ROBOTS AND SOCIETY

Field deployment of *Wolbachia*-infected *Aedes aegypti* using uncrewed aerial vehicle

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Over the past 50 years, there has been a marked increase in diseases like dengue fever, chikungunya, and Zika. The World Mosquito Program (WMP) has developed an approach that, instead of attempting to eliminate vector species, introduces *Wolbachia* into native *Aedes aegypti* populations through the release of *Wolbachia*-infected mosquitoes. Using this approach, a randomized controlled study recently demonstrated a 77% reduction in dengue across a treatment area within Yogyakarta, Indonesia. Existing release methods use the ground-based release of mosquito eggs or adults that are labor-intensive, are logistically challenging to scale up, and can be restrictive in areas where staff safety is a concern. To overcome these limitations, we developed a fully automated mosquito dosing release system that released smaller cohorts of mosquitoes over a wide area and integrated it into an uncrewed aerial vehicle. We established the effectiveness of this system using an aerial mark, release, and recapture approach. We then demonstrated that using only the aerial release method, we can establish *Wolbachia* infection in a naive *Ae. aegypti* population. In both cases, the use of aerial releases demonstrated comparable outcomes to ground-based releases without the required labor or risk. These two trials demonstrated the feasibility of using an aerial release approach for large-scale mosquito releases.

INTRODUCTION

Aedes aegypti is a medically important arthropod vector, responsible for transmitting serious arboviral diseases, such as dengue fever, chikungunya, yellow fever, and Zika. The geographical spread of this species has greatly increased because of rising trends in globalization and urbanization (*1*). The warmer and wetter conditions caused by climate change also prolong the length of the transmission season and expand the geographical range favorable for viral replication and mosquito vector survival (*2*, *3*). As a result, approximately 53% of the global population lives in areas that are environmentally suitable for dengue transmission, with the vast majority in Asia, followed by Africa and the Americas, affecting more than 100 countries (*4*, *5*). Global dengue incidence has increased 30-fold in the past 50 years, with an estimated 390 million cases per year and approximately 10,000 annual deaths reported (*5*, *6*), causing considerable economic burden on the government, local communities, and health care systems.

Effective prevention and control of dengue epidemics requires an integrated approach because there is no single effective method (*7*). One population reduction strategy is the use of the sterile insect technique (SIT), whereby irradiated sterile male mosquitoes are released into the field to mate with wild females and temporarily induce population sterility as nonviable progeny are produced (*8*). Another conceptually similar but methodologically different strategy is the use of the incompatible insect technique (IIT), which relies on the natural phenomenon known as cytoplasmic incompatibility (CI) induced by *Wolbachia* that results in nonviable offspring when *Wolbachia*-infected males mate with wild females that either do not carry *Wolbachia* or do not harbor the same *Wolbachia* strain (*9*, *10*). The combined IIT-SIT technique has been shown to effectively suppress mosquito populations in open-field trials for *Aedes albopictus* (*11*) and *Ae. aegypti* (*12*). However, these techniques require continual release of large numbers of males into the area, and efficacy has not been demonstrated at scale (*13*, *14*).

An alternative approach that circumvents the issue of continual release is the introgression of a disease refractory factor into a target *Ae. aegypti* population. This can be achieved by releasing a mix of *Wolbachia*-infected females and males and allowing *Wolbachia* to naturally introgress into a target population (*15*–*22*). The *w*Mel strain of *Wolbachia*, isolated from *Drosophila melanogaster*, has three core traits when introduced in *Ae. aegypti*: first, maternal inheritance from mother to all offspring regardless of infection status of the father; second, CI wherein *Wolbachia*-infected males induce infertility when they mate with uninfected females; and third, inhibition of viral replication in the host mosquitoes (*16*, *21*). The World Mosquito Program (WMP) has introduced *Wolbachia* into target *Ae. aegypti* populations in 14 countries [Mexico, Colombia (*19*, *23*), Brazil (*24*–*26*), Australia (*21*, *22*), Vietnam (*27*), Indonesia (*17*, *20*), Fiji (*18*), Kiribati (*18*), Vanuatu (*18*), New Caledonia (*28*), Sri Lanka, Honduras, and Laos] where the releases have either been completed or are currently underway. These releases demonstrated that *w*Mel *Wolbachia* can be established in local *Ae. aegypti* populations and persists for more than 10 years after release (*29*). Moreover, a randomized controlled trial in Yogyakarta, Indonesia resulted in a 77% reduction in dengue infections and an 86% reduction in dengue hospitalizations throughout *Wolbachia* treatment areas relative to untreated control areas (*17*).

Public health outcomes are highly influenced by population demographics. This includes greater adverse outcomes for women, children, and people of lower socioeconomic backgrounds when suffering from dengue infections (*30*–*32*). The *Wolbachia* method is not dependent on any specific characteristics of underlying population demographics. Because *Wolbachia* is an area-based public health intervention, once established in an *Ae. aegypti* population, it provides equal protection to all individuals therein. To maximize

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protection, individuals must consistently remain in *Wolbachia*-infected areas. Therefore, the scalability of *Wolbachia* deployment becomes crucial, especially across large and often difficult areas.

Wolbachia-infected mosquito releases of eggs and adult stages have involved a variety of methods, including directly by project staff and by community groups such as schoolchildren, businesses, and individual householders (*21*). However, ground-based releases are anticipated to scale in a highly linear way with respect to the size of a release. Hence, releases over large areas have many logistic challenges, including the large workforce and number of vehicles required, traffic congestion, and safety risks. This was further compounded by the coronavirus disease 2019 pandemic. We have been exploring options to ensure that the *Wolbachia* method remains viable at scale while also reducing risks to personnel.

Adult mosquito releases via air are being considered as a potential solution. It is hoped this will provide an efficient and low-cost method of scaling up deployment and thus the establishment of *Wolbachia*-infected mosquitoes over areas larger than 100 km^2. Aerial release strategies using small aircraft have already been used by area-wide integrated pest management programs in the attempt to eradicate the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (*33*, *34*) and tsetse fly *Glossina austeni* (Diptera: Glossinidae) (*35*). Recently, a small-scale, uncrewed aerial vehicle (UAV) aerial release of sterile *Ae. aegypti* has been trialed in Brazil that showed homogenous dispersal of approximately 200,000 high-quality males across 0.2 km^2 (*36*). Here, we report the successful outcomes from two open field trials in Fiji showing the feasibility of using a UAV to release *Wolbachia*-infected *Ae. aegypti* and providing a robust solution to a scalable population replacement implementation strategy. In the first trial, we demonstrated similar dispersal uniformity between UAV-based release and ground release methods. In the second trial, we established *Wolbachia* infections in the native *Ae. aegypti* populations over a 2-km^2 trial site in Fiji using UAV-based release strategy alone.

RESULTS

Adult release mechanism design and laboratory validation of mosquito dosing and viability

The UAV-mounted adult release system was designed to release 150 ± 50 mosquitoes per dose. The release mechanism contains four mosquito storage canisters, each capable of carrying approximately 40,000 adult mosquitoes, totaling a maximum capacity of 160,0000. Mosquito dosing was done in two stages, with the first stage separating mosquitoes into batches of 1500 and the second stage subdividing the mosquitoes into groups of 150, which are then propelled through the output area during release. To avoid mosquitoes waking up and clumping inside the release mechanism, which could compromise dosing accuracy and mosquito viability, temperature and humidity control units consisted of ice packs, silica beads, and fans, and temperature and humidity sensors were incorporated to maintain the internal environment at 7° to 10°C with relative humidity (RH) between 60 and 80%, all enclosed within an insulation foam box (Fig. 1A). The electronics consisted of a number of custom-designed printed circuit boards and an onboard Linux-based computer.

