Annual report MRCU - June 2017
Friendly *Aedes aegypti* project in West Bay

Introduction

A collaborative project has been established between the Mosquito Research and Control Unit (MRCU) and Oxitec to deliver a pilot operational program of Oxitec's innovative control system for *Aedes aegypti*, by sustained release of the OX513A *Ae. aegypti* males (also known as Friendly *Ae. aegypti*). The objective of the project is to demonstrate the efficacy of the technology in an operational setting as a precursor for full integration into the MRCU operations across the Cayman Islands. The current project started in July 2016, and this report provides results up to June 25, 2017.

Oxitec's approach is based on a genetically modified strain of *Ae. aegypti* (OX513A) developed in 2002, which has two additional genes; a self-limiting gene, and a fluorescent marker. When OX513A male mosquitoes, that cannot bite and spread diseases, are released they seek out and mate with wild females. All the offspring fathered by the OX513A males inherit the two additional genes. The self-limiting gene causes descendants to die before adulthood; hence repeated releases of Oxitec males cause reductions in the successive generations. The fluorescent marker enables dynamic adaptive management of the release rate to ensure suppression of the local *Ae. aegypti* population.

Oxitec delivers this solution in conjunction with local teams and adapted to local circumstances including the use of complementary vector control tools such as breeding-site reduction, public education and chemical insecticides. Projects begin with a period of community engagement and infestation monitoring before starting releases of Friendly *Ae. aegypti*. The location of release points and number of Friendly *Ae. aegypti* released are adapted in response to the local infestation as informed by weekly monitoring.

In the Cayman Islands, the Friendly *Aedes aegypti* project was announced on May 5th 2016 followed by an extensive program of community engagement activities that ensured the majority support of Caymanians. The first release was conducted on July 28th 2016 at the Public Health Clinic of West Bay in presence of Timothy McLaughlin, the Public Health surveillance officer. The production and releases were ramped up and OX513A males started to be distributed in the treatment area mid-August. In parallel, the monitoring in the treatment and comparator areas, started in 2015, continued in order to evaluate the suppression of the local *Ae. aegypti* population and enable dynamic adaptive management of the programme.

Community engagement

The Friendly *Aedes aegypti* project started with an intense community engagement program utilising a wide variety of methods adapted to the local conditions and culture. Communication strategy was developed in collaboration and with support from public services. Both the Mosquito Research and Control Unit and the Public Health Department provided resources to help spread information and answer questions about the project.

A number of activities were conducted including door-to-door visits in the release area, information booths and distribution of leaflets in several locations on Grand Cayman, a public meeting, radio and television talk shows as well as public service messages in newspapers and on radio.

A public opinion survey conducted in July 2016 revealed that 80% of the population of Grand Cayman had heard about the project and that 69% supported the Friendly *Aedes aegypti* project, validating the strategy followed. Activities to reach out to the community have continued since the beginning of the releases and will continue throughout the project.

Releases

The first release of OX513A *Ae. aegypti* males was conducted on July 28th 2016. The production was ramped up to operational schedule, three batches per week, from the beginning of August 2016. Consequently, full operational releases effectively started in mid-August with average level of circa 300,000 OX513A males per week (from weeks 2016-33 to 2016-43, Figure 1A). There was a temporary drop in target production during weeks 2016-44 and 2016-45 (Figure 1A). This was due to disruption in transportation of egg shipments from the UK due to Hurricane Matthew.

Subsequently the release rate increased by approximately 33% from circa 300,000 to 400,000 OX513A males per week between weeks 2016-47 and 2017-10. Increase in production from initial rates was in response to the high mosquito population, due to seasonality, and the corresponding below target percentage of fluorescent larvae recovered from ovitraps. Afterwards, the release rate averaged 250,000 males per week until now as the population levels dropped due to the dry season and suppression. The percentage of fluorescent larvae is a proxy for the fraction of wild females mated with OX513A males; a target of over 50% is recommended for operational deployment. The production was increased by moving the maturation and storage of the adults into a nearby facility and increasing production space.

The quality of the males released was confirmed by the quality control processes conducted in the production facility ensuring that the OX513A males were in optimal conditions to find and mate with the wild *Ae. aegypti* females.

