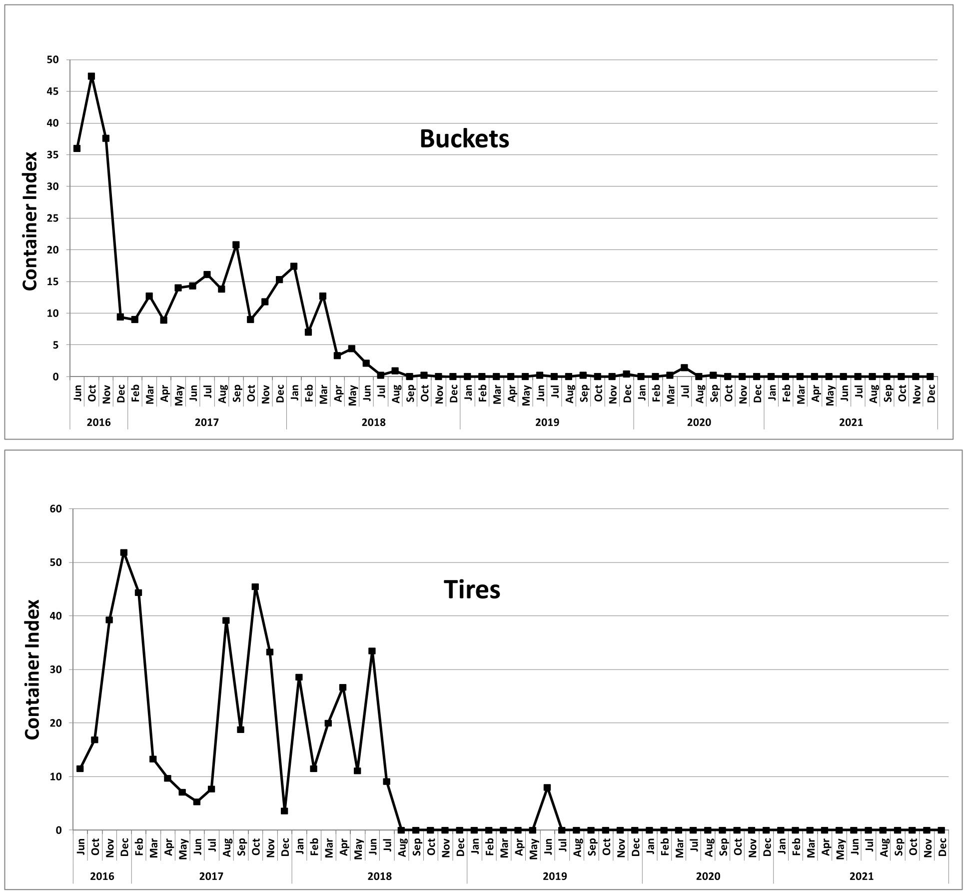


Fig. 7. (*continued*).

turnover in MV's resident population. Twenty-seven long-established households left MV after suffering the ravages of two hurricanes and unemployment due to COVID-induced factory layoffs during 2020. The vacated houses were occupied by families from outside MV, who knew nothing about the *Ae. aegypti* control program and lacked the community commitment of long-term residents. The volunteers responded by focusing their attention on educating the new residents and gained their full cooperation within a few months.

4. Discussion

The project's proof of concept was successful. The toolkit worked, and the community implemented it effectively and sustainably. The key elements of the project strategy – a multi-method toolkit with biological control at the core, larvicides in a limited but crucial role, targeting complete container coverage, adapting different methods to different larval habitats, transforming larval habitat into egg sinks, committed community volunteers doing house-to-house monthly monitoring, recognizing and accommodating the various ways that people use their water storage containers and respecting family preferences for different control methods – proved effective and essential for success. As citizen scientists fleshing out and administering the toolkit, the volunteers addressed the central research question "What are the details that will enable this strategy to function effectively?" Many of those details are in the results presented above.

The virtual elimination of *Ae. aegypti* production in observable larval habitats was the most definitive practical result of the study. Taking pupae as indicators of the production of adult mosquitoes (Fig. 9), there was very little known production after August 2018 (except for the setback when monthly monitoring was suspended during COVID lockdown in 2020). The few pupae that were seen were almost entirely in *pilas*. While ovitraps and BG traps did not provide the precision necessary for estimating how much the adult population was reduced, the results (Tables 6–7) showed that *Ae. aegypti* dropped to low numbers but fell short of the project goal of complete local elimination.

A noticeable vulnerability of the program was an increase in the number of *pilas*, and to a lesser extent drums and cisterns, observed to contain *Ae. aegypti* larvae whenever all-too-frequent disasters disrupted monthly monitoring (Fig. 6–7). Control in wells, tires, and buckets was more resilient to lapses. After *Ae. aegypti* larvae disappeared from wells, tires, and buckets in 2018, larvae were almost never observed in them again. Encouragingly, control in *pilas*, drums, and cisterns always recovered within a few months after a setback, larvae disappearing completely from drums and cisterns and declining to a small number in *pilas*. The broad lesson was that a program must be resilient to be sustainable, and resilience is achievable.

At the end of 2018, the volunteers declared MV to be free of dengue, Zika, and chikungunya (Fig. 10), a conclusion in line with the disappearance of recognizable cases of these diseases based on symptoms reported by Monte Verde residents during the monthly monitoring (Fig. 11). Integrated control projects with copepods in Bangkok, Thailand (Kittayapong et al., 2008) and source reduction, larvicides, and massive adult trapping in Puerto Rico (Barrera et al., 2019) had a similar experience, in which dengue disappeared without complete elimination of the mosquitoes.

Were MV's *Ae. aegypti* program and the virtual elimination of *Ae. aegypti* production responsible for the disappearance of the diseases? The data in this study are not sufficient to confirm that connection. Zika and chikungunya dropped to low levels in the entire region after 2016 (Table 8), and the same would be expected in MV regardless of its *Ae. aegypti* program. The evidence for a connection is strongest for dengue, whose absence in MV was a striking contrast with the large number of

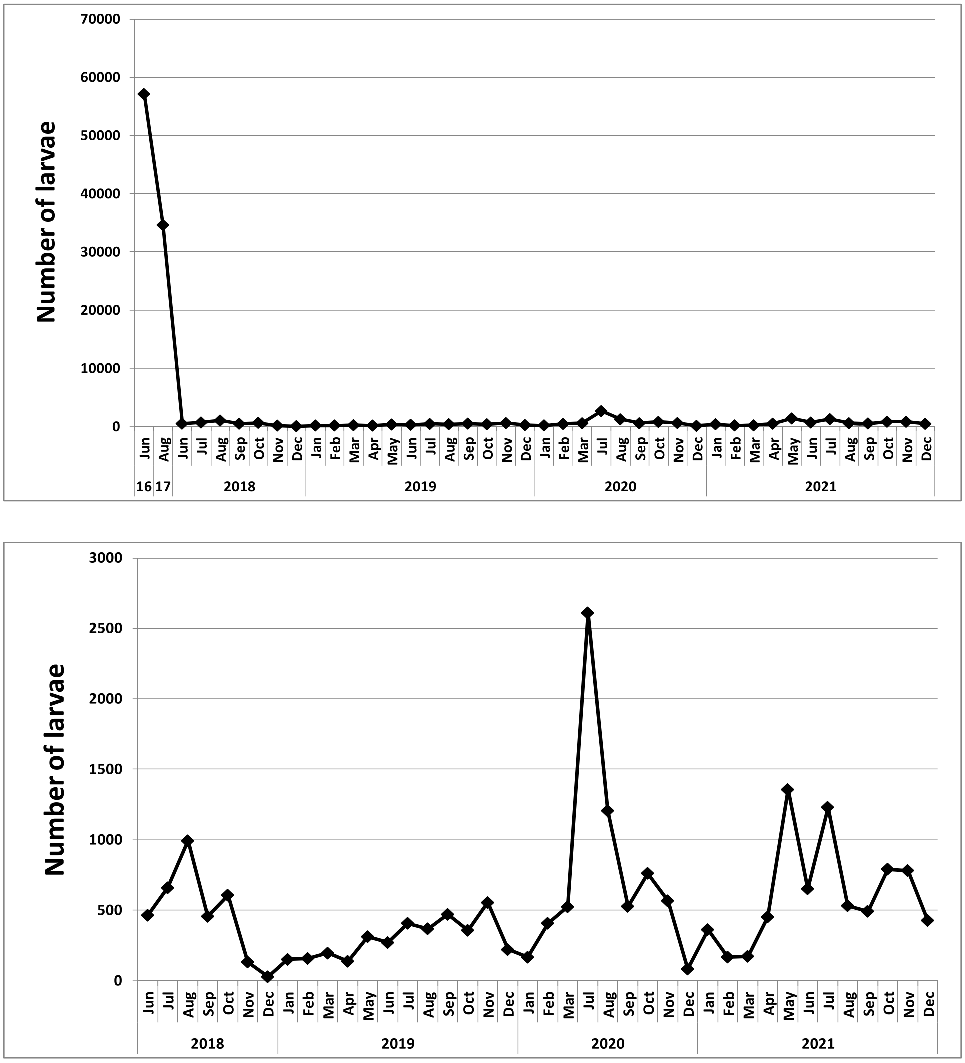


Fig. 8. Total number of mosquito larvae observed in monthly monitoring during June 2018 – December 2021. The top graph includes the only larval counts conducted in 2016 and 2017, showing the reduction by 2018.

dengue cases in similar nearby communities during a massive outbreak in the region during 2019 (Table 8 and Supplement 3). Dengue cases during that outbreak were conspicuous in the 2019 San Antonio baseline survey (Table 2).