The initial laboratory tests were performed to determine the mosquito output quantity and consistency by specifically testing the dosing unit of the adult release mechanism in the cold room at 4°C. Our tests showed that the average dose was 203 mosquitoes (SD = 37.58, SEM = 6.644, $n = 32$) and 200 mosquitoes (SD = 48.55, SEM = 9.176, $n = 28$) per output in replicates 1 and 2, respectively (Fig. 1B). Next, we determined whether the release mechanism could reliably release mosquitoes at warmer temperatures and higher humidity, conditions more closely resembling those of potential release environments. The full release mechanism including the internal temperature and humidity control was tested at 26°C and 70% RH. We showed that the release mechanism released an average of 149 mosquitoes (SD = 37.95, SEM = 7.172, $n = 28$) and 152.2 mosquitoes (SD = 38.07, SEM = 7.070, $n = 29$) per output in replicates 1 and 2, respectively (Fig. 1B). These numbers met the design target of 150 ± 50 mosquitoes per dose.

In addition, we also assessed mosquito mortality and longevity over a 2-week period after mosquito dosing and release by the release mechanism because the strong negative effects of this mechanical process on mosquito viability would render this method unusable for deployments. We found that there was no statistically significant difference in the immediate mortality of mosquitoes that were dosed and ejected from the release mechanism when compared to the immobilization control (mosquitoes that were cold-immobilized but not loaded into the release mechanism) and the packing control (mosquitoes that were cold immobilized and packed inside the storage canister of the release mechanism but did not undergo the dosing and release processes) [$P > 0.05$, ordinary one-way analysis of variance (ANOVA)] (Fig. 1C). No statistically significant difference was observed in female longevity of the ejected mosquitoes compared to those of the immobilization, packing, and rearing controls ($P > 0.05$, log-rank Mantel-Cox test) (Fig. 1D). We noticed that the immobilized control males showed a significant reduction in longevity compared with those of the ejected males and the other two control groups, rearing and packing controls ($P < 0.05$, log-rank Mantel-Cox test) (Fig. 1E). However, because the ejected group that underwent the same immobilization procedure did not show a statistically significant decline in longevity, this suggests that the short-lived immobilized mosquitoes are caused by factors other than the cold immobilization and the mechanical processes of the release mechanism. Together, these data showed that the release mechanism had no notable adverse effects on mosquito viability.

Community engagement

Before commencing the field trials, we sought approval from the local communities. In UAV trial I, prerelease surveys of 50 households in Nakasi assessed overall comfort, familiarity with WMP, the *Wolbachia* method, and proposed UAV deployments. Seventy-two percent of households felt positive (36 of 50) about using UAV technology to release *Wolbachia*-infected mosquitoes, 26% felt neutral (13 of 50), and 2% felt negative (1 of 50). Awareness was high, with 86% having heard of WMP's *Wolbachia* method and 66% aware of the proposed UAV deployment.

In UAV trial II, prerelease surveys of 103 residents in Nausori were assessed. A notable 99% of participants both were comfortable with and accepted the proposal (102 of 103). In addition, awareness was substantial, with 95% having heard of the *Wolbachia* method, 92% aware of WMP, and 89% familiar with the proposed UAV deployment.

Field trial I—Comparing mosquito dispersal by aerial and ground release methods

After the laboratory validation of the release mechanism and obtaining promising results on dosing consistency and mosquito viability, we integrated the adult release mechanism into the UAV, a DJI

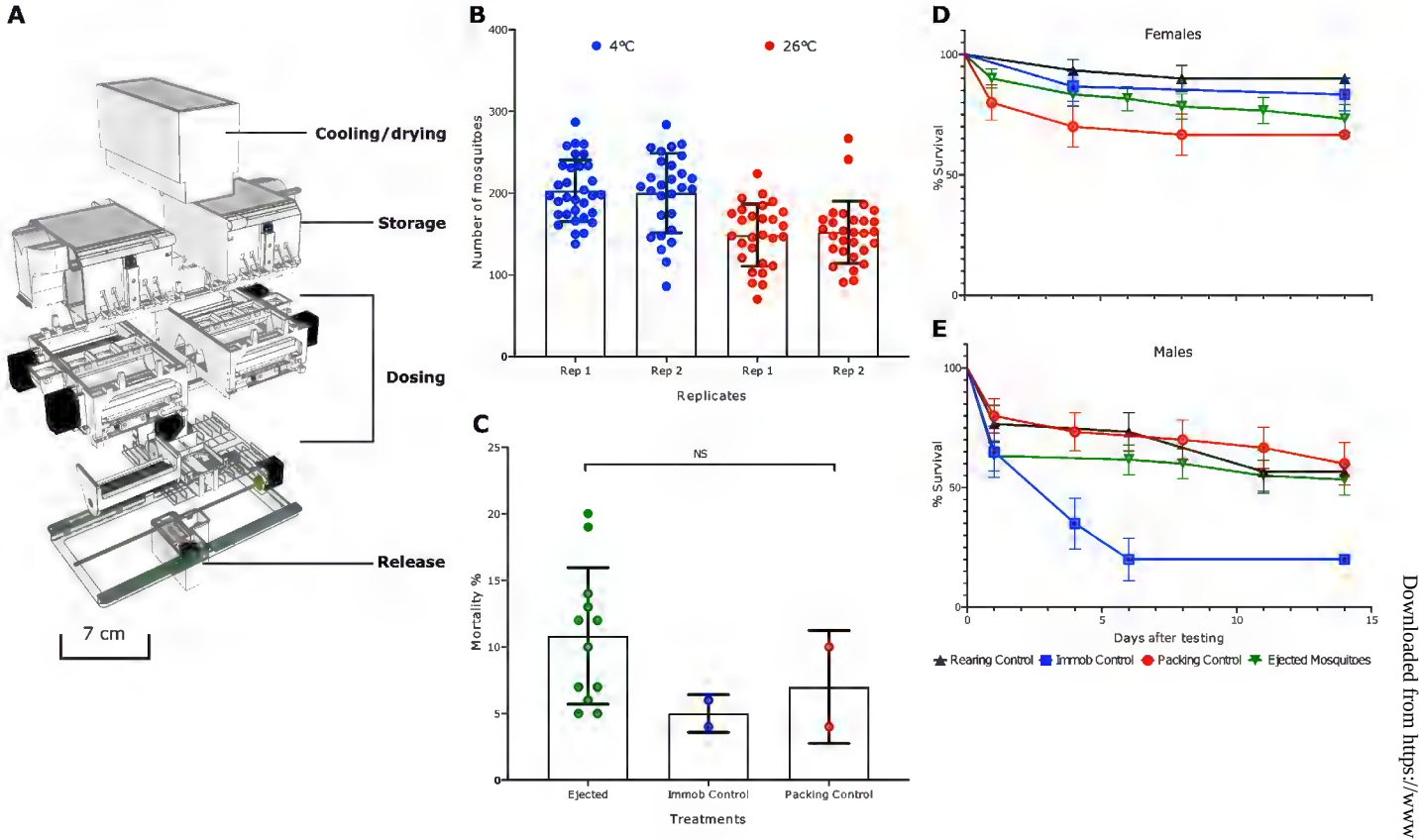

Fig. 1. Mosquito dosing and quality from the automated release mechanism. (**A**) Adult release mechanism design. Scale bar indicates 7 cm. (**B**) Number of mosquitoes released by the release mechanism. Data are presented as means ± SD ($N = 28$ to 30). (**C**) Mosquito mortality. Duplicate samples of approximately 80 mosquitoes were collected immediately after cold immobilization at 4°C for 11 min (immobilization control, blue dots) and retrieved from the release mechanism storage canister after 1.5 hours of storage (packing control, red dots). Mosquito mortalities were compared with those of mosquitoes collected from the release mechanism output (ejected, green dots), and statistical analysis was performed using ordinary one-way ANOVA (NS, not statistically significant, $P > 0.1$). Data are presented as means ± SD ($N = 2$ to 12). Mosquito longevity over 14 days was monitored for (**D**) females and for (**E**) males. Triplicates of 10 males (except duplicates of 10 males were monitored for immobilization control) and 10 females were maintained at 26°C, 65% RH, and a 12:12-hour light:dark cycle in a climate-controlled room. The number of dead mosquitoes was counted every second day. Statistical analysis was performed using a log-rank (Mantel-Cox) test. Data are presented as means ± SE ($N = 3$). For females, a small significant difference in longevity was observed for packing controls ($P < 0.05$). Immobilized control males showed a significant reduction in longevity compared with other males ($P < 0.01$).

