RESEARCH ARTICLE

REVISED Using models and maps to inform Target Product Profiles and Preferred Product Characteristics: the example of *Wolbachia* replacement

[version 3; peer review: 1 approved, 3 approved with reservations]

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Abstract

Background

The global prevalence of diseases transmitted by *Aedes aegypti* mosquitoes, such as dengue, Zika and Yellow Fever, is increasing, but development of promising new mosquito control technologies could reverse this trend. Target Product Profiles (TPPs) and Preferred Product Characteristics (PPCs) documents issued by the World Health Organization can guide the research and development pathways of new products and product combinations transitioning from proof of concept to operational use.

Methods

We used high resolution global maps of the case and economic burden of dengue to derive programmatic cost targets to support a TPP for *Wolbachia* replacement. A compartmental entomological model was used to explore how release size, spacing and timing affect replacement speed and acceptability. To support a PPC for a hybrid suppress-then-replace approach we tested whether *Wolbachia* replacement could be achieved faster, more acceptably or at a lower cost if preceded by a mosquito suppression programme.

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Results

We show how models can reveal trade-offs, identify quantitative thresholds and prioritise areas and intervention strategies for further development. We estimate that for *Wolbachia* replacement to be deployable in enough areas to make major contributions to reducing global dengue burden by 25% (in line with 2030 WHO targets), it must have the potential for cost to be reduced to between $7.63 and $0.24 (USD) per person protected or less. Suppression can reduce the number of *Wolbachia* mosquitoes necessary to achieve replacement fixation by up to 80%. A hybrid approach can also achieve fixation faster and potentially improve acceptability, but may not justify their cost if they require major new investments in suppression technologies.

Conclusions

Here we demonstrate the value dedicated modelling can provide for interdisciplinary groups of experts when developing TPPs and PPCs. These models could be used by product developers to prioritise and shape development decisions for new *Wolbachia* replacement products.

Keywords
mosquito, dengue, model, arbovirus, policy, intervention, Wolbachia, cost

Any reports and responses or comments on the article can be found at the end of the article.

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REVISED **Amendments from Version 2**

The second version of this manuscript has been updated in response to the reviewers' comments.

The motivation for and process of TPP development has been expanded in the introduction. Throughout the text, all statements that the hybrid suppress-then-release scenarios constitute cost savings have been removed, the importance of mosquito surveillance to ensure suppression achieved has been added, and subsequent updates made to Table 3. Furthermore, the analysis exploring the impact of seasonal changes on release ratio for *Wolbachia* release and hybrid suppress-then-release scenarios has been moved to Supplementary Figure 7.

The discussion has been edited to acknowledge that while egg releases every two weeks were modelled after the Yogyakarta RCT, adult releases weekly is possible and could accelerate time to fixation. Additionally, a conservative estimate of the fitness cost of *Wolbachia* infection was selected and, consequently, the decline in mosquito population from pre- to post-*Wolbachia* release is likely an over-estimation. Finally, an alternative use case considered in the TPP, to have larger distances between release sites, has been further clarified and the text now highlights the important role of other methods of dengue control, including vaccines, to reach the WHO 2030 goals in areas where *Wolbachia* implementation may be challenging or less cost effective.

All updated code and Supplementary Figures and Tables can be found on GitHub: https://github.com/katietiley/Wolbachia_TPP_PPC.git.

Any further responses from the reviewers can be found at the end of the article

Introduction

The *Aedes aegypti* mosquito is the principal vector of dengue, Zika, yellow fever and chikungunya viruses. Dengue incidence has been rising and the WHO Global Vector Control Response 2017 – 2030 reports an annual 96 million cases, 1.9 million DALYs and 9,110 deaths[1]. Vaccines are only available for yellow fever and are not currently widely used for dengue, though there are other dengue and chikungunya vaccine candidates in clinical trials[2,3]. There are no drugs available to combat these infections and so there is a reliance on prevention through vector control. Effective control of this vector is difficult to achieve and sustain given the mosquito's high reproductive rate and adaptation to urban habitats, with an egg stage that can survive desiccation and a larval phase that can develop in small, temporary water volumes (e.g., water containers and roof gutters). The rapid growth of cities has also favoured this mosquito[4]. As a result, existing vector control tools alone have generally been unable to sustainably control *Ae. aegypti* or the diseases it transmits over the long term. A range of novel technologies are under development[5], including biocontrol through use of *Wolbachia* spp. for population replacement or reduction/suppression, the release of genetically modified mosquitoes (such as Oxitec's 1st generation self-limiting technology (1gSLT)[6]), and other forms of sterile insect technique (SIT).

Ae. aegypti mosquitoes infected with *Wolbachia* strains show reduced rates of virus dissemination, making them less capable of transmitting arboviruses[7]. *Wolbachia* infection is also dominantly maternally inherited and leads to inviable progeny when *Wolbachia* males and wild-type females mate. This means that *Wolbachia* can be used to either replace the existing mosquito population with a lower competence phenotype by releasing females (or males and females) or suppress the existing population by releasing only males.

Wolbachia population replacement involves regular releases of *Wolbachia*-infected mosquitoes into a wild mosquito population over a period of several months. Modelling has shown that once a critical proportion of mosquitoes in the population have *Wolbachia*, prevalence should continue to increase to fixation without further releases, but below this threshold *Wolbachia* prevalence may decline (possibly to zero) once releases stop due to fitness costs associated with released mosquito strains[8]. Operationally, the chance and speed of exceeding this threshold and achieving self-sustaining coverage defined as the percentage of *Ae. aegpti* population infected with *Wolbachia*, can be achieved by: increasing the number of releases, decreasing the time gap between releases and increasing the ratio of *Wolbachia*-infected *Ae. aegypti* in relation to wild-type *Ae. aegypti* in each release. All of these options increases cost and can also lead to undesirable temporary increases in the *Ae. aegypti* mosquito population which should be addressed during community engagement to avoid it becoming could be a key barrier to community acceptability[9,10]. It should be noted that in practice, Wolbachia frequencies may fluctuate seasonally and still decline to zero after reaching fixation depending on environmental variables such as temperature, rainfall, and physical barriers[11,12].

A growing range of entomological, epidemiological and modelling evidence supports the widespread, long-term effectiveness of *Wolbachia* replacement[13–15], and research continues to identify environmental conditions associatedwith spatially and temporally heterogeneous Wolbachia establishment[11]. This includes a randomised controlled trial (RCT) of *wMel Wolbachia* in Yogyakarta City, Indonesiawhich demonstrated a 77% reduction in dengue incidence and an 86% reduction in hospitalizations[16]. To date, however, *Wolbachia* replacement programmes have only been conducted in specific mid-sized cities or specific neighbourhoods of cities. Thirteen countries have implemented replacementprogrammes at various levels of scale, with 12 through the World Mosquito Program (WMP) and an independent programme in Malaysia[17,18]. Meanwhile, China (with *Ae. albopictus*),Singapore, and the USA have so far chosen to use suppression-based programs due to perceived greater compatibility with their existing intensive and long-term efforts to suppress mosquito populations[19–21].

These novel technologies (*Wolbachia* replacement, *Wolbachia* suppression, 1gSLT and SIT) are subjects of ongoing development, evaluation, demonstration and scale-up in various high-burden programmatic and private settings. While not currently practiced, in theory, combining a prior programme of mosquito suppression followed by *Wolbachia* population replacement could offer community acceptance or dengue incidence reduction advantages[9,22].