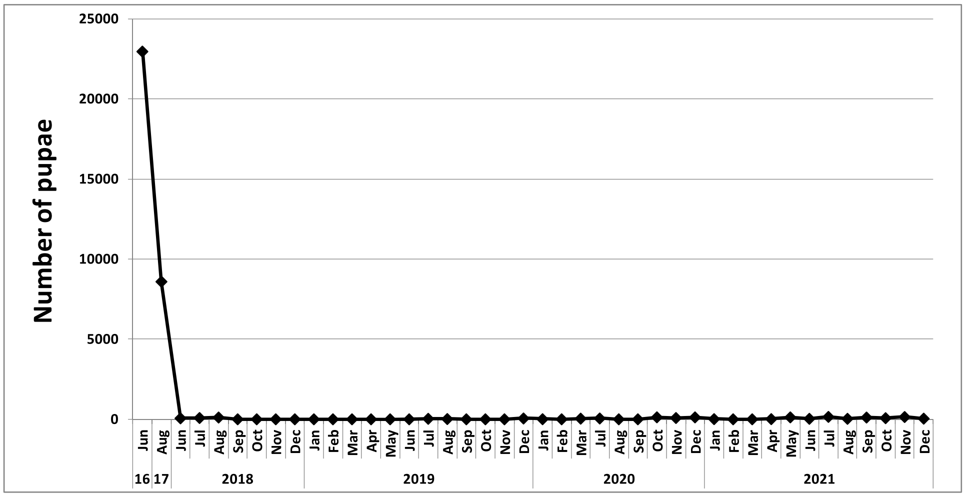
A rigorous conclusion about the responsibility of MV's *Ae. aegypti* program for the disappearance of the diseases will require a multi-site study that includes additional communities, using more accurate disease assessment procedures and a research design elucidating the connections between the control program, the mosquitoes, and the diseases. In Puerto Rico, Barrera et al. (2014a, 2014b) provided an example of a rigorous design for assessing program effectiveness at multiple sites where they documented reductions in *Ae. aegypti* with CDC autocidal gravid traps (AGTs). The study started with a baseline period monitoring AGT catches at several control sites and then switched different control sites to AGT intervention at staggered intervals until all the sites were under AGT intervention. This design allowed comparisons of intervention sites with control sites as well as "before and after" comparisons at the same sites.

4.1. Ingredients for success

Why was MV's control program successful at eliminating *Ae. aegypti* production from known larval habitats? "Ingredients for success" conspicuous in community-based environmental success stories around the world (Marten et al., 2005, (Marten, 2012b), (Marten, 2015)) provide insights into MV's toolkit design and control program as a whole.

Outside stimulation and facilitation generated shared community awareness and commitment. OBH facilitators stimulated a shared awareness and understanding of the problem and solutions, introducing game-changing ideas and encouragement for dealing with the problem. MV volunteers said they volunteered because they felt abandoned by government. OBH's educational meetings, medical clinic, and involvement with the school made a big impression. What the volunteers learned about *Ae. aegypti*, the diseases, and biological control was a revelation for them. They had virtually no prior knowledge about these matters, and volunteering offered an opportunity to learn more, which was a personal reward in itself. Residents said they were motivated to cooperate with the volunteers because attention from the volunteers made them feel better about themselves. Everyone's understanding and commitment deepened as they became active participants. With a shared ownership of the project, the community moved forward with its own manpower and financial resources.

Enduring commitment of local leadership. Through example, the volunteers became trusted and persistent leaders who inspired the deep-rooted and continuing community commitment and participation necessary for success. Residents said they were impressed with the competence of the volunteers and looked forward to their visits. There were plenty of problems, but the monthly monitoring routine provided



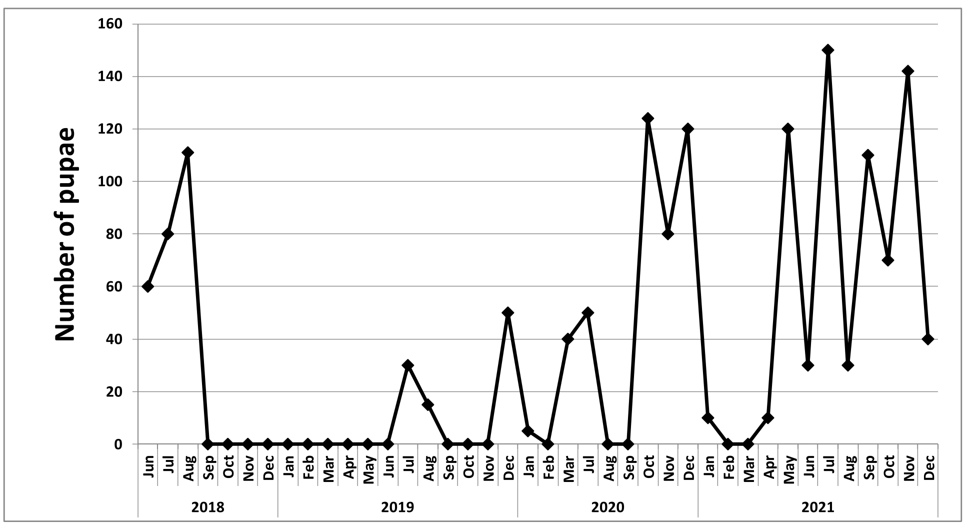


Fig. 9. Total number of mosquito pupae observed in monthly monitoring during June 2018 – December 2021. The top graph includes the only pupal counts conducted in 2016 and 2017, showing the reduction by 2018.

Table 5
Summary of Container Index changes displayed in Fig. 7 for different phases of the project.[1]

	Pilas	Drums	Buckets	Cisterns	Wells	Tires
Initial deployment (Jun–Dec 2016)	42→25	38→23	40→10	47→17	15→25	40–50
Refining the toolkit (Jan–Jun 2017)	25→20	23→18	9→15	15→38→27	23→5	12→5
Political turmoil (Jul 2017–Jan 2018)	35→5→21	34→5→18	20→2→17	47→8→25	18→7→20	40→2→30
Full participation (Feb–Aug 2018)	14→6	11→4	7→1	22→3	1→2	12→0
No production[2] (Sep 2018–Apr 2019)	3→1	0.5→0.3	0	7→0→2.4	0	0
Water problems (May 2019–Mar 2020)	3→6→3	0.3→1.5	0	0→2.4	0	0
COVID lockdown (Jul–Dec 2020)	18→3	6→1	1→0	12→0	0	0
Resident turnover (Jan –Dec 2021)	1→6→1	1→1.7→0.3	0	0	0	0

[1]→ indicates changes during each phase.
[2]No mosquito pupae observed in known *Ae. aegypti* larval habitats in Monte Verde.

the structure and consistency necessary to deal with them. Patience and respect were paramount.

Compatibility of the toolkit with the local community. The toolkit was adapted to realities of how people used their water storage containers, how turtles, copepods, and tilapia could survive and thrive under those conditions, what effort was reasonable to expect from families to reach a high level of container coverage, and the need to keep it as simple as possible. The use of turtles, copepods, and tilapia was straightforward and relatively simple once the details were worked out. The monthly monitoring routine was a simple and effective vehicle for volunteers and residents to tailor practical application of the toolkit to each household in a way that became sustainable habit.

Social and ecological diversity. Diversity provides more choices, enriching the possibilities for good choices. The ecological diversity of biological control and other methods in the toolkit provided the choices that each family needed to fit the right method to each of its containers. Social diversity came from MV residents, OBH facilitators, and the scientific advisor all working together to craft solutions from a broad pool of backgrounds, talents, and ideas. “Social memory,” learning from the past, added to the diversity by providing choices that had proved

Table 6
Results of ovitrap surveys in Monte Verde.

	Ovitraps[1]	%positive[2]	Eggs[3]	Eggs/trap[4]
June 2016 (baseline)	244	93.9±1.5	17,695	72.5±4.8
October 2016	60	85.0±4.6	2,828	47.1±7.0
August 2017	220	50.5±3.4	6,134	27.9±2.0
March 2018	242	39.7±3.2	1,569	6.5±0.8
July 2018	216	59.7±3.3	3,084	14.3±1.6
November 2018	241	40.2±3.2	2,375	9.9±1.1
May 2019	214	65.9±3.2	2,834	13.2±1.4
February 2020	170	49.4±3.7	1,133	6.7±1.0
July 2020[5]	222	88.3±2.0	7,202	32.4±2.0
November 2020	177	40.3±3.7	1,614	9.2±1.2
February 2021	157	60.0±3.9	2,138	13.8±1.7

[1]Number of ovitraps.
[2]Percentage of ovitraps containing *Ae. aegypti* eggs (±standard error).
[3]Total number of eggs.
[4]Average number of eggs/ovitrap including ovitraps with no eggs (±standard error).
[5]Increase in eggs/ovitrap associated with lapse in monthly monitoring during COVID lockdown.

Table 7
Average number of mosquitoes captured per trap-week by BG Sentinel traps in Monte Verde.[1].

	2017	2021
Aedes aegypti	3.99±0.98	0.74±0.17
Culex[2]	0.91±0.16	3.04±0.70
Coquilletiddia	0.99±0.41	0.26±0.10
Other[3]	0.13±0.05	0.53±0.17
Total	6.02±1.15	4.56±0.78

[1]June-September in 2017 (100 trap-weeks) and 2021 (57 trap-weeks).
[2]*Culex quinquefasciatus* and other *Culex* species.
[3]*Mansonia, Uranotania, Anopheles*, and mosquitoes of unknown identity.

effective and sustainable while withstanding the test of time. Because the scientific advisor had participated in Vietnam's *Ae. aegypti* program (Nam et al., 1998), as well as a pilot project during the 1990s in El Progreso, a city fifty km from Monte Verde (Leontsini et al., 1993; Marten et al., 1994b; Fernández et al., 1998), he was able to apply practical lessons from those experiences, and OBH drew upon prior community organizing experience for safe water in Honduran communities.

Overcoming social obstacles. There can be numerous social obstacles to success. For example: community participation is thwarted by competing demands for people's time, attention, and energy; people who feel threatened by innovation or other change take measures to suppress it. The commitment of the volunteers as a team motivated them to make the necessary time for their work. A key to participation by residents was the strong personal working relationship with volunteers. The local community council and numerous others might have perceived the *Ae. aegypti* control project to be a threat to their authority, but from the very beginning, the project made a point of securing and maintaining the support and involvement of community leaders and relevant government officials.

Rapid results, powerful symbols, and "success breeds success." Positive results cascaded through the community and stimulated everyone's commitment to achieve even more. From the beginning, people could see that mosquito larvae disappeared from wells and containers soon after turtles, copepods, or tilapia were introduced. Turtles particularly, with their charisma and voracious appetite for mosquito larvae, became a symbol that consolidated community commitment and action. Then, when the diseases disappeared, success itself became a symbol, as demonstrated by a "*zona libre*" sign proudly proclaiming MV to be free of the diseases (Fig. 10). This pride inspired other community projects such as trash cleanup, which in turn fed back to reinforce commitment to the *Ae . aegypti* program.

Building resilience. Resilience is the ability to continue functioning and sustain gains in the face of uncertainties and disruptions. A community's adaptive capacity, its ability to evolve and respond to challenges with prudent experimentation while learning from successes and mistakes, is central to resilience. The autonomy of the project with respect to various lines of political authority bolstered its adaptive capacity by protecting its flexibility. Resilience was also enhanced by avoiding dependence on resources that were unavailable or unreliable. Biological control was doable and sustainable with MV's limited financial resources. Success required strong community participation, which MV was able to provide. The main dependence was on a small quantity of larvicide. The robustness of the volunteers enabled rapid recovery of container coverage after setbacks due to political turmoil, COVID lockdown, and other disturbances. By the time OBH ended its intensive facilitation in March 2019, the volunteers had developed the competence and confidence to continue on their own.