M600 Pro Hexacopter, and tested it under real-world conditions (fig. S1). As part of the integration, a custom Android-based software app was developed to enable the uploading of missions and flight plans to the drone and release system, as well as the real-time monitoring of mechanism status and control via a radio telemetry connection.

This trial involved paired aerial and ground releases of *Wolbachia*-infected *Ae. Aegypti*, marked with distinct colored fluorescent powders, corresponding to release method and release week. The releases were carried out once per week for 4 consecutive weeks over a 1-km^2 area in Nakasi (Suva, Fiji) from November to December 2018 (Fig. 2A). To compare dispersal, we examined both the quantity and the spatial distribution of mosquitoes recaptured by traps associated with each release method. Furthermore, we performed quality assessment on the aerial- and ground-released mosquitoes to discern potential fitness effects arising from the entire release process, encompassing mosquito handling to deployment, specifically looking at mosquito viability, physical integrity, and longevity.

Out of the four aerial releases conducted, two were considered successful (weeks 1 and 3), whereas the remaining two were partially successful (weeks 2 and 4) because of technical issues encountered in the field with the release system. Furthermore, video recordings from the output camera were used to monitor the release's success. There was a noticeable inconsistency in aerial release quantities, ranging from 0 to 300 mosquitoes, despite the 150 ± 50 mosquitoes per release point. Because of the substantial variation in the release quantity coupled with the lack of reliable means to quantify release output, it was difficult to accurately estimate the total weekly aerial release numbers and to determine the aerial recapture rate. In contrast, for ground releases, an average of 127 mosquitoes (SD = 40.7, SEM = 7.415) were released per release point, with a weekly estimated release total of 20,320 mosquitoes. Ground release coverage was 100% for all four releases (Table 1).

Trap positivity and mosquito recapture numbers appear to heavily depend on the release's success. In the two successful aerial releases, weeks 1 and 3, the number of positive traps and the total number of recaptured mosquitoes were very similar to those of the ground release recapture. In the first release, 87% of release points were successfully achieved by aerial release (note that output video was not recorded for the first flight segment, but video footage for

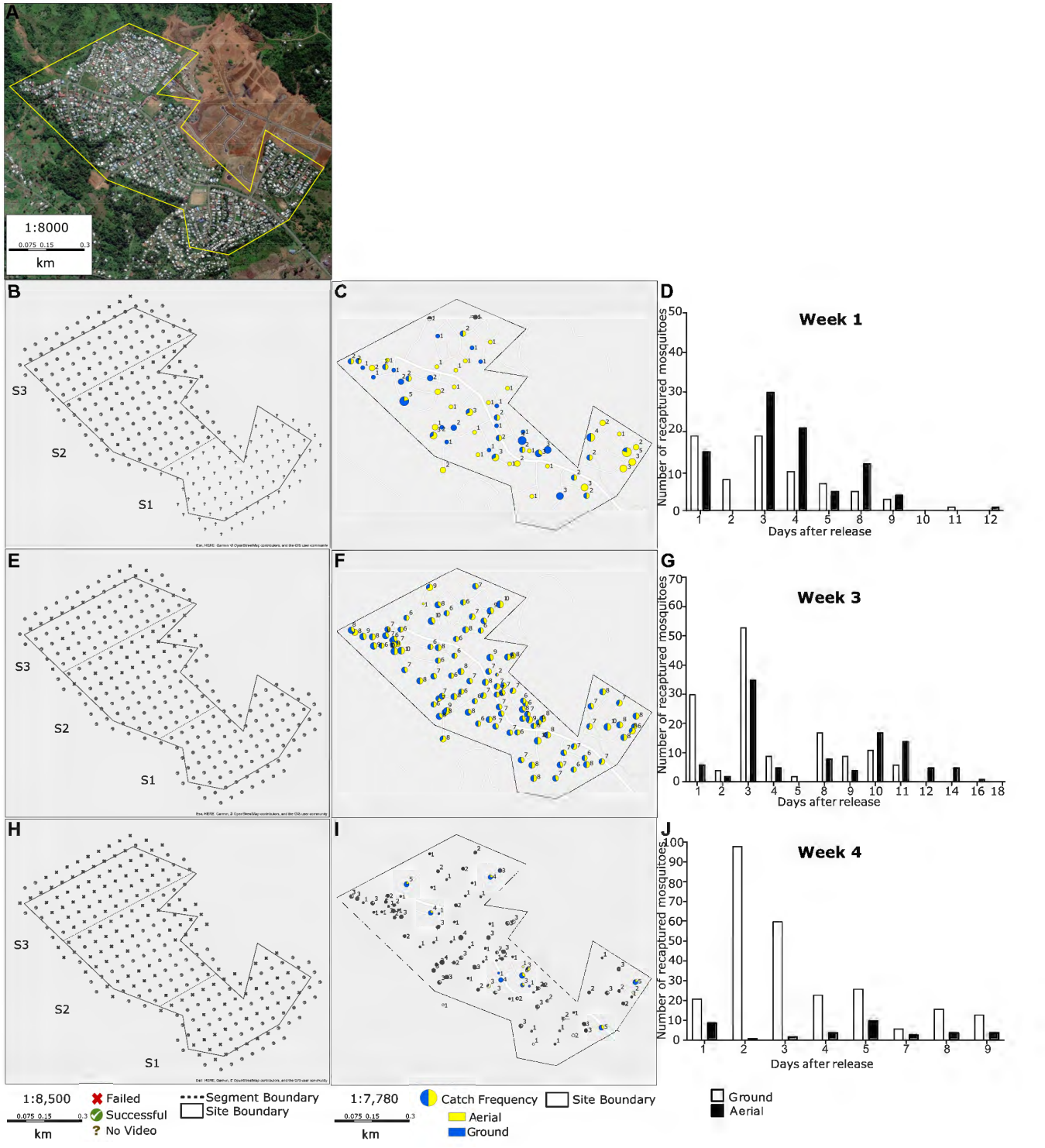

Fig. 2. Trial I release outcome and mosquito recaptures. (**A**) Satellite image of the release site in Naski, Fiji, with the release boundary shown in yellow. Successful release outcomes are shown in (**B**) to (**D**) for week 1 and (**E**) to (**G**) for week 3, and an example of a partial release in week 4 is shown in (**H**) to (**J**). Aerial release coverages are shown in (B), (E), and (H). The number of released mosquitoes was visually estimated from the mosquito mass size in video footage from the release mechanism. A release point was manually scored as successful (green tick) if it had more than 10 mosquitoes released. Red crosses indicate unsuccessful or missed release points, and brown question marks indicate release points with missing video footage. S1 to S3 indicate flight segments. Total catch for ground- (blue) and aerial-released (yellow) mosquitoes over a total of 9 to 16 days of field monitoring are shown in (C), (F), and (I). Each pie chart represents a positive trap. The dividends represent the proportion of aerial- and ground-released mosquitoes in a given trap. The total catch size in a trap is reflected by the size of the pie chart, and the exact catch number is indicated by the number next to the pie chart. Daily *Ae. aegypti* recaptures after each paired release are shown in (D), (G), and (J). The numbers of ground release recaptured mosquitos are represented by white bars, and the numbers of aerial release recaptured mosquitos are represented by black bars.