Date	**Quantity**
27 Jun 2016	150g
01 Aug 2016	180g
05 Sep 2016	180g
03 Oct 2016	157g
17 Oct 2016	138g
31 Oct 2016	216g
28 Nov 2016	270g
05 Dec 2016	135g
19 Dec 2016	216g
23 Jan 2017	216g
20 Feb 2017	270g
20 Mar 2017	270g
10 Apr 2017	234g
22 May 2017	504g
Total	**3136g**
Permit	**3140g**

Table 1: Date and quantity of eggs shipped from the UK to Cayman Islands.

Egg shipments

Egg shipment were typically sent every month, except in October due to Hurricane Matthew. The quantities of eggs shipped were increased from October to support the additional production (Table 1).

Egg quality on arrival was assessed upon arrival on island and some shipments were identified as not conforming to the standard expected. An investigation was conducted to identify the cause of the decrease in quality; it revealed that the storage conditions in UK and Grand Cayman could be improved for better preservation of the eggs. Consequently, storage conditions in the UK and in Grand Cayman have been optimised in terms of temperature and humidity to ensure an optimal production of males for release.

Quality Assurance

After larvae rearing, pupae are separated from larvae. Male and female pupae are separated based on their size difference using a calibrated wire sorter. The sex sorting efficiency is determined as in-process control. As acceptance criterion, sorting efficiency of ≤ 2 females / 1000 pupae is defined. If this criterion is not met, the batch is resorted and retested until the criterion is met. OX513A male batches that comply with the sorting criterion are used for setting up release pots.

On May 12th 2017, MRCU collected a release pot from production batch MRU0128 and screened it for the presence of females. Nine females were found in the pot by MRCU agent. Oxitec was informed about this event after release of the male batch on May 13th 2017.

Review of batch documentation for male release of batch MRU0128 showed that all production steps and in-process controls were completed as defined. Two pools of sorted male pupae were generated, P1M and P2M. Both were tested for sex sorting efficiency on May 10th 2017 and complied with no female pupae in 1000 pupae for each pool. On May 12th 2017 the site lead and project manager reviewed and approved the in process control. Males of batch MRU0128 were released on May 13th 2017.

Sex sorting and determination of sex sorting efficiency are highly manual and operator-dependent processes. Due to the highly operator-dependent nature of sex sorting and efficiency determination, this event has revealed the need for additional control measures which have been implemented:

1) Double check of pupae sex sorting efficiency by Quality Assurance or Project Management.
2) Extension of the In-Process Control on release pots: Determination of live female adults in one release pot per batch, acceptance criterion ≤ 2 live female adults / 1000 release pot (≤ 1 live female adults / 500 release pot).
   If the acceptance criterion is not met, the sampling is increased to a total of four release pots. The overall acceptance criterion is ≤ 8 live female adults / 4 1000 release pots (≤ 4 live female adults / 4 500 release pots).
   If the overall acceptance criterion is not met, the batch is discarded and not released.
3) Yearly retraining of staff on applicable procedures.

Since the additional measures have been in place, MRCU has continued checking release pots and found no females in the release pots checked since May 12th 2017 (3 batches). Oxitec has found no or ≤2 females in the release pots since May 12th 2017. This indicates that the sex sorting is performed to the expected standard and ensuring no more than 2 females are released per 1,000 males.