Supplement 4 provides more details from discussions with MV volunteers and residents about why the project was successful.

4.2. Why didn't Ae. aegypti disappear?

Why did *Ae. aegypti* fail to disappear when the production from known larval habitats was for all practical purposes zero?" There are several plausible explanations, none of which by itself seems to provide a fully satisfying answer to this question:

Lapses due to disruption by calamities. Social and environmental disruptions can be particularly severe in a marginal community like Monte Verde. Violent political protests, failures in the water supply, delivery delays for supplies such as larvicides, COVID lockdown, and residential turnover may have interfered with continuously sustaining the level of container coverage necessary for complete local elimination to run its course. Adult mosquitoes and eggs on the surface of containers can survive for months (Brown et al., 2017), even after larvae and pupae disappear, bridging gaps between periodic lapses.

Hidden larval habitats. The production of *Ae. aegypti* from hidden sites seems a plausible explanation for continuation of the population. The well-known surge in *Ae. aegypti* during the rainy season suggests the creation of larval habitats by rainwater in inconspicuous places. However, observable larval habitats created by rainwater in bottles, rain gutters, flowerpots, or trash contained few pupae during the baseline survey (Table 1) and none during the monthly monitoring. Intensive searches for additional possibilities such as bamboo or other plants collecting rainwater, latrines, or the drains of kitchen sinks or *pilas* did not reveal *Ae. aegypti* larvae. Moreover, if the quantity of hidden sites is not large, the contribution of those sites to the mosquito population should be nullified by the large number of wells and water storage containers that were transformed into egg sinks by biological control.

Refuges inside Monte Verde. As the *Ae. aegypti* population declined during 2018, larvae persisted in small clusters of positive *pilas* and drums at adjacent houses, sometimes continuing from one month to the next. Once the observed larval population was small, the few containers with larvae were generally not clustered or continuing from one month to the next, though some rooming houses may have provided ongoing refuge. As *pilas* were for all practical purposes the only larval habitat in MV observed to contain pupae after pupae disappeared from drums, a small number of poorly managed *pilas* scattered throughout MV may also have provided refuge.

Influx from the surrounding area. Mosquitoes can fly into MV from surrounding areas. While *Ae. aegypti* might be expected in the factory zone to the north, there were no eggs in ovitraps placed between the factories and MV. The natural vegetation and wetland immediately to the south are not *Ae. aegypti* larval habitat. Because the Las Colinas residential area to the east of MV had an average of 15.1 eggs/ovitrap in the March 2020 survey (Table 2), influx from there seems at least part of the explanation for continuing presence of *Ae. aegypti* in MV. However, it

Fig. 10. Volunteers proudly displaying their sign proclaiming Monte Verde to be a “dengue, Zika, and chikungunya free zone.”.

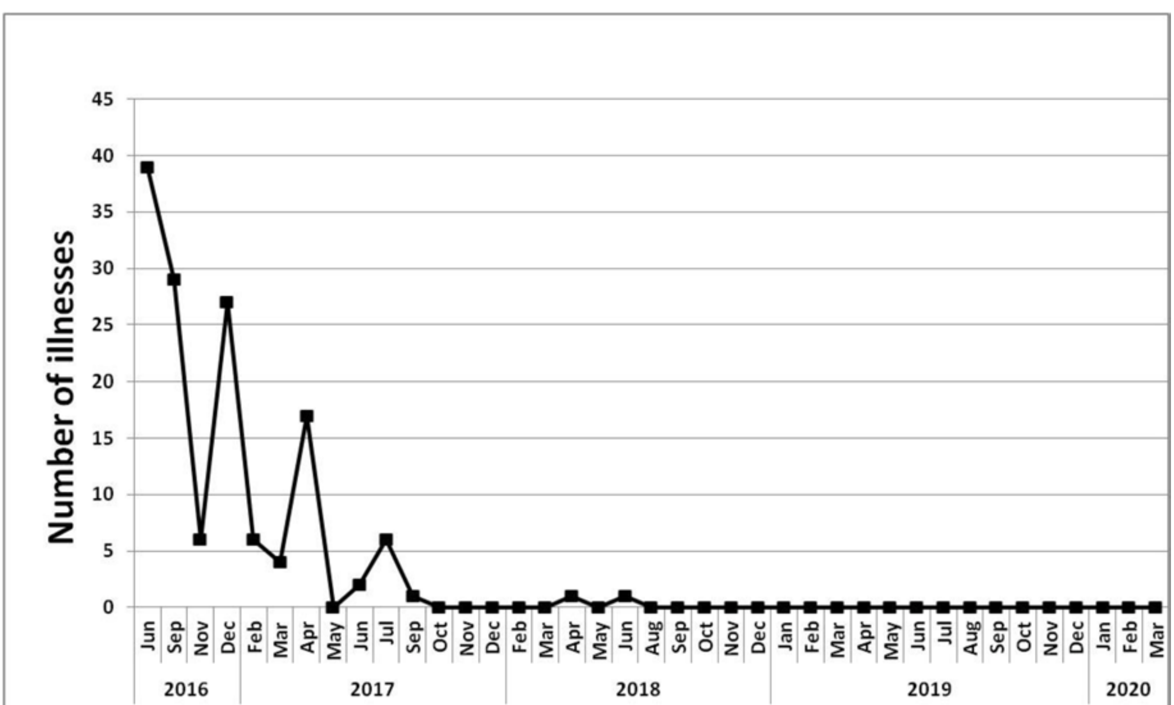


Fig. 11. Number of febrile illnesses with characteristic symptoms of dengue, Zika, or chikungunya reported by Monte Verde residents during monthly monitoring. Supplement 3 provides details about the data underlying this graph.

Table 8
Number of reported cases of dengue, Zika, and chikungunya in Honduras.[1].

Year	Dengue[2]	DHF[3]	Zika	Chikungunya
2014	42,753	2309	0	76
2015	44,834	1062	56	76,791
2016	22,961	313	31,468	17,692
2017	5217	126	120	532
2018	7942	1172	358	185
2019	112,798	19,435	240	219

[1]Source: PAHO (2019). Reported cases are a small but unknown fraction of actual cases.
[2]All dengue cases (including Dengue Hemorrhagic Fever).
[3]Dengue Hemorrhagic Fever.

does not appear to be the entire explanation because *Ae. aegypti* eggs in ovitraps, larvae in water storage containers, and adults in BG traps were spread across the community and not concentrated near Las Colinas. Because Las Colinas was a clear source of mosquitoes, MV volunteers planned to assist Las Colinas to eliminate *Ae. aegypti* production so the two communities could pursue local elimination together, but the plan was suspended with the onset of COVID.

4.3. Strengthening the toolkit

If we think of sustaining MV's elimination of *Ae. aegypti* production, extending the program to other communities, and possibly striving for complete local elimination, MV's experience points to a need for an even more powerful and resilient toolkit to compensate for inherent limitations of community participation and disruptions caused by social and environmental disturbances. The following improvements will be helpful:

- Strengthening the entire suite of methods for *pilas*. Nearly all the known remnants of *Ae. aegypti* production in this study were in *pilas*, and *pilas* were where production increased most during program lapses.
- A capacity to identify problem houses early on and attend to them vigorously.
- Inexpensive and labor-efficient routines for monitoring adult mosquitoes to assess program effectiveness on a house-to-house and community scale. A routine for community volunteers to routinely and reliably recognize and distinguish larvae and adults of *Ae. aegypti* and other mosquito species of public health importance.
- Clarifying quantitatively how complete the control coverage of larval habitats must be to achieve local elimination and how much reduction in the adult mosquito population is necessary for effective disease control.
- Enhancing incentives for motivating volunteers to join and continue with the program, while passing expertise from volunteer experience to new volunteers as turnover occurs.
- Ascertaining why complete local elimination did not occur and adjusting the tool kit or program procedures accordingly.
- Identifying avenues for improving the toolkit, and control program as a whole, by examining the program through a lens of "ingredients for success."
- Supplying baby turtles by farming a native species or harvesting it sustainably from the wild (see Supplement 1).

A particularly promising strategy for strengthening the toolkit is to increase the oviposition attraction of egg sinks created by biological control. Using the Containers-Inhabiting Mosquito Simulation Model (Focks et al., 1993), Marten concluded that container coverage should be at least 90% to drive a population to zero, but in fact mosquitoes disappeared in Vietnamese villages where the coverage of larval habitats with copepods was substantially less (Nam et al., 1998). The difference between model prediction and actual experience in Vietnam may have been because copepods increased the oviposition attraction of containers (Marten and Reid, 2007), an attraction that MV's oviposition study confirmed not only for copepods but also turtles, fish, and some larvicide formulations (Table 3).

Research on bacteria that attract *Ae. aegypti* oviposition has raised the possibility of treating water storage containers and wells with an attractant after converting them to egg sinks. Bacteria isolated from leaves of select tree species decomposing in water can render the water ten times more attractive to *Ae. aegypti* oviposition than plain water (Ponnusamy et al., 2008, 2015). Slow-release tablets for these bacteria were effective in lethal ovitraps in pilot projects in Peru and Thailand (Paz-Soldan et al., 2016). A substantial effort, including laboratory research beyond the capacity of communities like MV, will be necessary to adapt this technology for practical use in water storage containers and wells. An attractant should be affordable, ideally produced locally, and not reduce the water quality in a storage container.

4.4. Dissemination of Monte Verde's success to other communities

MV's success is an inspiration for similar communities to do the same. They do not have to wait for international donors to bring them high-tech solutions, nor is it necessary to wait for government. They can do it for themselves if they are shown how to use biological control and receive technical and moral support to become operational. The time required to repeat MV's success should be less than happened at MV because many details for the toolkit and how to mobilize community participation have already been worked out.

OBH and the MV volunteers have embarked on helping others to replicate what they have achieved. MV serves as a demonstration site, and as a team the volunteers have assisted several other communities with the training they need to develop their own programs. The team began in 2019 by initiating contact with a few communities suggested by government regional health offices, starting with the local school, conducting educational meetings with parents, and organizing a project for school children to do a baseline survey similar to the one at MV when the project began. These activities were suspended in March 2020 with COVID lockdown and began to resume again in 2021 as restrictions relaxed.