Table 1. Summary of the release's success and mosquito recollection data for field trial I. Successful release points are represented as a percentage of planned release points. The number of traps that caught marked mosquitoes and the total number caught during the monitoring period are listed for each release event. The normalized by release point values in brackets were calculated by dividing either the number of recaptured mosquitoes or the number of positive traps by the number of successful release points.

	Aerial				Ground			
Release	No. of successful release points (out of 270)	Successful release points (%)	No. of positive traps (normalized by release point)	Number recaptured (normalized by release point)	No. of successful release points (out of 154)	Successful release points (%)	No. of positive traps (normalized by release point)	Number recaptured (normalized by release point)
1	154*	87*	46 (30)	88 (0.57)	154	100	36 (23)	72 (0.47)
2	114	42	35 (31)	62 (0.54)	154	100	67 (44)	191 (1.24)
3	221	82	52 (24)	102 (0.46)	154	100	60 (39)	141 (0.92)
4	109	40	30 (28)	37 (0.34)	154	100	90 (58)	263 (1.71)

*Aerial release data were not recorded for flight segment 1. Successful release percentage was calculated as an average of segments 2 and 3.

flight segments 2 and 3 showed that 154 of 277 release points were successful) (Fig. 2B). As a result, 46 traps captured a total of 88 aerial-released mosquitoes across the three flight segments, whereas 36 traps captured a total of 72 ground-released mosquitoes (Fig. 2, C and D, and Table 1). The third aerial release was successful, with 82% successful release points (221 of 270 release points were successful) (Fig. 2E and Table 1). As a result, 52 traps were positive for aerial release and captured 102 mosquitoes, whereas 60 traps were positive for ground release and captured 141 mosquitoes (Fig. 2, F and G, and Table 1).

The second and the fourth weeks of aerial releases performed below expectations because of several factors. These included phase change material (PCM) leakage leading to mosquito clumping and release output blockage, a loss of communication between the release mechanism and the drone caused by a faulty communication cable, and onboard software issues resulting in the malfunction of the release mechanism. As expected, the number of aerial positive traps and recapture numbers were significantly lower compared with those of the ground release recapture. In week 4, only 42% (109 of 270) of planned aerial release points were successful, predominantly in flight segments 1 and 2 (Fig. 2H and Table 1). As a result, just 30 traps recaptured a total of 37 aerial mosquitoes, and these traps were mainly located in the flight segments that had successful releases (Fig. 2, I and J, and Table 1). In contrast, 90 traps were positive for ground-released mosquitoes, capturing a total of 263 mosquitoes, substantially surpassing the counts observed in the aerial release (Fig. 2, I and J, and Table 1).

Overall, the mean ground release recapture rate was 0.82% (SD = 0.40%). Because the aerial release recapture rate could not be estimated, we directly compared the number of the recaptured mosquitoes from each release method and adjusted for successful aerial release points; no statistical difference between mosquitoes caught in ground or aerial release areas could be detected (Fisher's exact test; $P = 0.456$). However, this is likely limited by the comparably low sample sizes for naturally highly variable data.

Mosquito marking not only facilitated the distinction between release methods but also enabled the differentiation between release weeks. This capability allows us to assess the field longevity of released mosquitoes for both aerial and ground releases. Mosquitoes from both release methodologies were able to survive for at least 8 days after release, with mosquitoes from aerial releases caught up to 15 days after release and mosquitoes from ground releases caught up to 11 days after release (Fig. 2, D, G, and J).

Field trial I—Labor requirements by aerial and ground release methods

The procedures of preparing and releasing mosquitoes vary considerably between the two release methods. In preparation for each ground release, a team of two staff members dedicated 3 hours to aliquot approximately 20,320 adults into 160 release tubes. The subsequent field deployment involved three staff members, including one driver and two field staff, and was completed in approximately 3 hours. A total of 6 hours was required for the ground deployment.

Conversely, for each aerial release, a team of two staff members dedicated 30 min to chill, collect, and pack approximately 40,000 adults into the adult release mechanism. The subsequent flight operation was done by three staff members (two pilots and one field staff) and took approximately 1.5 hours to complete three flights. Thus, a total of 2 hours was required for the aerial deployment. This means that despite requiring identical staffing, the aerial release method was more time efficient, from mosquito handling to releases, when compared with ground deployment for covering the same release area in this particular field site.

Field trial I—Postrelease mosquito quality

To assess mosquito fitness after each release, quality assessments were performed on both aerial- and ground-released mosquitoes. After each release, mosquitoes were examined for immediate mortality and physical damage. Mosquito longevity was also monitored over 6 days in the insectary after releases 2, 3, and 4. Although mosquitoes used in aerial releases were subjected to chilling, compaction, and mechanical separation, the immediate survival rate of the aerial-released mosquitoes was not statistically different from that of mosquitoes from the rearing control for releases 2 and 4 (fig. S2, B and D). Aerial-released mosquitoes in week 1 showed a statistically significant but small decrease in survival compared with the rearing control, with an average survival of 88.82% compared with 97.57% in the rearing control ($P = 0.0236$; fig. S2A). There appears to be a slightly more prominent decrease in mosquito survival for

week 3 aerial-released mosquitoes (average = 68.60%) compared with the rearing control (average = 91.40%, $P = 0.0360$); however, those mosquitoes had similar survival (average = 68.60%) compared to the cold immobilization control (average = 74.85%, $P > 0.5$; fig. S2C). This suggested that the reduced survival of aerial-released mosquitoes might be the result of the immobilization process specific to that week that was not seen in other releases. The mosquitoes from both aerial and ground releases were examined further for evidence of physical damage, and no statistically significant differences were observed between these groups of mosquitoes (fig. S3). We did, however, observe that scales present on the mosquito scutum were missing in almost all mosquitoes examined; this was likely to be attributed to the high cage-rearing density and not the release methods because mosquitoes that remained in the insectary had similarly high levels of missing scales (fig. S3). Furthermore, mosquito longevity did not appear to be impaired by the aerial release process, either [log-rank (Mantel-Cox) test; fig. S4]. Together, these results suggest that aerial-released mosquitoes exhibit survival, longevity, and physical intactness similar to those of the ground-released and rearing control mosquitoes.

Field trial II—Aerial release's success

We established *Wolbachia* in an *Ae. aegypti* population across a 2-km^2 area in Nausori, Fiji, from April to September 2019 via the aerial release of *Wolbachia*-infected mosquitoes using our UAV-based release system (Fig. 3A). To monitor *Wolbachia* establishment, a total of 33 Biogents-Sentinel (BGS) traps were deployed at 12 traps per km^2 inside the release area, and 9 traps were located up to 500 m outside (fig. S5A). Informed by field trial I, the release mechanism underwent major mechanical, electronic, and software upgrades to improve field performance and reliability. Throughout the field operation, from preparation to completion of the flights, the internal temperature of the release mechanism remained consistently stable, ranging from 7° to 10°C for at least 2 hours (fig. S6). Furthermore, an output sensor was incorporated to allow us to estimate the number of mosquitoes released per release point. We also dynamically adjusted the mosquito release grids for this trial to accommodate a range of factors, including weather permissiveness, mosquito availability, and occasional malfunctioning of the release mechanism (Fig. 3B).