Development and transition to scale of new products and strategies can be accelerated by the development of internationally recognised Target Product Profiles (TPPs) and Preferred

Product Characteristics (PPCs) documents[23]. TPPs provide specific quantitative guidance on the key characteristics a product must (minimum target), or should ideally (preferred target), meet when developed into a deployable mass market product. PPCs identify broader areas of unmet need and aim to stimulate new products or product combinations that can address these needs. In early 2022 the WHO convened a Technical Advisory Group (TAG) to develop a draft TPP for *Wolbachia* replacement and a draft PPC for a hybrid mosquito suppression then *Wolbachia* replacement strategy. The TPP for *Wolbachia* replacement began with the development of a "use case characterisation", following which specific TPP criteria were established under the categories of product performance, product characteristics, production and delivery and intellectual property. For each of these, a minimum and preferred target was established with the former intended to inform a go / no go product development decision point. A combination of different types of evidence from the field, laboratory and modelling studies were used to inform these targets, with the modelling work focused on release and cost-related characteristics. The final WHO TPP was published February 2022[24]. TAG members decided that a core premise of the TPP and PPC was that they should closely align with the WHO's strategy and goals to control dengue globally. As such the WHO's goal to reduce dengue incidence by 25% by 2030 (2010 – 2020 baseline[25]) provided a basis to understand the scale and range of settings in which these TPPs, PPCs and the products they ultimately produce are relevant. Computational models can play a key role in the development of TPPs and PPCs due to their ability to generalise beyond areas where data have been collected and make predictions if aspects of the product were to change. Here we describe a dynamic compartmental entomological model and a global geospatial economic model that we developed and used to explore how operational and economic aspects of *Wolbachia* replacement are likely to change once the technology is used at scale.

Methods

Global dengue cost model

The global dengue cost model aims to produce high spatial resolution estimates of the economic costs of dengue that would be averted by *Wolbachia* replacement. These were conservatively estimated to be composed of the direct medical cost of treatment of dengue patients and emergency (outbreak) vector control costs. In the absence of primary data on willingness to pay for *Wolbachia* replacement programmes, these averted costs were assumed to represent an appropriate proxy.

A high resolution (5km × 5km at the equator) map of symptomatic dengue case burden was obtained from Bhatt *et al.*[26], which estimates the spatial distribution of the 96 (67 – 196) million episodes estimated to occur each year. An average direct medical cost per symptomatic case (2013 USD) was derived for each country from Shepard *et al.*[27], considering the different costs of hospitalised and ambulatory cases and the country-specific distribution of symptomatic cases among these two different treatment settings. Direct medical costs include the costs of specific medicines and staff time required to treat a dengue patient and a portion of infrastructural costs and is the most relevant measure of what governments need to pay to treat cases of dengue illness each year. All costs were inflated from 2013 to 2020 USD using World Bank country GDP deflators with a maximum capped value of a two-fold increase[28].

A literature review on the cost of vector control in dengue endemic countries was conducted and identified studies with national and subnational estimates of vector control costs for 17 countries. Twenty studies included costs of routine vector control activities and seven studies included costs of vector control during dengue outbreaks (supplementary file 1 in Data Availability). All vector control cost values were converted back to local currencies using the exchange rate at the time of the costing, inflated to 2020 using country GDP deflators from the World Bank[28], and then converted to 2020 US dollars using 2020 exchange rates published by the World Bank[29]. To make predictions of per capita routine vector control costs for countries without costing data, a Poisson generalized mixed linear model was fit to the costing data with national GDP per capita (log scale) as a covariate and national-level random effects. Predictions were then made for all countries globally using World Bank GDP per capita figures from 2020. For countries where this data was missing (some small Caribbean and Pacific Island nations), global median GDP per capita was assumed. Of the seven studies identified that included costs of vector control during dengue outbreaks, five studies gathered information on both routine and outbreak vector control activities. We assume that implementing a *Wolbachia* release program will not avert routine (principally preventative) vector control costs because *Wolbachia* replacement is unlikely to eliminate dengue in most settings and additional vectors (e.g., *Ae. albopictus*) and nuisance biting mosquitoes will still drive a need for routine vector control activities. Instead, it was assumed that the implementation of *Wolbachia* replacement will significantly limit the size of outbreaks and their required vector control response and thus cost. These studies suggested that during outbreaks, the monthly cost of vector control increases by 20–50%. Three scenarios were explored where additional avertable outbreak costs composed 35% of routine monthly vector control costs for a duration of three months every year, with a sensitivity analysis exploring lower (20%) or higher (50%) values.

Total annual averted costs were estimated assuming *Wolbachia* replacement results in a 70% reduction in symptomatic cases (and their associated costs) and 100% of emergency (outbreak response) vector control costs. This is based on a conservative interpretation of the 77% effectiveness of *wMel Wolbachia* measured in the Yogyakarta trial[16] and the expectation of variable effectiveness across areas with different transmission intensities[15]. While *wMel Wolbachia* replacement has been shown to be stable in *Aedes* mosquito populations for over ten years in Australia[14], it is unclear how many future years of averted dengue costs would be appropriate to consider when estimating government or other funder willingness

to pay. We therefore estimate total averted costs for three-, five- and ten-year time horizons and assume that these costs represent the maximum price a government or funder would be willing to pay for *Wolbachia* replacement in a given setting. To quantify uncertainty around these thresholds, the analysis was repeated with values from the upper and lower bounds of the case burden[26] and economic burden[27] estimates and with 50% and 20% avertable vector control outbreak proportions respectively.

Next we identified which areas (5km x 5km pixels) would need to be targeted to reach the WHO goal of reducing global dengue burden by 25% in the most net cost efficient manner. For a generic environmental intervention, where cost of the intervention only depends on area covered, this would involve targeting areas with the highest density of dengue costs. However, because the cost of *Wolbachia* programmes have been shown to depend on the human population density and per capita GDP in the release area[30], this can change which areas are most important to prioritise from an optimal net cost perspective. To account for these variable implementation cost factors, each 5km × 5km pixel was ranked from highest to lowest based on a benefit (averted medical and outbreak costs) to cost (approximate *Wolbachia* programme cost estimate based on population density and per capita GDP from Brady *et al.*[30]) ratio. For clarity, the approximate *Wolbachia* programme cost from Brady *et al.*[30] only affects the ranking of pixels (i.e. targeting), not the TPP target cost estimates. Cumulative averted cases were then calculated and pixel selection ended when averted cases first exceeded 25% of the global total. The averted costs in the last, least cost-efficient pixel included in this subset then gave the cost threshold for *Wolbachia* replacement programmes, i.e., if *Wolbachia* replacement can be achieved at this cost (or lower) it will be possible, from the cost-efficacy perspective, to implement the intervention in enough areas to reduce the global burden of dengue by 25%. An alternative scenario was also calculated where it was assumed that *Wolbachia* replacement is only required to account for half of this global target, i.e., a 12.5% global burden reduction. Because such a global targeting approach prioritises countries with higher GDP, we also calculated a scenario where 95% of dengue endemic countries (defined as >10,000 symptomatic dengue infections a year as estimated by Bhatt *et al.*[26]) needed to achieve at least a 25% burden reduction through deploying *Wolbachia* replacement, to explore the cost threshold implication of a much wider deployment with improved equity between countries[31].