If a community wants to move ahead, MV volunteers can provide copepods and tilapia and assist the community with procuring turtles and appropriate larvicides. They can teach volunteers in that community how to use the toolkit, mobilize their neighbors for effective container coverage, and execute the monthly monitoring routine to fully implement the program. The MV volunteers are learning how to assess the suitability of a community for this kind of program, including key features such as the commitment of community leaders and the potential for an effective team of volunteers based on residents already active in community service. They are learning the value of a formal contract delineating community obligations and inputs necessary for program implementation.

Dissemination to other communities requires a whole new set of skills for MV volunteers, who have accomplished so much as social entrepreneurs in their own community. As they forge functional ties with other communities, deal with government officials on a range of issues, and refine a process of community organizing and education that delivers results under difficult circumstances, the volunteers are acutely aware of limitations in their formal education and prior experience outside their own community. Just as outside facilitation was essential for MV to develop and implement an effective program for itself, success with dissemination will require support and facilitation from organizations that foster the dissemination of community-based health programs.

Studies in humans and animals

There were no human subjects in this study. Animals were treated humanely.

CRediT authorship contribution statement

Gerald G. Marten: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing, Funding acquisition, Visualization, Supervision. **Xenia Caballero:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Project administration. **Arnulfo Larios:** Conceptualization, Methodology, Investigation, Data curation, Project administration. **Hilda Bendaña:** Conceptualization, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Bill Horan was responsible for initiating the project, participated in early project planning, and provided administrative support from Operation Blessing International in numerous ways. The current MV volunteers are Magdalena Alvarado, Olga Bueso, María Carranza, Obed Cruz, Dunia Girón, Naelson Larios, Denis Lemus, Joselin López, Melida Lozano, and Norma Lozano. Past volunteers were Neptalí Alvarado, Manuel Castellanos, Alexis García, Bayron Lemus, Sarahí López, Lurbin Lozano, Valeria Mejía, Wilmer Mejía, Rubén Perdomo, Manuel Pineda, Anthony Ramírez, Ana Reyes, Patricia Rosales, José Santamaría, and Amada Ventura. Danelia Castro, Alejandro Euceda, Ariana Hernandez, Ibeth López, Maria Fernanda Membreño, Zuri Murillo and, and Irving Odrid participated in the 2016 baseline survey. Jacky Umaña was an Operation Blessing Honduras facilitator during the first year of the project. Dennis Salomón López was in charge of epidemiology and contributed to community organization and development of the monthly monitoring during the first year, and Nancy Echeverría provided epidemiology expertise after that. Brendan O'Leary contributed to project planning, designed baseline surveys, analyzed results, and contributed to data analysis and reporting. Marco Suárez provided planning advice at the beginning of the project. Eduardo Suarez provided copepod species identifications. David Greenfield and Julio Mérida provided species identifications for local fish. Marlon López provided expert advice about tilapia management, and the Centro Nacional de Investigación Piscícola El Carao, Direccón General de Pesca y Acuicultura donated tilapia fingerlings to the project. Ross Kiester and Peter Paul van Dijk provided information about the red-eared slider turtle as an invasive species. The Health Vigilance Unit, Cortés Regional Health Center provided valuable government cooperation. Mario Howell helped with government statistics on dengue, Zika, and chikungunya. Philip Koehler and Roberto Peirera contributed pyriproxyfen-coated tiles for testing in MV. Clarke donated Natular larvicide, and Adapco donated BTI. The New Orleans Mosquito, Termite and Rodent Control Board helped to educate project staff on the basics of mosquito control, and Cynthia Harrison helped project staff to set up copepod cultures, provided training for mosquito monitoring methods, and identified mosquito species captured in BG sentinel traps. Josue Ramos identified larval and adult mosquitoes from BG traps, ovitraps, and water storage containers.

Funding

This work was supported by Operation Blessing International, Vulcan Foundation, Clarke Cares Foundation, and the EcoTipping Points Project. Vulcan and Clarke Cares Foundation were not involved in project design or manuscript preparation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2021.106269.

References

Achee, N.L., Gould, F., Perkins, T.A., Reiner Jr., R.C., Morrison, A.C., Ritchie, S.A., Gubler, D.J., Remy, T., Scott, T.W., 2015. A critical assessment of vector control for dengue prevention. PLoS Negl. Trop. Dis. 9 (5), e0003655.

Alvarado-Castro, V., Paredes-Solís, S., Nava-Aguilera, E., Morales-Pérez, A., Alarcón-Morales, L., Balderas-Vargas, N.A., Andersson, N., 2017. Assessing the effects of interventions for *Aedes aegypti* control–Systematic review and meta-analysis of cluster randomised controlled trials. BMC Public Health 17 (Suppl 1), 384.

Alvarado-Castro, V., Paredes-Solís, S., Nava-Aguilera, E., Morales-Pérez, A., Flores-Moreno, M., Legorreta-Soberanis, J., Jaimes-Néstor, E., Cockcroft, A., Andersson, N., 2019. Social capital is associated with lower mosquito vector indices–Secondary analysis from a cluster randomised controlled trial of community mobilization for dengue prevention in Mexico. Popul. Health Metrics 17, 18.

Barrera, R., Amador, M., Acevedo, V., Caban, B., Felix, G., Mackay, A.J., 2014a. Use of the CDC autocidal gravid ovitrap to control and prevent outbreaks of *Aedes aegypti* (Diptera–Culicidae). J. Med. Entomol. 51, 145–154.

Barrera, R., Amador, M., Acevedo, V.A., Hemme, R.R., Felix, G., 2014b. Sustained, area-wide control of *Aedes aegypti* using CDC autocidal gravid ovitraps. Am. J. Trop. Med. Hyg. 91 (6), 1269–1276.

Barrera, R., Harris, A., Hemme, R., Felix, G., Nazario, N., Muñoz-Jordan, J.L., Rodriguez, D., Miranda, J., Soto, E., Martinez, S., Ryff, K., Perez, C., Acevedo, V., Amador, M., Waterman, S.H., 2019. Citywide control of *Aedes aegypti* (Diptera–Culicidae) during the 2016 Zika epidemic by integrating community awareness, education, source reduction, larvicides, and mass mosquito trapping. J. Med. Entomol. 20 (10), 1–14.

Borjas, G., Marten, G.G., Fernández, E., Portillo, H., 1993. Juvenile turtles for mosquito control in water storage tanks. J. Med. Entomol. 30, 943–946.

Bouzid, M., Brainard, J., Hooper, L., Hunter, P.R., 2016. Public health interventions for *Aedes* control in the time of Zikavirus–A meta-review on effectiveness of vector control strategies. PLoS Negl. Trop. Dis. 10(12), e0005176.

Bowman, L.R., Runge-Ranzinger, S., McCall, P.J., 2014. Assessing the relationship between vector indices and dengue transmission–A systematic review of the evidence. PLoS Negl. Trop. Dis. 8 (5), e2848. https://doi.org/10.1371/journal. pntd.000-2848.

Boyce, R., Lenhart, A., Kroeger, A., Velayudhan, R., Roberts, B., Horstick, 2013. *Bacillus thuringiensis* israelensis(Bti) for the control of dengue vectors–Systematic literature review. Trop. Med. Int. Health 18, 564–577.

Brown, B.V., Borkent, A., Cumming, J.M., Wood, D.M., Woodly, N.E., Zumbado, M., 2009. Manual of Central American Diptera, 1. NRC Research Press, Ottawa.

Brown, H.E., Smith, C., Lashway, S., 2017. Influence of the length of storage on *Aedes aegypti* (Diptera: Culicidae) egg viability. J. Med. Entomol. 54 (2), 489–491.

Chan, K.L., 1973. The eradication of *Aedes aegypti* at the Singapore Paya Lebar International Airport. In: Proceedings, Symposium–Vector Control in Southeast Asia, 1st Southeast Asian Ministers of Education Organization Tropical Medicine Vector Control Workshop, 17-18 August 1972. Singapore. Sen Wah Press, Singapore, pp. 85–88.

Clark-Gil, S., Darsie, R.F., 1983. The mosquitoes of Guatemala–Their identification, distribution, and bionomics. Mosq. Syst. 15 (3), 151–284.

Devillers, J.F., 2020. Ecotoxicological effects of pyriproxyfen in aquatic ecosystems. Environ. Sci. Pollut. Res. 27, 16052–16068.

Elson, W.H., Reiner, R.C., Siles, C., Bazan, I., Vilcarromero, S., Riley-Powell, A.R., Kawiecki, A.B., Astete, H., Hontz, R.D., Barker, C.M., et al., 2020. Heterogeneity of dengue illness in community-based prospective study, Iquitos, Peru. Emerg. Infect. Dis. 26, 2077–2086.

Fernández, E.A., Leontsini, E., Sherman, C., Chan, A.S.T., Reyes, C.E., Lozano, R.C., Fuentes, B.A., Nichter, M., Winch, P.J., 1998. Trial of a community-based intervention to decrease infestation of *Aedes aegypti* mosquitoes in cement washbasins in El Progreso, Honduras. Acta Trop. 70, 171–183.

Focks, D., Haile, D.G., Daniels, E., Mount, G., 1993. Dynamic life table model for *Aedes aegypti* (Diptera–Culicideae)–Analysis of the literature and model development. J. Med. Entomol. 30, 1003–1017.

Han, W.W., Lazaro, A., McCall, P.J., George, L., Runge-Ranzinger, S., Toledo, J., Velayudhan, R., Horstick, O., 2015. Efficacy and community effectiveness of larvivorous fish for dengue vector control. Trop. Med. Int. Health 20 (9), 1239–1256.

Horstick, O., Ranzinger, S.R., 2015. Interim analysis of the contribution of high-level evidence for dengue vector control. Southeast Asian J. Trop. Med. Public Health 46 (Suppl 1), 131–137.

Invest, J.F., Lucas, J.R., 2008. Pyriproxyfen as a mosquito larvicide. In: Robinson, WH, Bajomi, D (Eds.), Proc Sixth Intern Conf Urban Pests. OOK-Press Kft, H-8200 Veszprém, Pápai út 37/a, Hungary, pp. 239–245.

Kay, B.H., Nam, V.S., 2005. New strategy against *Aedes aegypti* in Vietnam. Lancet 365, 613–617.

Kittayapong, P., Yoksan, S., Chansang, U., et al., 2008. Suppression of dengue transmission by application of integrated vector control strategies at sero-positive GIS-based foci. Am. J. Trop. Med. Hyg. 78 (1), 70–76.