The aerial release started on 30 April 2019 and continued for 2 weeks before the drone experienced a critical mechanical failure, resulting in the release trial being suspended for 5 weeks. The releases resumed on 18 June 2019 and continued for 14 weeks, with 1 missed week on July 23 because of mosquito availability. An average of 155 mosquitoes per hectare per week were released. This equates to approximately 31,000 mosquitoes per week across the whole release area (range = 6266 to 75,329, SD = 24,252). The cumulative release density was higher in flight segments 2 and 3 and lower in flight segments 1 and 4 (Fig. 3C).

Field trial II—Postrelease mosquito quality

Similar to the first trial, mosquitoes were collected after the flights to check for immediate survival and compared with that of the rearing and cold immobilization controls. In the majority (9 out of 14) of releases, mosquitoes collected at the end of the flights had survival rates ($P > 0.05$) similar to those of the rearing and cold immobilization controls. There appears to be a small effect of flight duration on mosquito survival, with an 8% drop in survival from flights 1 to 4 (average = 81.14% to 73.24%; Fig. 4A). This decline may be partly

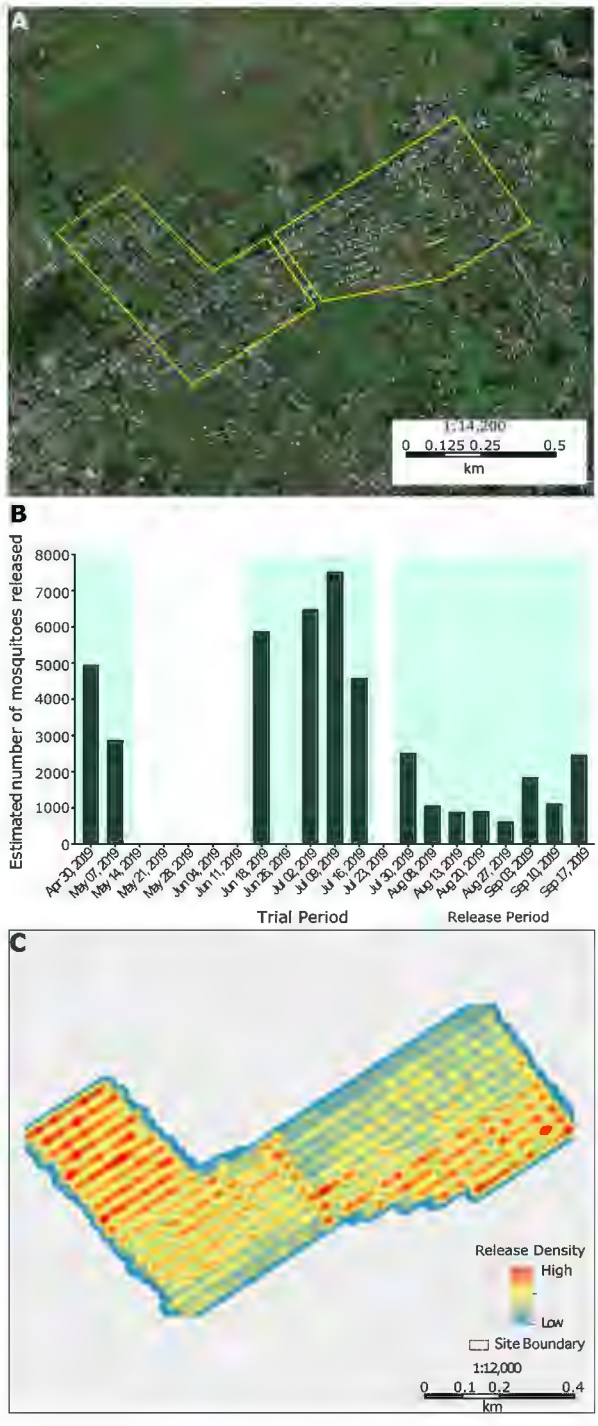

Fig. 3. Aerial release outcomes in UAV trial II (Nausori, Fiji) from 30 April to 17 September 2019. (**A**) Satellite image of the 2-km^2 release zone in Nausori, Fiji, with the release boundary shown in yellow line. (**B**) Estimated weekly aerial release numbers. The green shade indicates the release period. The weekly aerial release commenced on 30 April 2019 and continued for 2 weeks before suspending for 5 weeks because of drone failures. Aerial release resumed on 18 June 2019 and finished on 17 September 2019. Aerial release output sensor data were missing for 26 June; therefore, the number of mosquitoes was unknown, and on 23 July, the release was canceled because of an insufficient amount of mosquitoes for release. (**C**) Cumulative release density from 30 April to 17 September 2019 in a heatmap. Red indicates high release density, whereas blue indicates low release density.

attributed to the cooling and humidity control reaching their limits toward the end of the operation and partly to mosquitoes running out and a smaller number of samples collected for the later flights. In one of the releases (release date of 10 September 2019), a key structural component in the release mechanism was defective and resulted in high mosquito mortality, where fewer than half of the mosquitoes survived in the release mechanism. This result highlights the importance of having a durable release mechanism during the field operation to avoid mosquito mortality. Nonetheless, our data suggest that mosquitoes could be cold-immobilized, compacted, and dosed by the release mechanism without severe loss in viability as long as the release mechanism is functioning as intended.

When possible, collected mosquitoes were further examined for the blood-feeding rate, fecundity, egg hatch rate, and longevity in the laboratory after the release event for up to 12 days. When compared with the rearing control, mosquitoes collected from the release mechanism at the end of the flights did not show any statistically significant decrease in their blood-feeding rate, female fecundity, and egg hatch rate (Fig. 4, B to D). There was also no trend that aerial-released mosquitoes had shorter life spans (fig. S7).

Field trial II—*Wolbachia* establishment

To differentiate between release materials from the wild-type mosquitoes, aerial-released mosquitoes were marked with fluorescent dyes. To assess marking efficiency, we examined the mosquitoes under an ultraviolet (UV) lamp after the dusting procedure, revealing a success rate of 99.8% (table S1). Subsequently, 99.2% still retained the color dust after going through the release mechanism. *Wolbachia* screening polymerase chain reactions (PCR) confirmed that 99.3% of recaptured marked mosquitoes were *w*Mel positive. These data suggest our marking method was effective and reliable.

During the release period, a substantial percentage of recaptured mosquitoes were marked (median = 54.3%, range = 7.1 to 86.0%), and this proportion decreased markedly after the release was completed (Fig. 5). Marked mosquitoes were also caught by traps located at 250 and 500 m outside of the release area, indicating potential mosquito drift because of wind (fig. S8A). Furthermore, it was noticed that some of the recaptured mosquitoes, on the basis of the marking colors, could be traced back to releases that occurred up to 3 weeks prior, suggesting good field survival (fig. S8, B to D).

To determine *Wolbachia* establishment in the field, PCR screening of the recaptured mosquitoes that were unmarked showed a continuous increase in *Wolbachia* prevalence over the 14 weeks of releases (Fig. 5). The median *Wolbachia* prevalence was 71.4% (66.7 to 82.1%) in the 5 weeks after releases stopped. The final *Wolbachia* prevalence monitoring, undertaken in November 2020, showed that the majority of mosquitoes (17 of 29; 58.62%) were *w*Mel positive in the UAV deployment area 1 year after release.