Entomological model overview

An entomological *Wolbachia* replacement model was formulated and calibrated to the main evidence available at the time, namely the Yogyakarta RCT[16], which comprised nine to 14 egg releases every two weeks. This approach was also chosen to maximize consistency with other elements of the TPP. This compartmental mechanistic model follows *Aedes aegypti* population dynamics at egg, larvae, pupae, and adult stages, with pupae developing into female and male adults in equal proportion and each stage subject to a constant death rate:

$$\begin{aligned}
\frac{dO}{dt} &= \varphi F - \alpha_O O - \mu_O O \\
\frac{dL}{dt} &= \alpha_O O - \alpha_L L - \mu_L L \\
\frac{dP}{dt} &= \alpha_L \frac{L}{1+(\gamma L)^\beta} - \alpha_P P - \mu_P P \\
\frac{dM}{dt} &= 0.5\alpha_P P - \mu_M M \\
\frac{dF}{dt} &= 0.5\alpha_P P - \mu_F F
\end{aligned} \tag{1}$$

O denotes the number of eggs, L larvae, P pupae, M adult males, and F adult females. φ is the daily egg-laying rate of adult females. α_O denotes the rate at which eggs develop into larvae and the μ_O death rate of eggs. Similarly, α_L denotes the rate at which larvae develop into pupae and μ_L the larval death rate and α_P denotes the rate at which pupae develop into adults and μ_P the pupal death rate. μ_M and μ_F are the adult male and female death rates, respectively. Survival of larvae to pupal stage is density dependent, and using the flexible formulation proposed by Maynard Smith and Slatkin[32], includes the parameter γ which determines the density at which mortality remains proportionate and the parameter β the 'abruptness' of density-dependence.

These equations were then further developed to account for *Wolbachia* deployments, respectively impacting mating and larval survival:

$$\begin{aligned}
\frac{dO}{dt} &= \varphi F \frac{M + c_i M_W}{M + M_W} - \alpha_O O - \mu_O O \\
\frac{dP}{dt} &= \alpha_L \frac{L}{1+(\gamma(L+L_W))^\beta} - \alpha_P P - \mu_P P
\end{aligned}$$

Where, c_i denotes the failure rate of cytoplasmic incompatibility for the *Wolbachia*-infected adult males (M_W), and *Wolbachia*-infected larvae in the wild (L_W) also contribute towards larval competition.

While it is important that all wild-hatched larvae are subject to the same density dependence as they are occupying the same habitat, the introduced *Wolbachia*-infected eggs will be released in their own distinct larval habitat (self-contained release containers), therefore their survival is not impacted by the densities of wild-hatched larvae. The equations of released *Wolbachia* (rW) are as follows:

$$\begin{aligned}
\frac{dO_{rW}}{dt} &= RR(\dot{F} + \dot{M}) - \alpha_O O_{rW} - \mu_O O_{rW} \\
\frac{dL_{rW}}{dt} &= \alpha_O O_{rW} - \alpha_L L_{rW} - \mu_L L_{rW} \\
\frac{dP_{rW}}{dt} &= \alpha_L \frac{L_{rW}}{1+(\gamma L_{rW})^\beta} - \alpha_P P_{rW} - \mu_P P_{rW}
\end{aligned} \tag{2}$$

O_{rW} denotes the number of released *Wolbachia*-infected eggs, which is the product of the release ratio (*RR*) and the equilibrial adult population prior to control ($\dot{F} + \dot{M}$). L_{rW} denotes the number of *Wolbachia*-infected larvae resulting from released eggs, and P_{rW} denotes the number of *Wolbachia*-infected pupae resulting from released eggs. The aquatic-stage *Wolbachia*-infected *Ae. aegypti* that hatch outside of the release containers (subscript '*W*' instead of '*rW*') are tracked separately from those which are newly released. The wild-hatching *Wolbachia*-infected mosquitoes follow these dynamics:

$$\frac{dO_W}{dt} = \varphi F_W - \alpha_O O_W - \mu_O O_W$$
$$\frac{dL_W}{dt} = \alpha_O O_W - \alpha_L L_W - \mu_L L_W$$
$$\frac{dP_W}{dt} = \alpha_L \frac{L_W}{1+(\gamma(L+L_W))^{\beta}} - \alpha_P P_W - \mu_P P_W \quad (3)$$

Wolbachia-infected adult mosquitoes comprise those that have emerged from the wild combined with those emerging from release containers:

$$\frac{dM_W}{dt} = 0.5\alpha_P(P_W + P_{rW}) - \mu_M \varepsilon M_W$$
$$\frac{dF_W}{dt} = 0.5\alpha_P(P_W + P_{rW}) - \mu_F \varepsilon M_W \quad (4)$$

ε denotes the relative mortality of *Wolbachia*-infected adult mosquitoes compared to uninfected. A sensitivity analysis explored the impact of *Wolbachia* infection fitness costs on mosquito population dynamics under *Wolbachia* release scenarios by varying ε (Supplementary Figure 1). Parameter definitions and values are shown in Table 1.

Suppression

Wolbachia-infected egg release was also explored after first deploying suppression interventions. The suppression techniques

Table 1. Model parameters values.

Parameter	Description	Value	Reference
φ	Daily egg laying rate of adult females	500*(1/14)	Otero *et al.*, 2006[33]
M_{null}	Male uninfected adults	$M + c_i M_w$	-
F_{all}	Total female adults	$1+ F + F_w$	-
M_{all}	Total male adults	$1 + M + M_W + c_M M_s$	-
α_O	Daily rate eggs hatch into larvae	0.5	Marinho *et al.*, 2016[34]
α_L	Daily rate larvae develop into pupae	0.18	Marinho *et al.*, 2016[34]
α_P	Daily rate pupae develop into adults	1	Masters *et al.*, 2020[35]
μ_O	Daily mortality rate of eggs	0.01	Trpis, 1972[36]
μ_L	Daily mortality rate of larvae	0.1*	Couret *et al.*, 2014[37]
μ_P	Daily mortality rate of pupae	0.1*	Couret *et al.*, 2014[37]
μ_M	Daily mortality rate of adult males	1/14	Yakob *et al.*, 2008[10]
μ_F	Daily mortality rate of adult females	1/14	Yakob *et al.*, 2008[10]
γ	Determines the density at which mortality remains proportionate	1	Bellows, 1981[38]
β	Determines the 'abruptness' of density dependence	0.5	Bellows, 1981[38]
RR	Release ratio of *Wolbachia*-infected adults compared to total adult mosquitoes	Variable	Estimated
c_i	Proportion of cytoplasmic incompatibility that fails	0.012	Walker *et al.*, 2011[7]
c_m	Competitiveness of released sterilised males	0.5	Winskill *et al.*, 2014[39]
c_v	Proportion of adult population reached by adulticide	0.141	Estimated, described in adulticide section
ε	Relative mortality of *Wolbachia*-infected adult mosquitoes compared to uninfected	1.2	Joubert *et al.* 2016[40]
s	Adjustment parameter which matches average seasonal mosquito population to non-seasonal equilibrium mosquito population	2.09	Estimated, described in seasonality section

analysed were the release of 1st generation self-limiting technology (1gSLT), sterile insect technique (SIT), Male *Wolbachia* release, environmental management, larvicides, and adulticides. Each type of suppression was included as a function of time, *t*, so that a value which influences model dynamics is pulsed at specific times or maintained over a specific period. The efficacy of each method was based on evidence sourced from the literature, selected with a preference for large randomised-controlled trials, however, each suppression method works differently and trials to measure effectiveness vary with study design. Efficacy of a single burst of application was preferable but only found for adulticide. Studies measuring repeated concurrent applications were sourced for 1gSLT, SIT, and Male *Wolbachia* release, while environmental management and larvicides used an interrupted time series design. The impact of variations in study design are discussed in more detail below.