Lacey, L.A., 2007. *Bacillus thuringiensis* serovariety israelensis and *Bacillus sphaericus* for mosquito control. Am. Mosq. Control Assoc. Bull. 7, 133–163.

Lazaro, A., Han, W.W., Manrique-Saide, P., George, L., Velayudhan, R., Toledo, J., Ranzinger, S.R., Horstick, O., 2015. Community effectiveness of copepods for dengue vector control–Systematic review. Trop. Med. Int. Health 20 (6), 685–706.

Leonard, C.J., Marten, G.G., 1994. Copepods eating Ae. albopictus larvae. 1 min video. https://www.ecotippingpoints.org/resources/video-macrocyclops-albidus/index.html.

Leontsini, E., Gil, E., Kendall, C., Clark, G.G., 1993. Effect of a community-based *Aedes aegypti* control program on mosquito larval production sites in El Progreso, Honduras. Trans. R. Soc. Trop. Med. Hyg. 87, 267–271.

Marten, G.G., Reid, J.W., 2007. Biorational control of mosquitoes–Cyclopoid copepods. Am. Mosq. Control Assoc. Bull. 7, 65–92.

Marten, G.G., Che, W., Bordes, E.S., 1993. Compatibility of cyclopoid copepods with mosquito insecticides. J. Am. Mosq. Control Assoc. 9, 150–154.

Marten, G.G., Bordes, E.S., Nguyen, M., 1994a. Use of cyclopoid copepods for mosquito control. Hydrobiologia 292/293, 491–496.

Marten, G.G., Borjas, G., Cush, M., Fernandez, E., Reid, J.W., 1994b. Control of larval *Aedes aegypti* (Diptera–Culicidae) by cyclopoid copepods in peridomestic breeding containers. J. Med. Entomol. 31, 36–44.

Marten, G.G., Nguyen, M., Thompson, G., Bordes, E.S., 1997. Copepod production and application for mosquito control. In: New Orleans, LA–New Orleans Mosquito and Termite Control Board. New Orleans Mosquito and Termite Control Board, New Orleans, LA, pp. 1–43. http://gerrymarten.com/publicatons/pdfs/GM_Copepod-Production.pdf.

Marten, G.G., Brooks, S., Suutari, A., 2005. Environmental tipping points–A new slant on strategic environmentalism. World Watch Mag. 18 (6), 10–14.

Marten, G.G., Wolf, D., Hillstrom, H., 2020. The MV Story (Honduras)–Community Eradication of *Aedes aegypti* (the mosquito responsible for Zika, dengue fever, and chikungunya). 3 min video. http://www.ecotippingpoints.org/video/monte-verde/3min.html.

Marten, G.G., 1990a. Elimination of *Aedes albopictus* from tire piles by introducing Macrocyclops albidus (Copepoda, Cyclopidae). J. Am. Mosq. Control Assoc. 6, 689–693.

Marten, G.G., 1990b. Evaluation of cyclopoid copepods for *Aedes albopictus* control in tires. J. Am. Mosq. Control Assoc. 6, 681–688.

Marten, G.G., 2001. Dengue hemorrhagic fever, copepods, and biological control of mosquitoes in Vietnam. In: Marten, G.G. (Ed.), Human Ecology – Basic Concepts for Sustainable Development. Earthscan Publications, pp. 184–196.

Marten, G.G., 2007. Biorational control of mosquitoes–Turtles. Am. Mosq. Control Assoc. Bull. 7, 221–224.

Marten, G.G., 2012a. Using ovitraps to assess the quantity of mosquito larval habitat during local eradication with source reduction and ovitraps. J. Med. Entomol. 49 (3), 640–646.

Marten, G.G., 2012b. EcoTipping Points–Levers for sustainability. In: Silva Rivera, E, Vergara Tenorio, MC, Ernesto Rodríguez-Luna, E (Eds.), Casos Exitosos en La Construcción de Sociedades Sustentables. Universidad Veracruzana, Xalapa, Mexico, pp. 33–58. http://www.ecotippingpoints.org/resources/presentation-sustainable-societies.html.

Marten, G.G., 2015. Learn about ecotipping points–Lessons learned – ingredients for success. http://ecotippingpoints.org/about-etps.html#lessons.

Nam, V.S., Yen, N.T., Kay, B.H., Marten, G.G., Reid, J.W., 1998. Eradication of *Aedes aegypti* from a village in Vietnam, using copepods and community participation. Am. J. Trop. Med. Hyg. 59, 657–660.

Nam, V.S., Nguyen, T.Y., Phong, T.V., Truong, U.N., Le, Q.M., Le, V.L., Le, T.N., Bektas, A., Briscombe, A., Aaskov, J.G., Ryan, P.A., Kay, B.H., 2005. Elimination of dengue by community programs using *Mesocyclops* (Copepoda) against *Aedes aegypti* in central Vietnam. Am. J. Trop. Med. Hyg. 72, 67–73.

Oseguera, M., 2016. Industria de tilapia en Honduras–Situación actual retos y perspectivas. Consejo Hondureño de la Empresa Privada 1–46. http://www.agronegocioshonduras.org/wp-content/uploads/2019/09/Perfil-Rubro-de-Tilapia-Versi%C3%AF%C2%BF%C2%BDn-Final-Agosto-29-de-2016.pdf.

PAHO, 2019. Base de datos PLISA. Situación de Salud en las Américas–Indicadores básicos 2019. Departamento de Evidencia e Inteligencia para la Acción en Salud/Unidad de Análisis de Salud, Métricas y Evidencia. Pan Am. Health Organ.

Paz-Soldan, V.A., Yukich, J., Soonthorndhada, A., Giron, M., Apperson, C.S., Ponnusamy, L., Schal, C., Morrison, A.C., Keating, J., Wesson, D.W., 2016. Design and testing of novel lethal ovitrap to reduce populations of *Aedes* mosquitoes–Community-based participatory research between industry, academia and communities in Peru and Thailand. PLoS ONE 11 (8), e0160386. https://doi.org/10.1371/journal.pone.0160386.

Pecor, J., Robers, D., Harbach, R., Rejmankova, E., 2002. Mosquito studies in Belize, Central America, records, taxonomic notes, and checklist of species. J. Am. Mosq. Control Assoc. 18 (4), 241–276.

Ponnusamy, L., Xu, N., Nojima, S., Wesson, D.W., Schal, C., Apperson, C.S., 2008. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. PNAS 105 (27), 9262–9267.

Ponnusamy, L., Schall, C., Wesson, D.W., Arellano, C., Apperson, C.S., 2015. Oviposition responses of *Aedes* mosquitoes to bacterial isolates from attractive bamboo infusions. Parasites Vectors 8, 486.

Rueda, L.M., 2004. Pictorial keys for the identification of mosquitoes (Dipera: Culicidae) associated with dengue virus transmission. Zootaxa 589, 1–60.

Scott, C., Weaver, S.C., Costa, F., Garcia-Blanco, M.A., Ko, A.I., Guilherme, S., Ribeiro, G. S., Saade, G., Shi, P.Y., Vasilakis, N., 2016. Zika virus–History, emergence, biology, and prospects for control. Antivir. Res. 130, 69–80.

Sherman, C., Fernández, E.A., Chan, A.S., Lozano, R.C., Leontsini, E., Winch, P.J., 1998. Untadita–A procedure for maintaining washbasins and drums free of *Aedes aegypti* based on modification of existing practices. Am. J. Trop. Med. Hyg. 58 (2), 257–262.

Suárez, M.F., Marten, G.G., Clark, G.G., 1992. A simple method for cultivating freshwater copepods used in biological control of *Aedes aegypti*. J. Am. Mosq. Control Assoc. 8, 409–412.

Supplement for "Proof of concept for eliminating *Aedes aegypti* production by means of integrated control including turtles, copepods, and tilapia in Monte Verde, Honduras (Gerald G. Marten, Xenia Caballero, Arnulfo Larios, Hilda Bendaña)

Supplement 1: The introduced species issue

Prepared by Gerald G. Marten

Two of the animals in Monte Verde's biological control toolkit, tilapia and red-eared slider turtles, are not native to Honduras. What are the environmental implications of their use? What impact could escaped tilapia or turtles have on Honduran aquatic ecosystems?

It appears that the use of tilapia to prevent *Ae. aegypti* production does not create a threat to the environment beyond whatever might already exist. First of all, escape of tilapia or other fish from wells or water storage containers is very unlikely. Secondly, *Oreochromis niloticus* is already spread throughout Honduras in thousands of aquaculture ponds as a consequence of government policy to encourage small-scale aquaculture production, and tilapia is common enough in some lakes to be the basis for commercial fisheries (Oseguera 2016). *O. niloticus* does not appear to have caused conspicuous environmental damage in Honduras, where it is generally accepted as a food resource 0(SERNA 2010).

Although turtle escapes are unusual when a community knows how to manage the turtles, escapes do happen, and escaped turtles might make their way to natural aquatic habitats. Actually, *Trachemys scripta elegans*, which is native to southeastern United States, has already been disbursed through Central America by the international pet trade, though it is not at all common in Central America compared to closely-related native turtle species: *Trachemys venusta* on the Caribbean watershed and *Trachemys grayi* (= *Trachemys emolli*) on the Pacific watershed (McCranie 2018). More than 50 million baby *T. scripta elegans* have been shipped abroad from American turtle farms during the past 50 years, spreading this turtle around the world (https://www.cabi.org/isc/datasheet/61560) and resulting in it being listed among the planet's 10 worst invasive species (IUCN 2021). This reputation is based largely on the fact that it has thrived in Europe, where it has been blamed for the decline of native freshwater turtles, though the true extent of its responsibility for the decline is not clear because of other contributing factors (Fattizzo 2004). In fact, *T. scripta elegans* in Europe is most conspicuous in urban ponds that are not normally habitat for the native species.

There is no evidence so far of *T. scripta elegans* causing significant damage to native turtles or aquatic ecosystems in Latin America (Moll & Legler 1971, Ferronato et al. 2009). In fact, computer modeling has shown that much larger numbers of *T. scripta elegans* escapes or releases than expected from use for mosquito control would be necessary to establish viable populations of this turtle in nature (Banha 2017). Moreover, as native *Trachemys* species have coexisted for millennia with other genera of freshwater turtles in Latin America, introduced *T. scripta elegans* would be expected to coexist in the same way.