DISCUSSION

Here, we demonstrate that UAV-based deployment of *Wolbachia*-infected *Ae. aegypti* provides a viable alternative to ground-based implementations of the *Wolbachia* method in a low-density human population setting. With the expanding risk of vector-borne disease because of factors like climate change and increased global mobility (*4*, *5*), rapid development, expansion, and scaling up of effective interventions are critical. This requires continuous research, development, and refinement of potential solutions. Here, we report

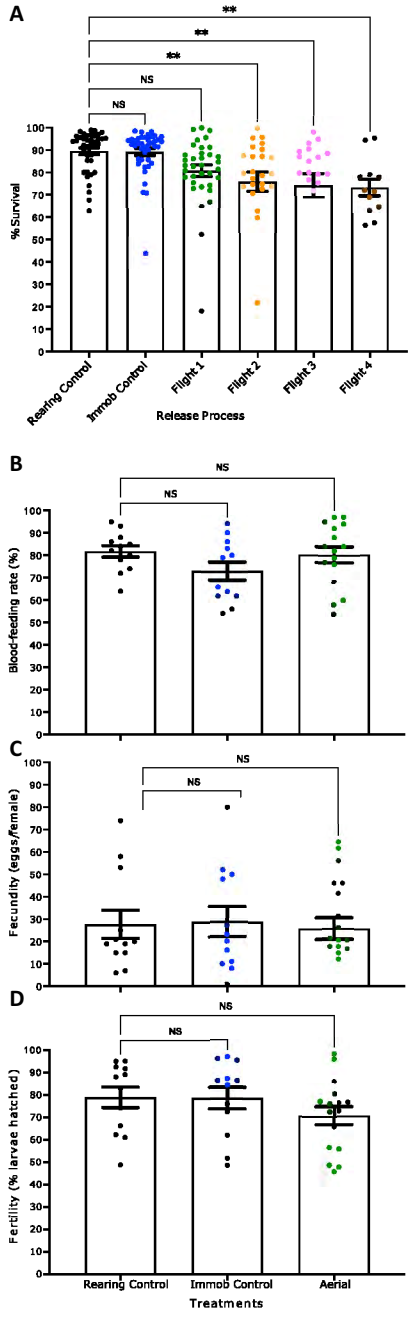

Fig. 4. Quality assessment of *w*Mel-infected *Aedes aegypti* in UAV trial II in Nausori, Fiji. Effects of chilling and mechanical processing of the mosquitoes by the adult release mechanism on (**A**) mosquito survival, (**B**) blood-feeding rate, (**C**) fecundity, and (**D**) fertility. Mosquitoes were sampled from the emergence cages (rearing control), after cold immobilization at 4°C for 30 min (immob control), and at the end of each flight (one to four). For mosquito survival tests, samples that contained 49 or fewer mosquitoes or mosquitoes that were recovered from the release mechanism to repeat a release on the following day were excluded from the analysis. To assess blood-feeding rate, fecundity, and fertility, mosquitoes from each flight were combined into one group, aerial. Females were allowed to blood feed, and the number of engorged females was counted and kept for oviposition. Fecundity is shown as eggs per female. Eggs were hatched in tap water, and larvae were counted 48 hours after hatching to determine fertility. Percentages of survival, blood-feeding rate, fecundity, and fertility of each group were compared with those of the rearing control, and statistical analysis was performed using ordinary one-way ANOVA (**$P < 0.05$). Data are presented as means ± SEM ($N = 12$ to 41).

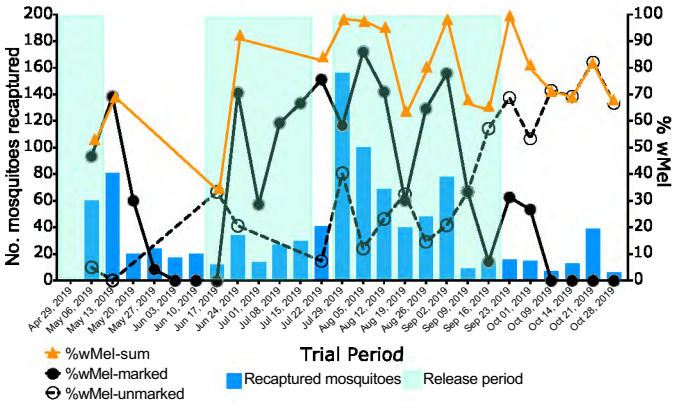

Fig. 5. *Wolbachia* establishment in UAV trial II in Nausori, Fiji. The green shade indicates the release period. The weekly aerial release commenced on 30 April 2019 and continued for 2 weeks before suspending for 5 weeks because of drone failures. Aerial release resumed on 18 June 2019 and finished on 17 September 2019. The weekly field monitoring started a week after the aerial release on 6 May 2019 and stopped after 28 October 2019. The final monitoring took place in November 2020 and showed that *w*Mel frequency was at 58.62% (17 of 29 *Ae. aegypti* were *w*Mel positive). The numbers of mosquitoes recaptured are represented by blue bars. Recaptured *Ae. aegypti* were PCR-screened for the presence of *Wolbachia*. The *w*Mel-positive *Ae. aegypti* were either marked or unmarked, representing the released or field materials, respectively. The solid line with a black circle depicts the percentage of marked *w*Mel-positive mosquitoes, whereas the dashed line with a clear circle depicts the percentage of unmarked *w*Mel-positive mosquitoes. The combined percentage of *w*Mel-positive mosquitoes, both marked and unmarked, is shown in the orange line.

the successful use of a scalable, discrete dosing mechanism coupled with an aerial release platform.

Although there is no perfect solution to this growing problem, the *Wolbachia* method is a proven powerful addition to the public health toolbox (*17*, *23*, *25*). Unlike traditional SIT methods, *Wolbachia* introgression requires the release of relatively low numbers of infected male and female mosquitoes for a defined period of time (*21*, *37*, *38*). Moreover, once established in a given mosquito population, *Wolbachia* has persisted for at least 10 years (*29*). This means that *Wolbachia* can be established within a target *Ae. aegypti* population of comparatively low numbers of mosquitos and provide long-term protection without the need for additional releases. Therefore, the *Wolbachia* method is well suited to UAV-mediated deployment, where payloads and flight times are limited.

We achieved *Wolbachia* establishment in a target *Ae. aegypti* population through UAV-mediated mosquito deployments. Despite the continuous influx of wild-type *Ae. aegypti* from the surrounding areas where *Wolbachia* was not deployed, the *Wolbachia* prevalence remained close to 60% 1 year after aerial release. The process of rearing *Wolbachia*-infected mosquitoes at high density, chilling, handling, and packaging did not negatively affect mosquito quality (figs. S2 to S4). This was critical because released *Wolbachia*-infected *Ae. aegypti* need to compete with the local mosquito population. These findings demonstrate that aerial-based deployment can be an effective alternative to existing ground-based deployment. However, like any new system, UAV deployment required extensive testing and refinement. For example, one important caveat when discussing aerial release is the possibility of release failure. Because of mechanical, electronic, and software issues of the early prototype release mechanism, two aerial releases performed well below expectations. For these partial aerial releases, very few aerial-released mosquitoes were recaptured compared with the ground releases (Fig. 2). Although subsequent refinement of the mechanism addressed this issue, it highlights the importance of a well-established trap and monitoring network that can detect issues, especially during the testing phase of a new release technology.

This work represents a custom-designed solution tailored to the *Wolbacha* method, which involves releasing small numbers of mosquitoes. This included the incorporation of an innovative microdosing mechanism capable of releasing 150 mosquitoes per release point and an output camera with a computer vision system to estimate the number of mosquitoes released at each release point, allowing for effective field release monitoring. Furthermore, the mechanism carries up to 160,000 mosquitoes in one full unit and stably maintains an internal temperature between 7° and 10°C for at least 2 hours in the field to complete four scheduled flights, each with around 15 to 20 min of flight time (fig. S6). This release mechanism is an improvement from the previously reported adult release system with a 50,000-mosquito carrying capacity and a refrigeration time of 15 min (*36*). The addition of an active dehumidification system helped to minimize moisture buildup and reduce the likelihood of mosquito clumping and blockage. Furthermore, the design philosophy behind our release mechanism is drone agnostic, allowing seamless integration with any drone (such as Freefly Alta X) or platform deemed optimal for the specific use case. The long-term vision is to integrate this release mechanism with a long–flight range drone, such as Wingcopter 198, to be able to release over large areas with minimum flights. Although UAV-mediated mosquito release has been demonstrated for SIT programs, this has been on a relatively small scale, around 200,000 mosquitoes released over 0.2 km^2 (*36*). Thus, increasing the area by an order of magnitude represents a substantial advancement in addressing the challenge in area-wide application.