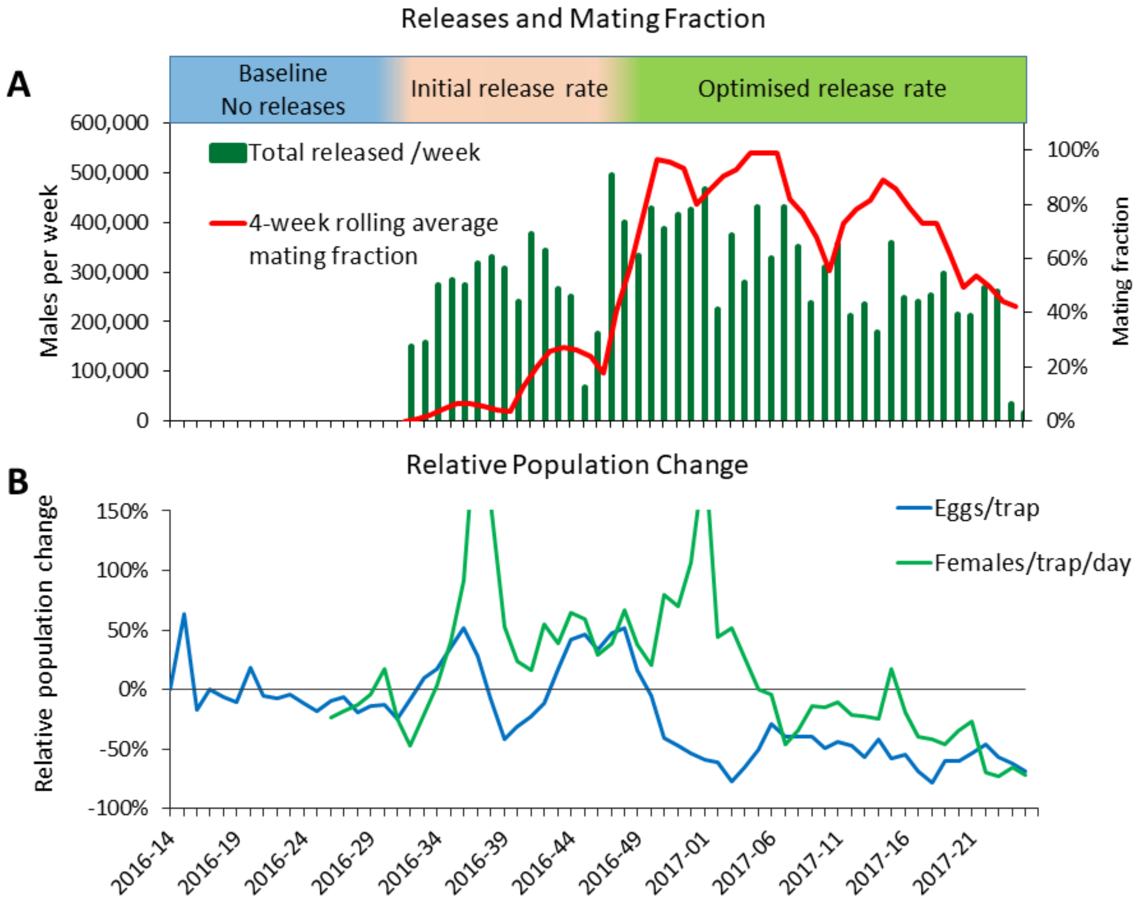

Figure 1: (**A**) Number OX513A males released per week and Mating Fraction (4-week rolling average of the percentage of marked fluorescent larvae) observed in the release area of West Bay. The dotted line indicates two missing data points; (**B**) Relative change in population in the release area relative to the comparator area for the number of eggs per ovitrap and number of females captured per trap per day in BG traps. A negative value indicates suppression in the release area.

Monitoring

Regular weekly ovitrap and adult surveillance were initiated before releases began and has been maintained to assess local population infestation in the release and the comparator areas. Ovitraps mimic natural breeding sites of Aedine mosquitoes and are used to collect eggs laid by females. They provide a sensitive measure of both presence (Ovitrap Index: proportion of positive traps) and abundance (Average number of eggs per trap) of the local population of *Ae. aegypti*. Adult surveillance is conducted using BG-sentinel traps that mimic the human host and attract and capture both male and female *Aedes aegypti* using visual and olfactory cues. They provide an indicator of the abundance (average number of females caught per trap per day) of the local population of *Ae. Aegypti.*

Within the release area, ovitraps can additionally be used to assess mating fraction (proportion of the wild females mated with OX513A males) by detecting the fluorescent marker in the larvae hatched from the eggs collected. This key metric allows dynamic adaptive management of the releases to respond to the local population density thereby ensuring efficiency. For operational use, we target over 50 % mating fraction for rapid suppression.