If there is any damaging interaction between *T. scripta elegans* and native turtles, it would probably be "genetic pollution" due to hybridization with closely-related *T. venusta* and *T. grayi*, rather than the establishment of distinct populations of *T. scripta elegans* that displace native species. Genetic pollution from a relatively small number of escaped turtles may not be a serious concern when compared to the massive scale of genetic pollution that already exists in native fauna around the world after centuries of transporting wild and domesticated animals. Nonetheless, genetic pollution of a local native turtle population could conceivably disrupt its adaptation to local environmental conditions. For example, the sex ratio of baby turtles depends on egg incubation temperature (Ewart 1994), and the sex ratio response of *T. scripta elegans* to temperature may be out of tune with the Honduran climate. On the other hand, genetic pollution could enhance the genetic diversity of native species, thereby increasing their capacity to adapt over the longer term to changing environmental conditions such as global climate change and urban expansion.

One way to prevent escaped *T. scripta elegans* from impacting natural ecosystems would be to render the turtles incapable of reproduction when they are used for *Ae. aegypti* control. Neutering procedures remain to be developed for baby turtles.

All things considered, the most desirable way to provide a large-scale supply of turtles would be local farm production of a native Honduran species. This would require significant capital and intensive technical support to do it properly and under the best of circumstances would take at least several years to begin supplying a usable number of turtles. Another possibility for supplying native turtles would be to sustainably harvest wild baby turtles if a native species such as *T. venusta* or *T. grayi* can provide an adequate supply. Older turtles that are taken out of service for mosquito control could be returned to the wild or a turtle farm for reproduction.

Because it appears that bringing *T. scripta elegans* to Honduras for *Ae. aegypti* control will not have a significant detrimental impact on native turtle species or Honduran ecosystems, the most practical option for the short term seems to be continued use of imported *T. scripta elegans*, while watching closely for any harmful environmental impacts and working simultaneously on native turtle alternatives to make importation unnecessary in the longer term.

References

Banha F., Gama M., Anastácio P.M., 2017. The effect of reproductive occurrences and human descriptors on invasive pet distribution modelling: *Trachemys scripta elegans* in the Iberian Peninsula. Ecol Model 360, 45-52.

Ewert M.A., Jackson D.R., Nelson C.E., 1994. Patterns of temperature-dependent sex determination turtles. J Exp Zool 270, 3-15.

Fattizzo, T., 2004. Distribution and conservational problems of *Emys orbicularis* in Salento (South Apulia, Italy). Biologia, Bratislava 59/Suppl 14, 13-18.

Ferronato B.O., Marques T.S., Guardia I., Longo A.L.B., Piña C.I., Bertoluci J., Verdade L.M., 2009. The turtle *Trachemys scripta elegans* (Testudines, Emydidae) as an invasive species in a polluted stream of southeastern Brazil. Herp Bull 109, 29-34.

IUCN, 2021. *Trachemys scripti elegans*. Global invasive species database, Intern Union Cons Nat, http://www.iucngisd.org/gisd/species.php?sc=71.

McCranie J.R., 2018. The lizards, crocodiles, and turtles of Honduras: systematics, distribution, and conservation. Bull Mus Comp Zool 15(1), 1-129. https://doi.org/10.3099/0027-4100-15.1.1

Moll E.O., Legler JM, 1971. The life history of a neotropical slider turtle, *Pseudemys scripta* (Schoepff), in Panama. Bull Los Angeles County Mus Nat Hist Sci 11, 1-102.

Oseguera M., 2016. Industria de tilapia en Honduras: situación actual retos y perspectivas. Consejo Hondureño de la Empresa Privada, 1-46. http://www.agronegocioshonduras.org/wp-content/uploads/2019/09/Perfil-Rubro-de-Tilapia-Versi%C3%AF%C2%BF%C2%BDn-Final-Agosto-29-de-2016.pdf

SERNA (Secretaria de Recursos Naturales y Ambiente), 2010. Informe de País: convención sobre diversidad biológica. República de Honduras, 1-185. https://www.cbd.int/doc/world/hn/hn-nr-04-es.pdf

Acknowledgment

Ross Kiester and Peter Paul van Dijk provided information about the red-eared slider turtle as an invasive species.

Supplement for "Proof of concept for eliminating *Aedes aegypti* production by means of integrated control including turtles, copepods, and tilapia in Monte Verde, Honduras (Gerald G. Marten, Xenia Caballero, Arnulfo Larios, Hilda Bendaña)

Supplement 3: Disappearance of the diseases

Prepared by Gerald G. Marten

Two "mobile brigade" clinics in Monte Verde (MV) provided robust assessment of the diseases. Immediately after the June 2016 baseline survey at the beginning of the project, a two-day clinic staffed by MV volunteers working side-by-side with a group of physicians and other health professionals that had previously participated in Operation Blessing Honduras "Mobile Brigade" medical clinics in other communities. The clinic was for all illnesses and 670 MV residents attended. Although there were no blood analyses for dengue, Zika, or chikungunya, participating physicians devoted particular attention to whether patients displayed symptoms of these diseases. 39 patients were diagnosed by the physicians to have dengue, Zika, or chikungunya.

A second clinic was held in May 2018 with 310 MV residents attending. Patients had common colds, bronchitis, anemia, high blood pressure, vaginal infections, and fungal toenail infections, but there were no diagnoses or other indications of dengue, Zika, or chikungunya.

The monthly monitoring from September 2016 to March 2020 included questions to every family about febrile illness during the previous month. A professional epidemiologist helped to decide what to record: symptoms including headache, body pain, joint pain, and rash, which are known to be characteristic of dengue, Zika, and chikungunya and detectable even in mild dengue cases (Elson 2020); symptoms indicating respiratory or digestive infections, conjunctivitis, and other febrile illnesses that were sometimes common in MV but clearly not dengue, Zika, or chikungunya; whether the sick person went for medical treatment; and whether there was a physician diagnosis.

Each month's results were summarized as the number of cases with characteristic symptoms for each disease: fever, headache, and body pain for dengue; fever, headache, and rash (including "red eye") for Zika; fever, headache, and severe joint pain for chikungunya. The tabulation was conservative to avoid counting other illnesses as a disease transmitted by *Ae. aegypti*. While we were confident that counted cases were dengue, Zika, or chikungunya, we also recognized that assignment of a case to one of these diseases in particular was not very reliable. Only blood analysis could provide a reliable diagnosis, and blood analysis virtually never happened for MV residents, even with medical treatment.

The conservative count from monthly monitoring data did not include cases of dengue, Zika, and chikungunya that lacked characteristic symptoms such as body pain, joint pain or rash strongly enough for MV residents to mention them to a volunteer during a monthly monitoring visit. We therefore added another tabulation, which corresponded to the general belief in MV that any illness with fever and headache and no other dengue symptoms was probably dengue unless there were symptoms to suggest some other disease. We counted the number of illnesses each month with only fever and headache and no other symptoms. This tabulation included not only “milder” cases of dengue, Zika, or chikungunya that were missed by the first tabulation, but also illnesses with only fever and headache that were due to some other disease.

Figure 1 shows results from the “conservative” tabulation of illnesses with symptoms characteristic of dengue, Zika, or chikungunya reported by MV residents during the monthly monitoring. While there were large month-to-month fluctuations during 2016-2017, there was also a steady decline from a large number of cases in 2016 to no cases in October 2017, and except for two cases of dengue in 2018, no cases during the 31 months from October 2017 to the end of the record in March 2020. Symptoms indicated that 44% of the illnesses during 2016-2017 were Zika, 42% chikungunya, and 14% dengue. Five illnesses in 2018 with severe joint pain characteristic of chikungunya were not counted for Figure 1 because they were considered relapses of chikungunya contracted during 2016-2017.

Figure 2 shows monthly monitoring illnesses with only fever and headache and none of the other symptoms for dengue, Zika, or chikungunya. Figure 2 resembles Figure 1, having far more illnesses during 2016-2017 (averaging 7.4 cases/month) than during 2018-2020 (averaging 0.6 cases/month). While it is likely that some of the illnesses in Figure 2 were not dengue, the similar time pattern in Figures 1 and 2 suggests that at least some of the illnesses with only fever and headache were dengue, in agreement with the prevailing perception in MV that fever and headache are definitive dengue symptoms. We do not know how many, if any, of the relatively small number of illnesses in Figure 2 that continued into 2018-2020 were in fact dengue.

How much was MV’s *Ae. aegypti* program responsible for the disappearance of recognizable cases of dengue, Zika, and chikungunya seen in Figures 1? This question cannot be answered with complete confidence because Illnesses were assessed on the basis of symptoms, without confirmation from laboratory tests, and comparable data for dengue, Zika, and chikungunya were not collected at control communities over the same time period.

Nonetheless, a partial answer is possible by comparing data from MV with government statistics for dengue, Zika, and chikungunya in the surrounding area. Because the government did not routinely release statistics on these diseases, we used a Pan American Health Organization report (PAHO 2019) as the source for Honduran national statistics and obtained a few numbers for Cortés Department and San Pedro Sula and Choloma municipalities from the government’s regional health office and newspaper articles based on government sources. The number of cases reported in all government statistics was a substantial underrepresentation of actual cases because people did not seek medical attention unless a case was severe, but the figures could at least indicate higher and lower numbers for each disease during different time periods.

Table 1 shows what was happening nationally with dengue, Zika, and chikungunya during 2014-2019. When the project began in 2016, Honduras was in the middle of the same Zika epidemic that had overwhelmed much of Latin America. Although Zika was receiving most of the attention, the nation was also in the middle of a chikungunya epidemic that began the previous year, and endemic dengue was present in full force. Choloma municipality reported 728 dengue cases, 1720 Zika cases, and 708 chikungunya cases during 2016.

Zika and chikungunya dropped to very low levels during 2018-2019 (Table 1). Dengue declined during 2017 and increased during 2018 enough to record 915 cases of Dengue Hemorrhagic Fever in Cortés Department alone. There was a major dengue epidemic throughout Honduras in 2019 with 112,798 cases of dengue and 19,425 cases of Dengue Hemorrhagic Fever reported nationally, 17,551 reported cases of dengue in San Pedro Sula and Choloma municipalities combined, and 3163 cases of Dengue Hemorrhagic Fever in Cortés Department. Regional health workers observed large numbers of dengue cases everywhere in nearby communities similar to MV (Nancy Echeverría, personal communication). Quantitative data from such communities would have been desirable, but the MV project's small budget did not have the scope to monitor other communities, and even the capacity were there, many months of effort were required to obtain government approval to collect data in such communities.

The baseline survey in San Antonio during August 2019 was the main source of information about illnesses in a similar community. Residents reported 37 illnesses with strong dengue symptoms during the previous month. Some of the people with dengue symptions sought medical attention and were confirmed by physicians to have dengue. The high incidence of dengue in San Antonio was a stark contrast with the absence of dengue in MV throughout 2019 and probably reflected what would have happened in MV without its *Ae. aegypti* program.