1gSLT. Release of 1gSLT adult males produce offspring with wild females of which only the males develop to adulthood from the pupal stage. 1gSLT was included in the model by pulsing adult males into a sterile adult male compartment M_S weekly, which then contributed to the production of sterile eggs, O_S, which developed through sterile larval and pupal compartments, L_S and P_S, contributing to a density dependant survival function. RR_{supp} denotes the release ratio for mosquito release suppression techniques. ε denotes the relative mortality of *Wolbachia*-infected adult mosquitoes compared to uninfected adult mosquitoes.

$$\begin{aligned}\frac{dO_S}{dt} &= \frac{\varphi F_{all}\, c_M M_S}{M_{all}} - \alpha_O O_S - \mu_O O_S \\ \frac{dL_S}{dt} &= \alpha_O O_S - \alpha_L L_S - \mu_L L_S \\ \frac{dP_S}{dt} &= \alpha_L \frac{L_S}{1+(\gamma(L+L_W+L_S))^{\beta}} - \alpha_P P_S - \mu_P P_S \\ \frac{dM_S}{dt} &= RR_{supp} f_{RIDL} - \mu_M \varepsilon M_S\end{aligned} \tag{5}$$

Parameters for the hypothetical fixed rate efficacy of 20%, 50%, and 80% were calculated by comparing the total adult population at model equilibrium with the minimum adult population reached after five weeks of application. The literature-derived efficacy values were 45% five weeks after the last suppression period and 70% ten weeks after the last suppression period[41], calculated by comparing the total adult population at model equilibrium to the total adult population after five- or ten-weeks of suppression which achieved the desired efficacy (summarised in Supplementary Table 1). A caveat of this approach is that the resulting minimum adult population is reached later than five- or ten-weeks, therefore, the maximum efficacy calculated in these scenarios is marginally greater than the literature value stated (shown in Supplementary Figure 2).

SIT. SIT involves releasing sterile adult males which produce sterile eggs (in the same manner of Equation 5) that do not develop further. SIT was included in the model by pulsing adult males into the sterile adult male compartment, M_S, which then contributed to the production of sterile eggs which then do not develop further.

$$\frac{dM_S}{dt} = RR_{supp} f_{SIT}\, 0.5\alpha_P P_S - \mu_M \varepsilon M_S \tag{6}$$

Parameters for the hypothetical fixed rate efficacy of 20%, 50%, and 80% were calculated by comparing the total adult population at model equilibrium with the minimum adult population reached after five weeks of application. The literature-derived efficacy values were 49% five weeks after the last suppression period and 77% ten weeks after the last suppression period[42], calculated by comparing the total adult population at model equilibrium to the total adult population after five- or ten-weeks of suppression which achieved the desired efficacy (summarised in Supplementary Table 1). A caveat of this approach is that the resulting minimum adult population is reached later than five- or ten-weeks, therefore, the maximum efficacy calculated in these scenarios is marginally greater than the literature value stated (shown in Supplementary Figure 2).

Male Wolbachia release. Male *Wolbachia* release involves releasing only *Wolbachia*-infected adult males, resulting in no offspring due to cytoplasmic incompatibility with the local non-*Wolbachia*-infected females. Male *Wolbachia* release was implemented in the model by pulsing *Wolbachia*-infected adult males into the *Wolbachia*-infected male compartment:

$$\frac{dM_W}{dt} = RR_{supp} f_{IIT}\, 0.5\alpha_P (P_W + P_{rW}) - \mu_M \varepsilon M_W \tag{7}$$

Parameters for the hypothetical fixed rate efficacy of 20%, 50%, and 80% were calculated by comparing the total adult population at model equilibrium with the minimum adult population reached after five weeks of application. The literature-derived efficacy values were 65% five weeks after the last suppression period and 92% ten weeks after the last suppression period[43], calculated by comparing the total adult population at model equilibrium to the total adult population after 5- or 10-weeks of suppression which achieved the desired efficacy (summarised in Supplementary Table 1). Similar to 1gSLT, a caveat of this approach is that the resulting minimum adult population is reached later than 5- or 10-weeks, therefore, the maximum efficacy calculated in these scenarios is marginally greater than the literature value stated (shown in Supplementary Figure 2).

Environmental management. Environmental management reduces the amount of egg-laying habitat which was simulated by manipulating the egg production rate:

$$\begin{aligned}\frac{dO}{dt} &= f_{EM}\, \varphi F \frac{M_{null}}{M_{all}} - \alpha_O O - \mu_O O \\ \frac{dO_W}{dt} &= f_{EM}\, \varphi F_W \frac{M + M_W}{M_{all}} - \alpha_O O_W - \mu_O O_W\end{aligned} \tag{8}$$

Parameters for the hypothetical fixed rate efficacy of 20%, 50%, and 80%, and the literature-derived efficacy of 47.4%[44], were calculated by comparing the total adult population at model equilibrium with the new population equilibrium reached after applying this technique for the duration of the simulation; this

emulates real-world scenarios in which application and outcome are typically long-term (summarised in Supplementary Table 1).

Larvicides. Larvicides were simulated by equally reducing the number of eggs, larvae, and pupae. Because the mode of action is identical and because the best measurement of effectiveness of an intervention that targets the aquatic stages of the mosquito came from a trial of predatory guppies, this effectiveness as measured by 45 was chosen to represent the effectiveness of larvicides.

$$\begin{aligned}
\frac{dO}{dt} &= \varphi F \frac{M_{null}}{M_{all}} - \alpha_O O - f_{LV}\mu_O O \\
\frac{dO_W}{dt} &= \varphi F_W \frac{M + M_W}{M_{all}} - \alpha_O O_W - f_{LV}\mu_O O_W \\
\frac{dL}{dt} &= \alpha_O O - \alpha_L L - f_{LV}\mu_L L \\
\frac{dL_W}{dt} &= \alpha_O O_W - \alpha_L L_W - f_{LV}\mu_L L_W \\
\frac{dP}{dt} &= \alpha_L \frac{L}{1+\gamma(L+L_W+L_S)^\beta} - \alpha_P P - f_{LV}\mu_P P \\
\frac{dP_W}{dt} &= \alpha_L \frac{L_w}{1+(\gamma(L+L_w+L_S))^\beta} - \alpha_P P_W - f_{LV}\mu_P P_W
\end{aligned} \tag{9}$$

Parameters for the fixed rate efficacy of 20%, 50%, and 80%, and the literature-derived efficacy of 44%[45], were calculated by comparing the total pupae population at model equilibrium with the new pupae equilibrium reached after applying this technique for the duration of the simulation; similar to environmental management techniques this emulates real-world scenarios in which application and outcome are typically long-term (summarised in Supplementary Table 1).