Through regular monitoring of the population in both the treatment and comparator areas, a sharp seasonal increase in *Ae. aegypti* population in early June 2016 was detected and maintained until November 2016 at the end of the rainy season when the population decreased in both areas. Average number of eggs per trap was approximately 5-fold higher in the high season compared to the preceding low season (mean of 7 and 7 eggs/trap in low season vs. 33 and 36 eggs/trap high season for release and comparator areas respectively). Infestation levels in the release and comparator areas remained comparable through pre-release baseline monitoring into high season when releases were initiated. Mating Fraction was assessed in the release area from initiation of releases with fluorescent larvae being detected in the ovitraps in August. Due to the pre-mated females present before the start of the releases - female *Ae. aegypti* mate only once in a lifetime of 2 to 4 weeks - it is expected that the fluorescence will increase in the 4 weeks of initial releases before stabilising. Although increasing from 5% in August/September to 28% in October/November, it did not go beyond the 50% fluorescent rate target (Figure 1A). From mid-November, onwards there was a sharp increase in mating fraction, averaging 77% (standard error of the mean [sem]: 4.7%) from week 2016-47 to 2017-23 well above the 50% target. This corresponded with both, an initial increase in release rate by approximately 33% and the start of the dry season resulting in seasonal drop in population of *Ae. aegypti*. Both these factors likely contributed to higher numbers of released OX513A males compared to local male counterpart.

Adaptive Management

In accordance with adaptive management approach to Oxitec's control strategy, a two-fold strategy was adopted to achieve the 50% fluorescence target, when this was not initially attained under the challenging conditions of seasonally driven high infestation rates; an increase in release rates, coupled with insecticide applications in an integrated vector control approach.

Increased release rate

The production unit was expanded in order to allow a 33% increase in total production in late 2016 and early 2017. In order to do this the maturation and storage of the adults was transferred to a nearby facility increasing overall production space.

Traditional control

During the rainy season, *Ae. aegypti* population levels are high and an integrated vector management (IVM) approach is desirable to achieve suppression. MRCU typically implements multiple methods to tackle *Ae. aegypti* populations including; house surveys, thermal-fogging and aerial pesticide applications particularly in response to dengue, chikungunya or Zika virus case reports. In response to the below-target mating fraction and high mosquito numbers, an IVM consisting of aerial applied larvicide and adulticide sprays was initiated. Both release and comparator areas would be exposed to the same larvicide and adulticide applications to ensure adequate assessment of the impact of OX513A releases (Table 2).

- Aerial larvicide was applied on October 20th 2016 in both areas; a second application was planned the following week for optimal impact on the population of *Ae. aegypti* but could not be conducted due to strong winds. Strong winds persisted throughout November and prevented a second application.
- Biweekly aerial application of adulticides were also planned. However, the high winds also prevented these scheduled applications of pesticide at the optimal time (1h before sunset) to target *Ae. aegypti* during November 2016. Six applications (during sunset) targeted at *Aedes taeniorhynchus*, the

black salt marsh mosquito, were conducted over both areas between October 29th 2016 and November 16th 2016 (this was part of the *Ae. taenyorhyncus* standard MRCU control).

Although larvicide and adulticides could not be applied in optimal conditions, it is likely that they contributed to the decrease of the *Ae. aegypti* population in both areas in November 2016 together with the start of the dry season (lower rainfalls, high winds and decrease in temperature).

Date	Treatment	Formulation	Target species
October 20, 2016	Larvicide	*Bacillus thuringiensis* (*Bti*)	*Aedes aegypti*
October 29, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
October 31, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
November 5, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
November 6, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
November 11, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*
November 16, 2016	Adulticide	Chlorpyrifos (Mosquito Mist 2)	*Aedes taeniorhynchus*

Table 2. Larvicide and Adulticide applications in the release and comparator areas conducted in 2016. The time of application of the adulticide is optimized for the species targeted as they have different activity periods. An application outside the peak activity period has a reduced effect on the species.

<u>Population suppression</u>

Aedes aegypti populations are subjected to substantial variations depending on meteorological conditions (temperature, humidity and rainfall). In Cayman, this is manifest by higher mosquito populations in the wet hotter season (May to November: avg. 28.3C, 130mm rainfall monthly) and lower in the colder dryer season (December to April: avg. 25.5C, 60mm rainfall monthly).

Consequently, it makes the assessment of the local population in terms of absolute number difficult to interpret over prolonged periods and seasons. The impact of the Oxitec programme is therefore assessed in terms of change in the population of the release area relative to a comparator site. This approach accounts for underlying seasonally driven effect and any impact of conventional controls that were applied to both sites. The ongoing change in the population of the release site relative to the comparator site is presented in Figure 1B as 4 week rolling average values.