What do the government statistics and San Antonio data tell us about how much MV's program was responsible for the apparent disappearance of the diseases? Part of the decline during 2016-2017 was undoubtedly due to the general decline of dengue, Zika, and chikungunya in the region during that period. However, the fact that the decline of dengue in MV went all the way to zero (or virtually zero) and stayed there, despite a significant dengue presence in the region during 2018 and a dengue epidemic during 2019, lends credibility to a conclusion that MV's control program contributed to the decline during 2016-2017 and could claim at least some responsibility for the absence of dengue after that.

Table 1. Number of reported cases of dengue, Zika, and chikungunya in Honduras.[1]

Year	Dengue[2]	DHF[3]	Zika	Chikungunya
2014	42,753	2,309	0	76
2015	44,834	1,062	56	76,791
2016	22,961	313	31,468	17,692
2017	5,217	126	120	532
2018	7,942	1,172	358	185

2019	112,798	19,435	240	219

1. Source: PAHO (2019). Reported cases are a small but unknown fraction of actual cases.
2. All dengue cases (including Dengue Hemorrhagic Fever).
3. Dengue Hemorrhagic Fever.

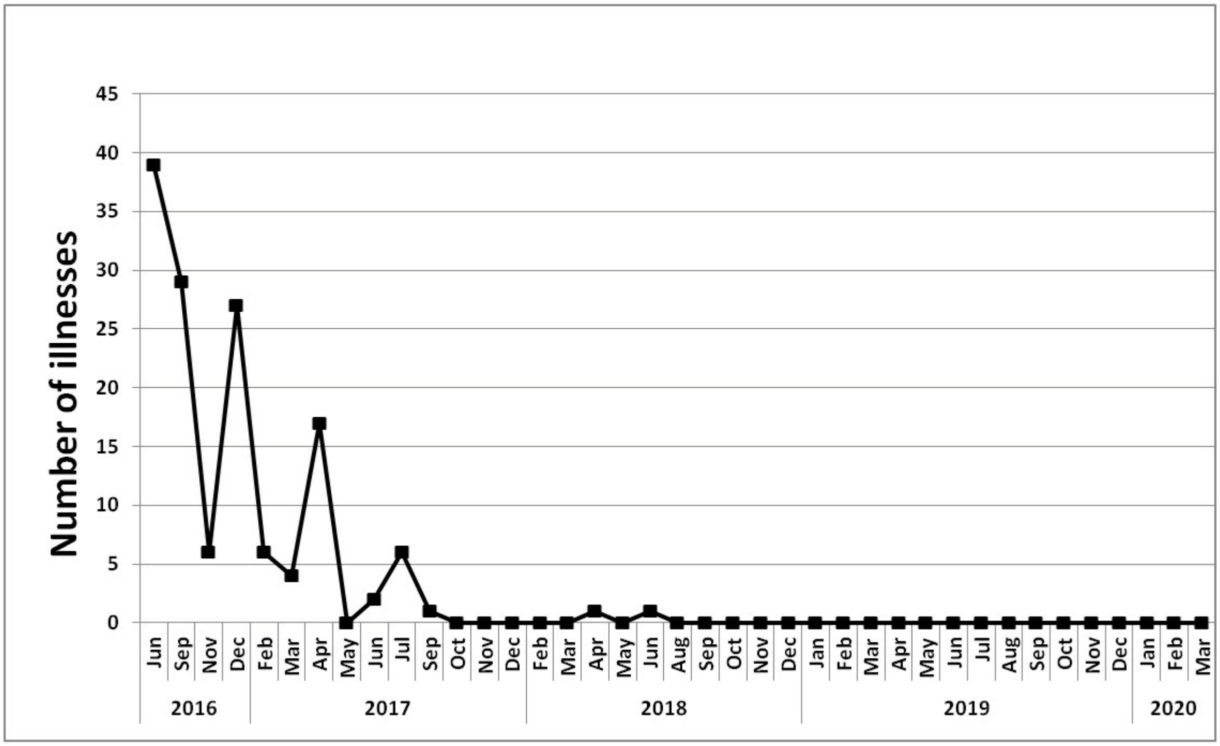


Figure 1. “Conservative” tabulation of the number of febrile illnesses with characteristic symptoms of dengue, Zika, or chikungunya reported by Monte Verde residents during monthly monitoring. The source for June 2016 is the medical brigade clinic.

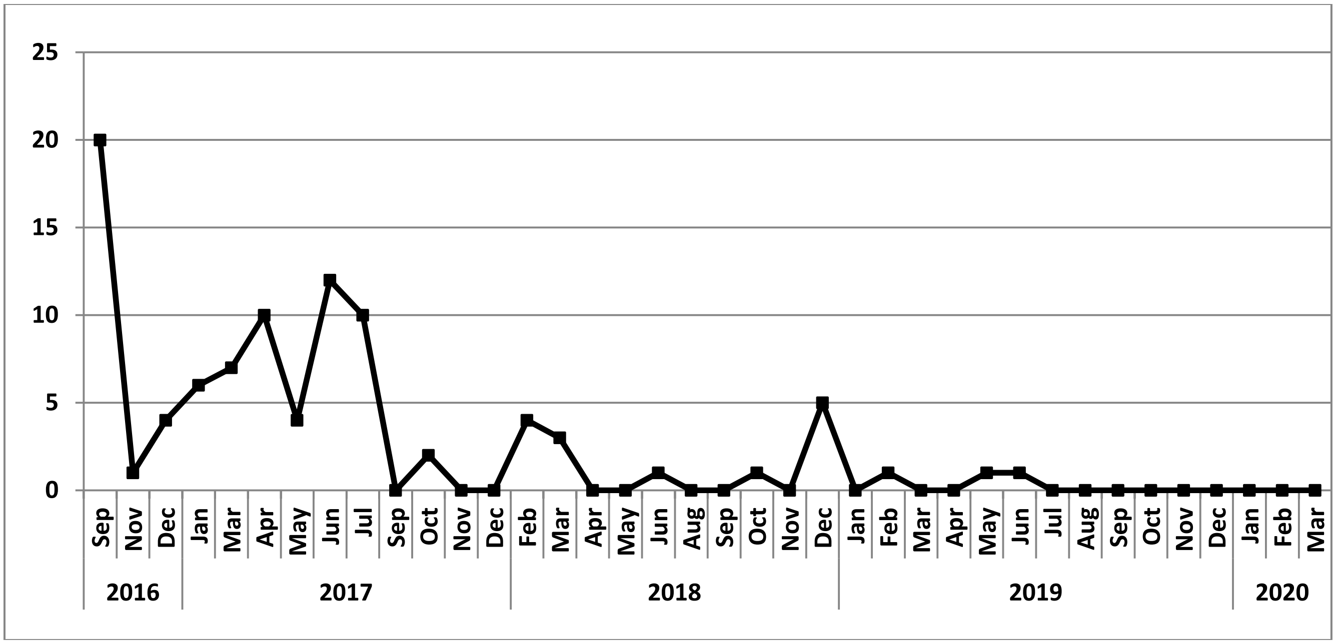


Figure 2. Number of illnesses with only fever and headache reported by Monte Verde residents during monthly monitoring. Some of these illnesses were probably not dengue, Zika, or chikungunya.

<u>References</u>

Elson W.H., Reiner R.C., Siles C., Bazan I., Vilcarromero S., Riley-Powell A.R., Kawiecki A.B., Astete H., Hontz R.D., Barker C.M, et al, 2020. Heterogeneity of dengue illness in community-based prospective study, Iquitos, Peru. Emerg Infect Dis 26, 2077-2086.

PAHO, 2019. Base de datos PLISA. Situación de Salud en las Américas: Indicadores básicos 2019. Departamento de Evidencia e Inteligencia para la Acción en Salud/Unidad de Análisis de Salud, Métricas y Evidencia. Pan American Health Organization, Washington, DC https://iris.paho.org/handle/10665.2/51543?locale-attribute=es

Supplement for "Proof of concept for eliminating *Aedes aegypti* production by means of integrated control including turtles, copepods, and tilapia in Monte Verde, Honduras (Gerald G. Marten, Xenia Caballero, Arnulfo Larios, Hilda Bendaña)

Supplement 4: Why was the project successful?

Why was community participation so effective?

One notable result of the project was the high level of community participation essential for effective *Ae. aegypti* control. How did this happen? The following are answers from volunteers and residents:

Interviews were conducted with project participants in 2020 to examine "Why was the project so successful?" In the end, "Why was community participation so good?" emerged as the central question. The scientific advisor interviewed the Operation Blessing Honduras facilitators, the facilitators interviewed volunteers, and the volunteers interviewed a sample of MV residents. Sometimes a single person was interviewed and other times a group of two or three. The interviews employed an "open-ended, semi-structured" discussion format, beginning with the central question after making sure that everyone was clear about it. Interviewers also had a short list of supporting questions that they wanted to cover, adding these questions to the discussion as needed. If an unexpected idea appeared, the discussion followed the idea and clarified it before returning to the main flow.

Volunteers

Why did people become volunteers? The educational meetings at the beginning of the project stimulated people with a history of community service for their church or MV's primary school to join the project. They said they felt abandoned by government. Attention from outsiders, including Operation Blessing's medical brigade and support for the school, made a big impression. The voracious appetites of turtles, copepods, and tilapia for mosquito larvae in demonstrations at the educational meetings offered these people intriguing possibilities for taking control of their lives and the well-being of their community. They were also keen on learning. What they learned about *Ae. aegypti*, the diseases, and biological control in the educational meetings was a revelation for people who previously were not aware of the connection between the insect larvae in the water storage containers and wells, the mosquitoes in their homes, and dengue, Zika, and chikungunya. Becoming a volunteer offered the opportunity to learn more, which was a personal reward in itself.

The volunteers felt that one major factor contributing to their success was the openness of MV residents, who from the outset welcomed their visits for monthly monitoring. The volunteers considered MV to be exceptional in this regard, pointing to the need for special measures to overcome low social solidarity when disseminating *Ae. aegypti* control to some other communities.

The volunteers said that the dramatic impact of turtles, copepods, and fish on mosquito larvae when they started using biological control at their own homes motivated them to extend the same to their neighbors. From there, a steady stream of small but tangible successes reinforced their commitment to do their best despite the heavy demands of the project. Continual improvement in community participation motivated them to confront every obstacle such as "problem houses" until they found a way to overcome it.