Adulticides. Finally, the deployment of adulticides through fogging and chemical spraying were simulated by pulses which manipulate the mortality rates of adult male and female compartments:

$$\begin{aligned}
\frac{dM}{dt} &= 0.5\alpha_P P - \mu_M M - f_{AD} c_v M \\
\frac{dM_W}{dt} &= 0.5\alpha_P (P_W + P_{rW}) - \mu_M \varepsilon M_W - f_{AD} c_v M_W \\
\frac{dF}{dt} &= 0.5\alpha_P P - \mu_F F - f_{AD} c_v F \\
\frac{dF_W}{dt} &= 0.5\alpha_P (P_W + P_{rW}) - \mu_F \varepsilon F_W - f_{AD} c_v F_W
\end{aligned} \tag{10}$$

Parameters for the fixed rate efficacy of 20%, 50%, and 80% were calculated by comparing the total adult population at model equilibrium with the minimum adult population after one application. Mani *et al.*[46] reported an initial 94% reduction in mosquito resting density from application of deltacide, a synergized mixture of pyrethroids, after which the population completely recovered within seven days (summarised in Supplementary Table 1). This combination of great suppression and swift recovery could not be replicated in the model by only manipulating adult mortality; this may be because some portion of the reduction in resting density was due to a repellent effect, which has been noted as a possibility by the source paper[46] or because recovery was due to recolonisation by neighbouring populations which is not modelled here. To fit this literature efficacy a parameter for 94% mortality rate was first calculated by comparing the total adult population at model equilibrium with the minimum adult population after one application and subsequently a coverage parameter, denoted as c_v, was fitted using the 94% efficacy parameter. The highest proportion of coverage was calculated which allowed 80% population recovery within three weeks of suppression using literature-derived efficacy; the assumption of this recovery speed was explored with a sensitivity analysis, shown in Supplementary Figure 3, and found to be minimally affected by changing the number of weeks taken for recovery.

Seasonality

Seasonality is defined by using a normalised and smoothed lowess curve of average monthly precipitation (sourced from www.meteoblue.com) to create a score, bounded by 0 and 1, for a seasonality profile with a distinct wet and dry season each year, produced using data from Rio de Janeiro. Within the model, this score influences γ within the density dependent function. The density-dependent seasonal function of larval survival is:

$$\frac{L}{1+(\gamma f_K s(L+L_W+L_S))^\beta} \tag{11}$$

The seasonality function, f_K, returns a precipitation score dependent on time, t, which affects the rate larvae develop and enter the pupal stage. s is a constant, calculated to ensure the average mosquito population in the seasonal model is within 0.5 of the non-seasonal model equilibrium which allows comparability to the non-seasonal analyses as suppression efficacy parameters and functions execute according to this average. This affects all wild-hatched model compartments; however, the released *Wolbachia*-infected eggs are introduced in containers which isolate them from the limits of rainfall dependent egg hatching and larval growth (seasonal population dynamics shown in Supplementary Figure 4). Placement of the seasonality function within the model was explored (Supplementary Table 2) in addition to the impact of temperature, rather than precipitation, on larval development (Supplementary Figure 5).

Release scenario analyses

Wolbachia coverage data was extracted from the report by Utarini *et al.*[16] using WebPlotDigitzer[47] and the intervention cluster-level results used as reference to calibrate the model. Simulations of *Ae. aegpti* population dynamics were undertaken to investigate the intervention conditions which would produce the desired *Wolbachia* coverage levels (>95%) in the mosquito population. Specifically, a range of release ratios (0.03 to 0.1, in increments of 0.01) and number of releases (9 to 14, pulsed every 14 days) were explored and their influence on the number of days until target coverage was achieved.

The influence of suppression efficacy (20%, 50%, 80%, and a literature derived efficacy) and the week of switch from suppression to *Wolbachia* release (1 to 10) was investigated in terms of the minimum release ratio (0.0025 to 0.4, explored in increments of 0.0025) necessary to reach *Wolbachia* target coverage within six months of the first *Wolbachia* release.

The seasonality model was run for 18 months and the initial six months burn in period needed for model calibration was discarded; five weeks of suppression followed by five rounds of *Wolbachia* replacement release were simulated, exploring minimum RR (0.0025 to 0.4 in increments of 0.0025) required to reach target coverage within six months of first *Wolbachia* replacement release.

Results

Exploring the sensitivity of *Wolbachia* replacement to key release characteristics

To explore how self-sustaining *Wolbachia* replacement can best be achieved and its sensitivity to various operational parameters we formulate, fit and simulate from an entomological dynamic compartmental model. By calibrating the release ratio parameter, our model showed a good fit to the mosquito release data from the Yogyakarta RCT[16] with replacement dynamics and coverage levels proceeding at a similar rate. The model reached 50% coverage after 121 days, compared to an average of 117 days observed in the RCT, and 90% coverage after 180 days, compared to an average of 239 days observed in the RCT (Supplementary Figure 6). As *Wolbachia* reaches fixation the model slightly overestimates final *Wolbachia* coverage, likely due to prevalence being suppressed in the RCT due to migration of uninfected adult mosquitoes from outside the release area which were not included in our model and would be reduced when implemented as a wide-scale blanket intervention.

During a *Wolbachia* replacement programme, our model predicts that the total adult mosquito population experiences a temporary exacerbation above baseline levels, followed by a decline before reaching a new equilibrium once *Wolbachia* fixation has been achieved (Figure 1A, 1B). Due to the fitness