Until week 2016-51, the *Ae. aegypti* populations naturally fluctuated in both areas but remained comparatively stable (relative change average 4% (sem: 4.5%) through the low and high seasons, even though populations had increased by a factor of 5 in the high season (7 and 7 eggs/trap in low season vs. 33 and 36 eggs/trap in the high season for the release and comparator areas respectively). This indicates that, before initiation of the project, populations in both areas were similar throughout seasons and subjected to similar weather conditions.

Following the high mating fraction from week 2016-47, steady and sustained *Ae. aegypti* population suppression was observed from week 2016-51 onwards (Figure 1A) in terms of eggs per trap. The one to two-month lag between sustained high mating fraction and impact on population is typical of the technology as the generation time for *Ae. aegypti* is 2 to 4 weeks.

The adult monitoring shows a similar trend, though of a lower amplitude initially, in terms of population suppression the 2017 but did not show any population suppression until week 2017-07. This delay might be the result of the difference in sampling between the two kind of traps: (1) the adult trap captures females that are looking for a host and a blood meal before they can lay eggs; (2) the ovitrap collects eggs from females that have already blood-fed and are ready to lay eggs. These two population of females might not have a linear relationship resulting in differences in the population change level recorded.

Comparing the eggs/trap ratios between the two areas over 2016 and 2017 wet seasons gives a 62% suppression in the release area (Figure 2, 0.92 vs. 0.35). This figure highlights the importance of monitoring a comparator area as the natural level of the *Ae. aegypti* population during the 8 weeks considered in 2017 in the comparator area (46 +/- 7.5; week 2017-18 to 2017-24) is higher than the 21 weeks average in 2016 (40 +/- 4.8; week 2016-24 to 2016-44) likely due to meteorological conditions and traditional control methods. This level of suppression is to be expected at this stage of the programme as the egg bank from last year is hatching due to the first rains. As the population was not under control at the end of last wet season the egg bank in the treatment area induced an increase of the population in the treatment area as well. However, it is likely that a part of the eggs was fathered by Oxitec males, hence the smaller increase in population than the comparator area, resulting in a higher level of suppression being recorded (Figure 3). This population will die and the remaining population in the release area should continue decreasing and result in a higher level of suppression in the coming months.

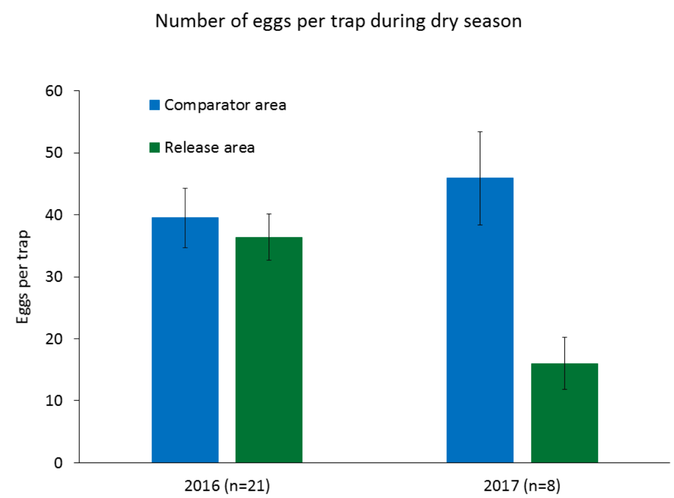

Figure 2: Average number of eggs collected in ovitraps in the release and comparator areas during the dry seasons of 2016 (before OX513A releases) and 2017 (after OX513A releases). A 65% suppression of the *Ae. aegypti* population was observed between the two periods.