Residents

Residents said that attention from the volunteers, as neighbors who cared about them, was a major reason for cooperating with the project. The attention from volunteers made them feel greater personal value. Residents were inspired to persist in their effort to prevent mosquito production at their homes because they were impressed with the competence of the volunteers and looked forward to their visits. Residents were also impressed with the effectiveness of biological control, particularly turtles. Some gave a turtle to relatives in another community who had larvae in their *pila* there.

Assessment of the project strategy

With the research question "What are the details that will enable this strategy to function effectively?" as a point of reference, the volunteers worked out those details as citizen scientists. The following list summarizes some of the things that were learned about the project strategy.

***Beginning with education and gaining the support of local leaders*.** The educational activities at the beginning of the project were the catalyst for everything that followed. From the very beginning, it was important to secure the support and involvement of local leaders: neighborhood government, managers of the local water system, religious leaders, and schools. The volunteers appreciated the importance of placing reasonable demands on the limited time and resources of leaders and everyone else in the community.

Multiple methods in the toolkit. The multiplicity of methods in the toolkit proved essential for success by accommodating the differences among different kinds of larval habitats, the various ways that people used their water storage containers, and family preferences with regard to control methods. Biological control was effective as long as people followed simple procedures to use it properly and the water supply system was managed so the use of water storage containers could be compatible with the biological control. Turtles were the most effective method for *pilas* and drums. They eliminated all mosquito production as long as they were rotated to *pilas* or drums wherever and whenever larvae were seen. Copepods, turtles, and tilapia were all 100% effective for wells, copepods seeing the most use because they could be introduced and forgotten with no need to provide care. Copepods were also effective in cisterns and tires lining wells as long as they were reintroduced periodically. Tilapia could fill in for most larval habitats if there was a family preference. Other methods were essential to manage containers for which biological control was not suitable or not working at the moment:

for example, larvicides, removing larvae with a net or pouring them out, or storing containers so there was no water in them when not in use.

Targeting complete container coverage* and transforming breeding sites into *egg sinks. The "100% container coverage" policy and the principle of transforming as many *Ae. aegypti* breeding sites as possible into egg sinks were keys to success. *Pilas* called for extra attention because they were the most important source of the mosquitoes, the most recalcitrant larval habitat for complete container coverage, and the most demanding for a strong toolkit and family habits to consolidate control. "Problem houses" emerged as a major obstacle to achieving complete coverage, pointing to the importance of identifying problem houses early on so they can be managed properly.

***Volunteers* and *monthly* monitoring.** The crucial role of volunteers unfolded as the project progressed. The monthly monitoring, in which the volunteers visited every house and inspected all potential *Ae. aegypti* breeding sites, emerged as a powerful and ongoing learning experience for volunteers and residents to work together at refining the toolkit and mobilizing community participation. The volunteers found it most effective to visit each house in a group of two or three. They believed that they were taken more seriously as a group, and volunteers could consult one another about technical issues and how to communicate with residents during the visit. Patience and persistence were paramount. It took time for volunteers to earn the confidence of their neighbors. It took time for people to work out how to use the biological control and other methods in their own household. Some households lost their container management habits when monthly monitoring was discounted during the first three months of COCID lockdown, pointing to the importance of maintaining monthly monitoring to reinforce the habits, even when recurrent disaster makes monitoring difficult.

Ingredients for success

For a more penetrating view of why the project was successful, we used a lens of "ingredients for success" that the EcoTipping Points Project distilled from environmental success stories around the world (Marten 2005, 2015). The same ingredients were equally conspicuous in MV and are listed below to show how they were manifested there. The ingredients provide insights into the design of MV's toolkit and its implementation by the community.

Outside stimulation and facilitation. While action at the local level is essential, outsiders can be a source of fresh ideas. A success story typically begins when people from outside a community stimulate a shared awareness about a problem and introduce game-changing ideas and encouragement for dealing with it. The MV project began when Operation Blessing Honduras held community meetings to make people aware of connections between the mosquitoes, larvae in wells and water storage containers, diseases, and what they could do about it. Operation Blessing did not give MV money, but it did provide some material assistance to the school, and it assigned two full-time facilitators and a scientific advisor to provide technical and organizational support and encouragement for the project.

Shared community awareness and commitment. Genuine community participation with a shared understanding of the problem and what to do about it, and shared ownership of the action that follows, is essential for success. The community moves forward with its own decisions, manpower, and financial resources. MV's shared vision began with the community meetings. A shared understanding of the problem and what to do about it developed greater depth among the volunteers as they proceeded with their work. The volunteers spread this understanding to their neighbors, whose understanding and commitment likewise deepened as they became active participants in the program.

Enduring commitment of local leadership. Trusted and persistent leaders inspire the deep-rooted and continuing community commitment and participation necessary for success. MV's volunteers provided group leadership, commitment, and persistence. There were plenty of problems, but they overcame them. The volunteers developed a constructive working relationship with their neighbors, and the monthly monitoring routine provided the structure and consistency necessary for success despite discouraging setbacks.

Co-adaption between social system and ecosystem and "letting nature do the work." The community's social system and ecosystem co-adapt and fit together, functioning as a healthy and sustainable whole (Marten 2001). Designing a toolkit around biological control was a way of organizing the aquatic ecosystems in wells and water storage containers for nature to do the work of preventing mosquito production. Turtles, copepods, and tilapia worked 24 hours a day as long as people kept them healthy. Community implementation of the toolkit happened when community perceptions, values, knowledge, technology, organization, and social institutions all evolved to create a "social commons" to mesh with the local "environmental commons" of water storage containers and wells that provided larval habitat. Social and environmental gains went hand in hand. The toolkit and the community organization to put it into use were crafted to fit the realities of social conditions and larval habitats by focusing on how people used their water storage containers, how turtles, copepods, and tilapia could survive and function under those conditions, and what effort was reasonable to expect from families for maintaining biological control.

Rapid results and "success breeds success." Quick "payback" helps to mobilize community commitment. Once positive results begin to cascade through the social system and ecosystem, normal social, economic, and political processes take it from there. Success breeds success. Although it took more than a year for MV volunteers and their neighbors to work out the details of the toolkit, and another year to reduce mosquito production nearly to zero, people were able to see tangible results within months after the project began. Mosquito larvae disappeared from many wells and water storage containers soon after turtle, copepod, or tilapia introductions began. Perhaps most important, the diseases declined rapidly and virtually disappeared from Monte Verde within a year after the project began. Once people saw that biological control really worked, they were inspired to devote the attention and effort necessary to maintain copepods, turtles, or fish in their water storage containers with a target of 100% container coverage.

A powerful symbol. Prominent features of MV's story represented the entire process in a way that consolidated community commitment and mobilized community action. The turtles, with their charisma and impressive ability to wipe out mosquito larvae, became a symbol for the entire project. Success itself also became a symbol, as embodied in a "*zona libre*" sign proudly proclaiming MV to be free of the diseases (Figure 1). This pride inspired other community projects such as trash cleanup, which in turn fed back to reinforce commitment to the *Ae. aegypti* program in a spiral of "success breeds success."

Overcoming social obstacles. There can be numerous social obstacles to success. For example: competing demands for people's time, attention, and energy; people who feel threatened by innovation or other change take measures to suppress or nullify it; dysfunctional dependence on or control by some part of the status quo prevents people from moving forward with promising initiatives. A key to achieving necessary community participation in MV was a strong, personal working relationship between the volunteers and their neighbors. Even when a few households chose not to use biological control, they took responsibility for eliminating mosquito production in their own way. "Closed" and "abandoned" houses were a particular challenge, but respectful assertiveness by the volunteers eventually led to contact with the owners and securing their cooperation. While MV's *Ae. aegypti* control program was separate from the local community council and could have been perceived as a threat to its authority. However, the volunteers managed to maintain a constructive relationship with the council.

Social and ecological diversity. Diversity provides more choices, enriching the possibilities for good choices. Social diversity came from MV residents, Operation Blessing Honduras facilitators, and the scientific advisor all working together to craft solutions from a broad pool of backgrounds, talents, and ideas. The ecological diversity of biological control and other methods in the toolkit provided the choices that each family needed to fit the right method to each of its containers while also accommodating family preferences. "Social memory," learning from the past, added to the diversity of choices by providing choices that had proved effective and sustainable while withstanding the test of time. Because the scientific advisor had participated in Vietnam's *Ae. aegypti* program, he was able to share practical lessons from Vietnam, and Operation Blessing Honduras drew upon its prior community organizing experience with Honduran communities for safe water.

Building resilience. Resilience is the ability to continue functioning and sustain gains in the face of uncertainties and disruptions. A community's adaptive capacity – its ability to evolve and respond to challenges with prudent experimentation, learning from successes and mistakes, and replicating success – is central to resilience. The autonomy of the project with respect to MV's community council and other lines of authority in Honduras bolstered its adaptive capacity by preserving its flexibility for experimentation and learning. Another important part of resilience is avoiding dependence on unavailable or unreliable resources. Because the biological control and other methods did not require much money, the toolkit was doable and sustainable in a community with MV's limited financial resources. However, successful use of the toolkit did require strong community participation, a resource that MV was able to provide. The robustness of the monthly monitoring enabled rapid return of container coverage to previous levels after setbacks due to political turmoil, water system breakdown, and larvicide depletion.

MV was dependent on facilitation from Operation Blessing Honduras during the first three years of the project, but with experience, the community developed the self-sufficiency and the volunteers developed the competence and confidence as “citizen scientists,” community organizers, and project managers to continue the project on their own. Then, after Operation Blessing’s facilitation finished, the project’s leadership in MV reinforced the ability to improve and adapt the toolkit by maintaining a working relationship with Operation Blessing and the scientific advisor, ensuring ongoing access to technical support that might be needed for dealing with future challenges.

References

Marten, G.G. 2001. Coevolution and coadaptation of human social systems and ecosystems. In Marten G.G., Human Ecology: Basic Concepts for Sustainable Development. Earthscan Publications, p. 96-105. http://gerrymarten.com/human-ecology/chapter07.html

Marten G.G., Brooks S., Suutari A., 2005. Environmental tipping points: a new slant on strategic environmentalism. World Watch Magazine 18(6), 10-14. http://gerrymarten.com/publicatons/pdfs/ETP_Strategic-Environmentalism.pdf

Marten G.G., 2015. Learn about EcoTipping Points: Lessons learned – Ingredients for Success. http://ecotippingpoints.org/about-etps.html#lessons

Figure 1. Volunteers proudly displaying their sign proclaiming Monte Verde to be a “dengue, Zika, and chikungunya free zone.”