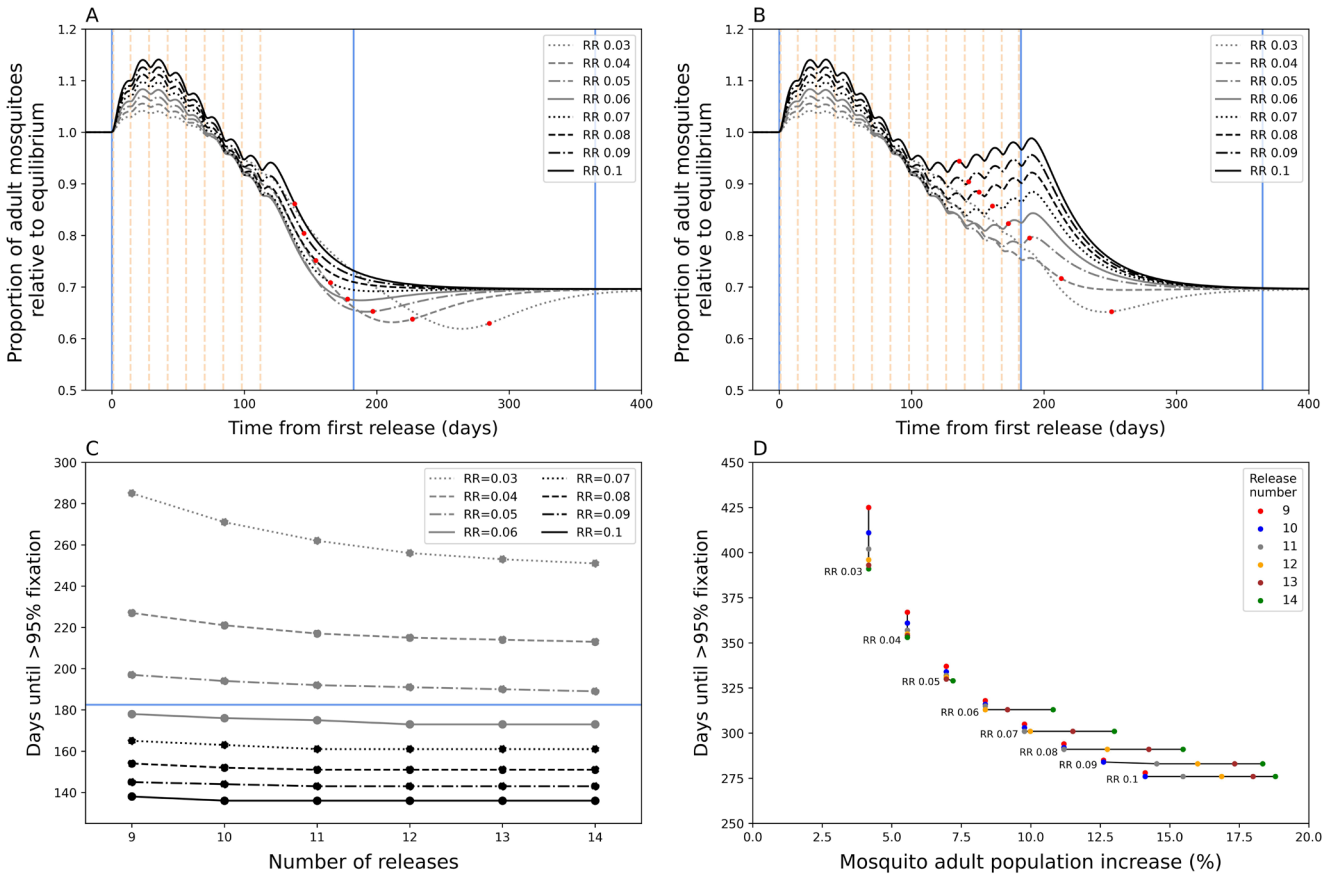

Figure 1. Mosquito dynamics of *Wolbachia* replacement. Figures **A** and **B** show the total adult mosquito population size over time during *Wolbachia* replacement after a nine (**A**) and fourteen (**B**) release (dotted vertical lines) round programme. Red dots indicate the date at which target coverage (>= 95% *Wolbachia* coverage) was first achieved. The blue lines show the time points from first release at 0 days, 6 months, and 1 year from left to right. **C**) Days until *Wolbachia*-infected adult mosquitoes reach target coverage for different numbers of releases and release ratios (RR). The horizontal blue line indicates 6 months. **D**) The percentage increase in total mosquito population for different numbers of releases and release ratios. This is calculated as the sum of the peak increase after initial release compared to the pre-release population equilibrium and the peak increase after target coverage is achieved compared to post-fixation equilibrium population, the latter excludes lower RR instances where the population at fixation is lower than the new population equilibrium.

cost of *Wolbachia* (conservatively modelled to be 20%[40], but highly variable depending on environment[48]), this new equilibrium mosquito population size is predicted to be lower than before *Wolbachia* release.

As expected, *Wolbachia* target coverage can be achieved faster by increasing RR and/or by increasing the number of releases (Figure 1C). Our model shows that increasing RR of each release will reduce time to target coverage more than increasing the number of release rounds, particularly at higher RR values. Above a RR of 0.06, increasing the number of release rounds has little additional effect on time to target coverage. Our model predicts that achieving *Wolbachia* target coverage within six months of the first release is possible with RR >= 0.06 with >= 9 releases. Higher RR and number of release rounds lead to ever diminishing decreases in time to target coverage. Increasing the RR from 0.06 to 0.1 is only predicted to increase time to target coverage by 40 days (nine release programme, Figure 1C).

Higher RRs and higher release round numbers also lead to disproportionately undesirable temporary exacerbation issues, particularly at higher values (Figure 1A,B,D). A doubling of RR from 0.05 to 0.1 could lead to an approximate doubling of exacerbation (6.96% to 14.11%) under a nine-release programme, but this could be up to 2.6 times more (7.20% to 18.80%) if the number of release rounds were increased to 14. This is because prolonged releases at high RR led to a secondary peak in mosquito abundance that prolongs the period of exacerbation (Figure 1B); in the fourteen-release programme this is still less than the original population, however, increasing number of releases could expect this secondary peak to eventually exceed the prior population size.

Overall, these simulations suggest the importance of balancing speed of *Wolbachia* replacement with the potentially negative consequences of temporarily exacerbating the mosquito population. In combination with other field evidence, this work supported the TPP's guidance on "time to achieve target coverage". The models suggested that a time to achieve coverage of less than 12 months was highly feasible (minimum TPP standard) and that a goal of 6 months (preferred TPP standard) was achievable. To counterbalance the issue of exacerbation, the TPP included a criterion for "community acceptability" that states that any increase in nuisance biting through the chosen release characteristics is "acceptable to local residents", recognising that the definition of "acceptable" is likely to be highly context specific.

Global cost targets for *Wolbachia* replacement

The TAG identified cost as a key reason limiting wider adoption of *Wolbachia* replacement and therefore a "mature product cost once implemented at scale" criterion was a key feature of the TPP. This cost criterion needed to be low enough to drive innovation and ensure a significant proportion of the global population at risk of dengue can benefit, but not too low as to exclude promising products from further development.

Because detailed data on willingness to pay was unavailable at the time of analysis, we developed a range of scenarios that assume willingness to pay is approximated by the costs of treating dengue cases and of vector control in response to outbreaks over a range of years (Table 2). Each scenario gave a theoretical cost each 5km × 5km area would be willing to pay for *Wolbachia* replacement. Areas that supported higher costs typically had higher dengue burden but were also heavily influenced by the cost of dengue treatment and prevention.

We estimate that to achieve a 25% reduction in the global burden of dengue, as per the WHO 2020-2030 goals, using only *Wolbachia* replacement targeted to the most cost-efficient areas, would require releases across 924,557km^2 in 73 countries

Table 2. The predicted target cost per person for *Wolbachia* replacement based on different assumptions about desired global impact (rows) and averted medical and outbreak control costs (assumed proxy of willingness to pay, columns). *Wolbachia* replacement would need to be at or below this cost to achieve each impact scenario in full. All values show median estimates in 2020 US dollars, brackets show model predicted uncertainty around the true value of this cost threshold at the 95% credible interval level).

Impact scenario	Required cost per person covered (10 years benefit)	Required cost per person covered (5 years benefit)	Required cost per person covered (3 years benefit)
12.5% global burden reduction	$7.63 (5.15 – 29.42)	$4.10 (2.77 – 15.83)	$2.54 (1.71 – 9.78)
25% global burden reduction	$4.33 (2.73 – 18.95)	$2.33 (1.47 – 10.20)	$1.44 (0.91 – 6.30)
12.5% national burden reduction	$0.98 (0.64 – 3.78)	$0.53 (0.34 – 2.03)	$0.33 (0.21 – 1.26)
25% national burden reduction	$0.72 (0.26 – 1.66)	$0.39 (0.14 – 0.89)	$0.24 (0.09 – 0.55)

(Figure 3). This corresponds to 34.7% of the urban (> 300 people per km^2) area at risk and all major dengue-endemic cities and just 1.7% of the total area at risk of dengue. If *Wolbachia* only needs to achieve half of the global 25% reduction, with other interventions responsible for the remaining half, *Wolbachia* releases would only need to be targeted to 255,459km^2 over 47 countries (Figure 2B and 2E). However, because these cost estimates are uncertain and because this approach prioritises high income countries where dengue treatment costs are high, we also include a third and fourth targeting scenario where 25% or 12.5% of the national burden must be reduced for the majority (95%) of dengue endemic countries (Figure 2C and 2F). These scenarios improve equity over dengue-endemic regions. A full list of cities and 2nd administrative units included under each targeting scenario, the costs each will support and additional contextual information (population, density, etc) is included in the following repository: https://github.com/katietiley/Wolbachia_TPP_PPC.git.