Adult delivery of mosquitoes via air is envisioned to be used in areas that are hard to service using conventional ground release or egg release systems. Typically, these are high-density urban areas that are either unsafe to enter or difficult to reach because of poor infrastructure. UAV-mediated aerial deployment provides a potential strategy to overcome these limitations. However, despite the technical feasibility demonstrated here, there remain ongoing limitations on implementation. Deployments are constrained by the regulatory requirements necessary to legally fly a high-payload UAV in urban areas (*39*, *40*). Urban areas in general, especially high-density urban areas, are subjected to strictly controlled airspace to ensure the safety and well-being of residents (*40*, *41*). One way to overcome this limitation is to significantly reduce the total weight of the adult release mechanism to under 2 kg, aligning with the payload capacity of currently approved drones from Matternet and Wing, which hold type certificates with a payload of 1 to 2 kg, and working with existing operators who already have approvals for flights over urban areas. Other drone delivery services include Amazon Prime Air, Zipline, and Flytrex, which are certified as operators for urban delivery in the United States. These flights are still limited to specific areas where the risk is deemed low enough, such as avoiding areas where there are likely to be groups of people. Nonetheless, they show that it is fully possible to safely use a light-payload drone above urban areas. Furthermore, it is imperative to develop thorough risk assessments, comprehensive risk mitigation, and contingency

plans for each planned aerial field release operation to enhance safety measures implemented during operations. The regulatory hurdle and safety consideration creates a highly site-specific balancing act between UAV and ground deployments.

This proof-of-concept study provides the basis for the further development of improved dosing mechanisms, long-range delivery platforms, and the associated mosquito-handling systems required. The release of biting mosquitoes in urban and semi-urban areas are complex endeavors that require effective community engagement, multiple release approaches, and extensive postrelease monitoring to be effective. We also sought to highlight that any UAV-based release system needs to be optimized and refined to ensure effective releases. Acknowledging the care needed, this work provides a framework and pathway to implementing the *Wolbachia* method through UAV-based systems.

MATERIALS AND METHODS

Mosquito rearing

Mosquitoes used in this study have been described in (*18*). *w*Mel-infected *Ae. aegypti* eggs were produced by WMP at Monash University (Melbourne, Australia) and shipped to Fiji at controlled temperatures ranging between 17° and 25°C using a specialized courier company (LabCabs International) (*42*). To produce adults for release, *w*Mel-infected *Ae. aegypti* were reared at a density of approximately 3750 larvae per liter and fed daily with a diet formulation consisting of 50% tuna meal (Ridley Aqua Feed), 35% beef liver powder (NOW Foods), and 15% brewer's yeast (NOW Foods) at 29°C and ambient humidity. Trays were flushed and refilled with fresh water as needed. When pupation reached above 50%, approximately 25,000 pupae or larvae were enclosed in an emergence cage (900 mm by 300 mm by 300 mm). Adults were maintained at 26°C ± 1°C and 70% ± 5% RH and fed with 10% sucrose solutions until they were 4 to 6 days old for release.

Mosquito marking and color detection for mark-release-recapture field studies

One day before the release, mosquitoes were marked with fluorescent powder (BioQuip) by creating small dust storms directly in the emergence cage or adult release tube using a 50-ml syringe. Approximately 5 ml of fluorescent dust was loaded at a time, and multiple dust storms could be created. Dust applications were repeated a few times to ensure even dust coverage across the whole cage. To check for the presence of dust on mosquitoes, mosquitoes were cold immobilized at 4°C for 10 min and visually inspected under a UV lamp (Woods Lamp, DLC Vet Suppliers, Australia) with a dissecting microscope in a dark room. Varying colored fluorescent powders were used to differentiate among individuals from different treatments and release stages.

Mosquito handling for aerial and ground release

To prepare mosquitoes for aerial and ground release, emergence cages containing 4- to 5-day-old mosquitoes were chilled at 4°C for 6 min to immobilize mosquitoes for collection. For aerial release, cold immobilization was done on the day of release. Cold-immobilized mosquitoes were collected from the cages and loaded directly into the canisters of the UAV release mechanism, which was then sealed, insulated, and transported to the UAV takeoff base. Mosquitoes were maintained at 7° to 10°C inside the UAV release mechanism during transportation and throughout flights.

For ground release, mosquitoes were cold-immobilized 1 day before the release day. Chilled mosquitoes were aliquoted into groups of 150 mosquitoes using 1.44-ml 3D-printed cubes and transferred into adult release tubes made of PVC pipes (diameter of 7 cm, length of 21 cm) that were secured with mesh at both ends with rubber bands. Cotton balls soaked with 10% sucrose solution were placed on the top of the tube, and mosquitoes were placed at 26°C ± 1°C and 70% ± 5% RH to recover. Adult release tubes were transported to the field by car the following day.

Regulation and approval

The work described herein occurred as part of WMP operations within Fiji and has been described in (*18*). Briefly, a desktop risk assessment was undertaken by the Biosecurity Authority Fiji for the *Wolbachia* method. This assessment held that implementation within Fiji would pose an acceptable risk. Stemming from this and with continued engagement with the Ministry of Health and Medical Services of Fiji and the Biosecurity Authority Fiji, approval of the operation was provided by the Fijian government. *Wolbachia*-infected *Ae. aegypti* were imported under authorization from the Biosecurity Authority Fiji. Mosquitoes used for this study were imported into Fiji under permit numbers SUV-02/18, SUV-10885/19, SUV-11304/19, and SUV-11638/19.

Community engagement

Community engagement and determination of public acceptance for the UAV deployment also occurred under the frameworks established as part of WMP Fijian operations (*18*). A communications and engagement team implemented the WMP's Public Acceptance Model (PAM) for obtaining community support for mosquito releases (*22*, *43*). To achieve this, the PAM used four core activities: raising awareness, public surveys, an issues management system, and a community reference group (CRG).

Awareness was generated as part of the overall Fijian community engagement strategy. This relied on newspapers, television and talkback radio, social media, visiting homes and businesses, mailings, and community meetings.

The CRG consisted of diverse members of the Fijian community who met on numerous occasions to provide independent oversight of WMP community engagement and deployment activities. Subsequent to prerelease surveys, a summary of all engagement activities was provided to the CRG for final approval. During both the prerelease and deployment periods, no complaints regarding WMP operations were received.

Aerial release mechanism for mosquitoes

An automated mechanism was developed to release adult mosquitoes in small discrete doses of approximately 150 each. The mechanism consists of mosquito storage canisters, a dosing unit, a release output, and a temperature and humidity control unit, all enclosed within an insulation foam box (Fig. 1A). The cooling system was powered by a series of custom-formed ice packs filled with water, and dehumidification was provided by rechargeable silica gel beads. The temperature was actively controlled within 6° to 10°C by a number of fans using a hysteresis control enabled by a series of temperature and humidity sensors placed throughout the mechanism. Humidity was similarly controlled with a fan that redirected the cooling air over the silica gel to extract the moisture and with a hysteresis controller to maintain values between 60 and 80% RH. A camera was

attached to the output area, coupled with a computer vision system to estimate mosquito release numbers and monitor the release's success. Control electronics and software were developed for a fully autonomous release system, and the mechanism was subsequently integrated into a DJI M600 Pro Hexacopter UAV (DJI, China) (fig. S1). The temperature and humidity stability of each storage canister and the dosing unit were measured throughout loading, transport to the release site, and flight. Temperature was maintained at the target range, 7° to 10°C.