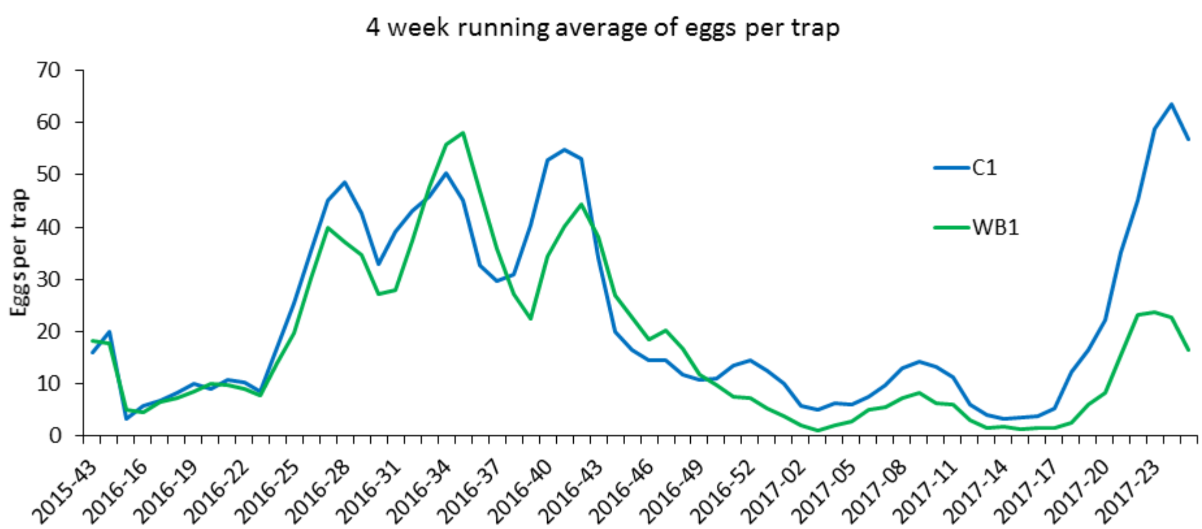

Figure 3: rolling average of the number of eggs per trap in the release (WB1) and comparator (C1) areas.

Conclusion

The sustained high mating fraction over the 50 % target during the last 7 months is a clear indication that the released males are mating a large majority of the wild females and competitively overwhelming the wild males in the release area. The impact on the wild population is already visible and we are now observing a 62% suppression of the *Ae. aegypti* population in the area after the start of the wet season (7 weeks). The monitoring of the release and comparator areas has enabled the evaluation to take into account seasonal variations to obtain an unbiased estimation of the suppression measured in the release area regardless of the natural decrease due to the start of the wet season.

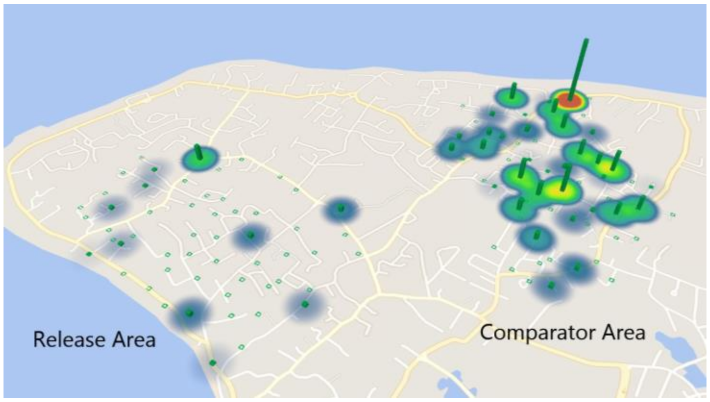

Figure 4: Heat map representing the number of eggs in individual ovitraps in the comparator and release areas in West Bay (60 in each areas) during week 2017-24.

This outcome is within expectations according to previous field suppression observed in Cayman Islands, Brazil and Panama with substantial suppression observed within 4 to 6 months following start of releases at an adequate rate. The observed level of suppression was limited during the dry season as the population level was low in the comparator area as well. However, the release area has shown consistent lower levels of infestation for the whole of 2017, confirming that the population has been suppressed. The map of the number of eggs in each individual trap in both areas show an important suppression in the release area for the last week of collection (Figure 4). The continuation of the releases and maintenance of a high mating fraction throughout the beginning of the wet season and the control of the egg bank from the end of the 2016 wet season are expected to consolidate the suppression of the *Ae. aegypti* population in the release area.

The adaptive management of the releases thanks to the fluorescent marker has enabled the programme to fit the local *Ae. aegypti* population requirements for suppression and the integration of traditional control methods. Integrated vector management using larvicides and adulticides, though difficult to evaluate, could have played a role in the rapid decrease of the populations in both areas in November 2016. The exact synergy between Oxitec and other control methods used by MRCU could be further evaluated in the future to optimise the impact on the wild population and reduce the cost of *Ae. aegypti* control.