For *Wolbachia* replacement to be implemented in enough areas to meet these impact targets, the cost of implementation must have the potential to ultimately be reduced to between $7.63 and $0.24 per person covered depending on scenario (Table 2). The cost thresholds identified in Table 2 represent the area with the lowest averted costs (assumed lowest willingness to pay) within the areas needed to reach each impact target. This means that many eligible areas, or even whole countries, could support higher programme costs, but ultimately *Wolbachia* replacement will need to be implemented at or below this cost threshold in order to reach the impact target. The distribution of these costs and benefits by country is shown in Figure 3 for the 25% global burden reduction impact target. This shows that while globally *Wolbachia* replacement will need to be achieved for $2.33 per person to meet the 25% impact goal, many countries could support higher costs with many high burden countries able to implement in a wide range of high burden areas above the $10 per person line.

Cost targets become lower (increasingly more ambitious for product development) as impact scenarios become more ambitious or, to a lesser extent, as the accepted duration of benefits becomes shorter (rows and columns in Table 2 respectively). To achieve a 25% national burden reduction in all dengue endemic countries would require a cost target ~ 10x lower than to achieve a 12.5% global dengue burden reduction, emphasising that even higher cost products could still have substantial global impact, but would be less equitable unless subsidised for countries with lower financial capacity for dengue prevention and treatment. Due to high uncertainty in estimates of the true burden of dengue[26] and its costs of treatment[27] and prevention, uncertainty around these cost thresholds is moderate with higher uncertainty around higher median cost thresholds.

Each of these scenarios and their respective cost targets were presented to the TAG for discussion and selection for the TPP. Recognising that the TPP minimum criteria should reflect the minimum cost for a product to be viable at substantial scale, TAG members selected the $2.33 per person covered target (corresponding to a 25% global burden reduction with five years of benefit, Table 2). This cost needs to include the programme of activities required to reach 90% coverage of *Wolbachia* in the release areas one year after starting releases. The TPP also makes allowances for a slower programme where 90% coverage is achieved over three years, but this must be achieved at a more stringent minimum TPP cost target of $1.44 per person covered (corresponding to a 25% global burden reduction with three years of benefit, Table 2). However, to challenge developers to meet the more equitable 25% national dengue burden reduction, the TPP preferred cost threshold was set at $0.24 per person covered (corresponding to three years of benefits in this scenario, Table 2). These decisions were also informed by evidence that the current World Mosquito Program cost base for *wMel Wolbachia* replacement is in the US$5-22 per person range[30] with a medium-term goal of achieving *Wolbachia* replacement for $1 per person[49]. Given that these TPP targets represent the lowest averted medical and outbreak control costs per person among all areas where releases are required, there are many areas that could support higher programme costs. Therefore, provided developers can demonstrate the prospect of achieving the TPP cost targets in future, there is

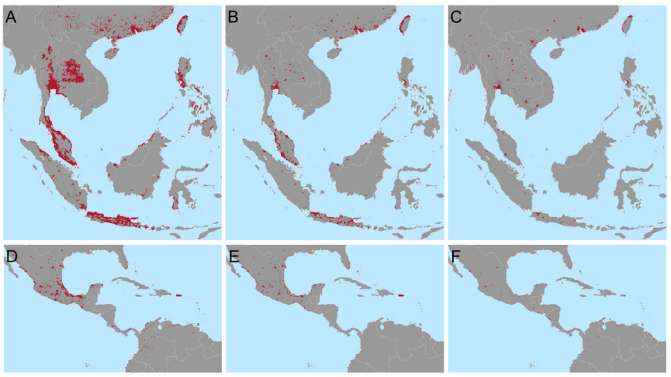

Figure 2. Targeting areas for *Wolbachia* replacement to meet different global and national goals in Southeast Asia (**A**–**C**) and Central America and the Caribbean **D**–**F**). Maps show the areas most cost efficient to target (red) to reduce the global burden of dengue by 25% (**A** and **D**) or 12.5% (**B** and **E**) or the national burden by 25% (**C** and **F**) based on the cost of treatment and prevention of current dengue burden. Predictions for other areas and lists of municipalities to be targeted are included in the following repository https://github.com/katietiley/Wolbachia_TPP_PPC.git.