Flight permissions and planning

Flight permission from the Civil Aviation Authority of Fiji (CAAF) was obtained to operate in the 1- and 2-km^2 areas in Nakasi and Nausori, respectively. Aerial releases were performed at altitudes of 50 m and 60 m above the take-off base in trials I and II, respectively, with flight speeds of 10 to 12 m/s during the releases. All flights were carried out by local residents from Pacific Flying Labs and Drone Services Fiji. All remote pilots were certified professionals. It is important to note that regulations are highly specific to individual countries and regions within each country. Moreover, UAV regulations are continually updated. Hence, it is recommended that anyone looking to undertake UAV flights consult with local experts.

Mosquito dispersal study design (field trial I)

The aim of this study was to compare the dispersal uniformity and field longevity of *Wolbachia*-infected mosquitoes released from the air to those released from the ground. Releases occurred between 19 November 2018 and 17 December 2018. Each week for 4 consecutive weeks, paired ground and aerial releases were conducted on the same day in a 1-km^2 area in Nakasi (18.0641°S, 178.5135°E; Fig. 2A). Nakasi is located along the Suva-Nausori corridor in the central division of Fiji, with approximately 6915 people in 1486 households (population data in 2017). The area was selected for the following reasons: First, the land was flat, which made flight planning more straightforward without the need to adjust flight altitude during a mission. Second, the majority of the houses did not have a gate, which allowed easy accessibility for ground releases and trap servicing. Last, the area was isolated by the surrounding plantation, which made it suitable as the initial study site for aerial release.

A release grid based on approximately 75 m was used for both aerial and ground releases. For ground release, 154 release points were evenly spaced across the study area, confined to roads, and excluded non–built-up areas (fig. S9A). Tubes of 150 adults were transported to the field using a car and released at each release point. For aerial release, we generated a flight plan consisting of 270 release points in straight, equidistant lines, with 90 release points in a 100-m buffer area (fig. S9B). A total of three flights (15 min each) were scheduled to treat the study area with a target of 150 mosquitoes released per release point.

Mosquitoes were marked a different color for each release method and for each release week. For the first and the third week of releases, aerial mosquitoes were marked yellow, and ground mosquitoes were marked blue. For the second and the fourth release, aerial mosquitoes were marked pink, and ground mosquitoes were marked blue-green (for mosquito marking method, see mosquito marking method above).

For field monitoring, a dense network of 100 BGS traps (Biogents AG, Germany) was deployed in a 100-m grid within the 1-km^2 release area (fig. S9C). Traps were serviced the day after each release and daily for 5 consecutive days. After the last release, traps were serviced daily for a total of 9 days, except Sunday. To minimize the time difference between the first and the last trap to recollect mosquitoes, trap servicing was completed within 4 hours. Recaptured *Ae. aegypti* were counted and checked for colors to identify the release method and field materials.

Wolbachia establishment study design (field trial II)

The aim of this study was to establish *Wolbachia* using the improved version of the release mechanism integrated into a DJI M600 Pro Hexacopter to release *Wolbachia*-infected mosquitoes over a 2-km^2 release area in Nausori (18.0249°S, 178.5614°E) (Fig. 3A). Nausori is a town located 9 km northeast of Nakasi. Approximately 3917 residents across 899 households resided in this selected area (population data as of 2017). This trial area was selected because it is flat and located on the other side of the Rewa River, separating this area from the rest of the concurrent WMP deployment across Suva at the time. In addition, during the peak months of February to May, the number of reported cases of suspected dengue ranges between 100 and 200.

The weekly aerial release commenced on 30 April 2019 and continued for 2 weeks. Unfortunately, in the second week of release, we experienced a hard landing because of the false triggering of the parachute from rain damage. As a consequence, the trial was suspended for 5 weeks before it resumed on 18 June 2019 and continued for another 14 weeks, with the last release taking place on 17 September 2019. The release was carried out in four flights. Each flight was approximately 16 min. We initially planned to release on the basis of a 55-m release grid with 468 release points (fig. S5B); however, because of variations in mosquito availability and weather conditions, we adjusted the release grid each flight and each week accordingly. Three colors of fluorescent powder, yellow, red, and blue, were used in rotation to mark the weekly released mosquitoes. For field monitoring, a total of 33 traps were deployed, with 24 traps placed inside the release zone at a density of 12 traps per km^2 and 9 traps placed 250 to 500 m outside of the release boundary (fig. S5A). Traps were serviced once weekly during the release period. After the release was completed, we continued the weekly service of the traps for another 3 weeks (1 to 14 October 2019). During the following 2 weeks (21 to 30 October 2019) of field monitoring, mosquito recollection was done using Prokopack aspirators (John W. Hock Company, USA). Long-term monitoring was conducted by the local Ministry of Health using a combination of BG traps and Prokopack aspirators. Collected *Ae. aegypti* were checked for colors to identify release materials, stored in 70% ethanol, and shipped to Monash University (Melbourne, Australia) for *Wolbachia* screening as described below.

Mosquito quality assessment

Triplicates of 100 to ~200 mosquitoes were collected at different stages throughout the rearing, preparation, and release process to examine the effects of marking, chilling, compaction, and mechanical separation on mortality, longevity, physical damage (trial I only), blood-feeding success, fecundity, and fertility (trial II only). In general, collected mosquitoes were placed inside BugDorm cages (30 cm by 30 cm by 30 cm, MegaView Science Co. Ltd.), and the number of dead mosquitoes was counted within 2 days of collection for mortality. Live mosquitoes in trial I were microscopically examined for missing or damaged antennae, legs, wings, and scales.

To measure longevity, approximately 100 live mosquitoes of mixed sex were placed inside BugDorm cages (300 mm by 300 mm by 300 mm) and fed with 10% sucrose, and total death was recorded daily for 6 days. The longevity tests were conducted with some modifications in the second field trial: Triplicates of 20 males and 20 females were kept separately in 30-ounce plastic cups with grated inner surfaces for resting spaces. Cups were covered with mesh and secured with rubber bands, and mosquitoes were fed with 10% sucrose using cotton balls. Death was recorded every second day except on the weekends for 12 days.

To determine blood-feeding success, 50 females and 15 males were sorted into BugDorm cages (300 mm by 300 mm by 300 mm) and fed on the arms of human volunteers for approximately 20 min. Blood feeding of mosquitoes on adult human volunteers was performed in accordance with Monash University Human Research Ethics permit number (CF11/ 0766-2011000387). The number of engorged females was counted the next day and kept for oviposition. Eggs were collected 3 days after blood meals, counted, and hatched to determine fecundity (eggs per female) and fertility (percentage of hatched larvae).

Wolbachia screening

Adult *Ae. aegypti* collected from the field in the second field trial were screened for *Wolbachia* at Monash University. Screenings were done using *Ae. aegypti*– and *w*Mel-specific primers and probes in Taqman qPCR assays as previously described (*22*).

Statistical analysis

Data analysis and visualization were undertaken using GraphPad Prism v9.3. To assess the effects of release processes on mosquito survival, blood-feeding rate, fecundity, and fertility, ordinary one-way ANOVAs were performed on three biological replicates for each experiment. To assess mosquito longevity, survival curves were compared using the log-rank (Mantel-Cox) test on three biological replicates for each experiment. To compare the number of recaptured mosquitoes between the ground and aerial release methods, Fisher's exact test was performed.

Supplementary Materials

This PDF file includes:

Figs. S1 to S9

Table S1

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