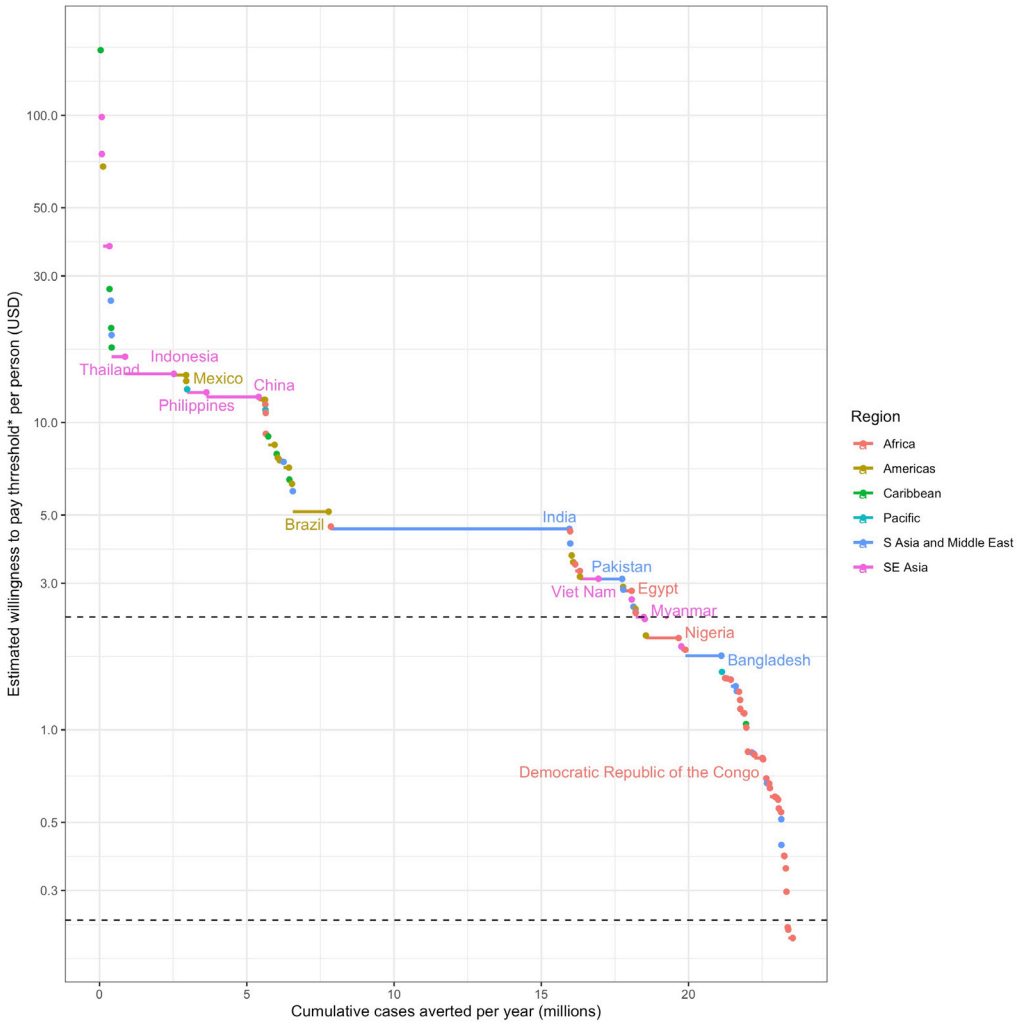

Figure 3. The cumulative contribution of each dengue endemic country to the 25% global reduction in dengue burden and the averted costs (willingness to pay proxy) per person covered at which it can be achieved. *The willingness to pay threshold is estimated by cumulative medical and outbreak response costs over 5 years for the TPP minimum and 3 years for the TPP preferred criteria. Horizontal dotted lines show the cost thresholds of $2.33 per person and $0.24 per person chosen for the TPP minimum and preferred criteria respectively. Only high burden countries are labelled.

scope to operate higher cost programmes before these targets are achieved.

Exploring the development of a hybrid "suppress then replace" approach

In addition to the draft TPP for *Wolbachia* replacement, the TAG was also tasked to develop a draft PPC for a hybrid mosquito population suppression followed by *Wolbachia* replacement approach. Reducing the natural mosquito population size could allow *Wolbachia* replacement programmes to achieve higher release ratios or achieve comparable release ratios by releasing fewer *Wolbachia* mosquitoes. To support the development of the PPC we developed a compartmental entomological model and simulated hybrid strategies with a range of suppression types to answer the questions: can hybrid strategies achieve coverage faster, improve community acceptance and reduce costs relative to a *Wolbachia* replacement programme alone?

Development of this model first involved fitting the model to the literature-derived efficacy estimates, which differ for each suppression method. Study design influences measurement of maximum suppression efficacy, time taken to reach maximum suppression, and time taken to recover to pre-suppression levels, which were all considered when fitting the model and making predictions for a standardised single application programme. When comparing a single suppression application with literature-derived efficacy adulticide achieves the greatest suppression but rapidly returns to pre-suppression levels (Figure 4). Other methods take longer to reach peak effectiveness, but also have longer durations of effectiveness, particularly insect release methods. Male *Wolbachia* release is the most effective insect-release method but 1gSLT has a longer-lasting effect. Finally, environmental management and larvicides were estimates to result in the least suppression when applied over a short time period as they typically require long consistent

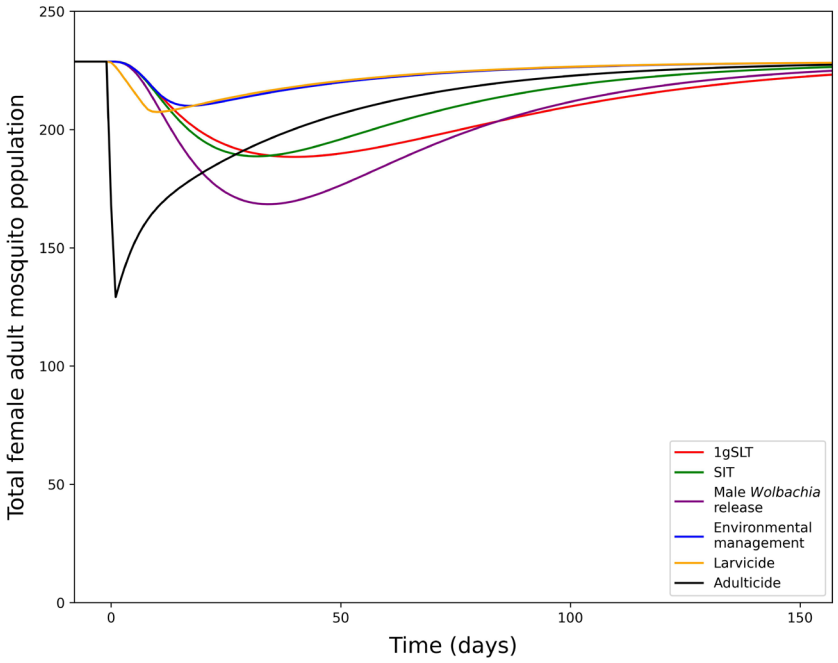

Figure 4. Dynamics of adult mosquito population after a 1-week suppression programme with literature-derived efficacy. Showing the total adult female mosquito population dynamics under 1-week suppression application with different methods.

periods of application to reach maximum suppression efficacy. With reductions in adult mosquito population size in the range of 8.18 – 43.51%, from a single application all methods of suppression were predicted to remove the ~1–10% mosquito population exacerbation seen in replacement only programmes.

Our model predicts that a prior suppression programme of five weekly rounds could reduce the number of *Wolbachia* mosquitoes required to reach target coverage within 6 months by 16–81% depending on suppression method used (Figure 5A, comparing literature-derived estimates). All insect release-based suppression methods gave greater reductions in required *Wolbachia* mosquitoes than conventional methods. This superiority is maintained even if the peak effectiveness is standardised across different methods of suppression (Figure 5A), suggesting longer-lasting suppression methods are preferable for hybrid approaches. It may seem counterintuitive to use mosquito killing methods at the same time as *Wolbachia* mosquitoes are being released, but if these mosquito killing methods do not disproportionately affect *Wolbachia* mosquitoes relative to the wildtype (as assumed in our model), suppression will still reduce the overall number of *Wolbachia* mosquitoes required for replacement. Among conventional suppression methods, adulticide outperformed environmental management and larvicide with reductions in required release ratio of 39%, 19% and 16% respectively, considering literature-derived efficacy.

Similar results were found when the number of *Wolbachia* release rounds were reduced (as opposed to reducing the release ratios per round) suggesting programmes could realise this benefit by reducing the number or density of *Wolbachia* releases. Conversely, programmes could choose to release the same number of *Wolbachia* mosquitoes, but now at much higher release ratios which would achieve *Wolbachia* target coverage faster. Insect release suppression methods could be used to decrease the time to target coverage by up to 80%, while conventional methods would only marginally improve speed (less than 20%), or not at all in the case of environmental management (Table 3).

By reducing the number of Wolbachia mosquitoes released and duration of the replacement phase of the hybrid strategy, the costs of this phase could be reduced. If these levels of suppression can be achieved at a lower cost than the savings of the replacement phase, then a hybrid strategy could reduce costs overall. Here we use models to estimate these maximum costs for suppression, beyond which a replacement-only programme would be preferable. If the baseline replacement programme can meet the TPP minimum cost target of $2.33 per person, then suppression would need to cost not more than $0.41 – $1.17 per person for mosquito-release suppression methods and $0.09 – 0.50 per person for conventional methods (Table 3).

A hybrid programme may be considered more acceptable from a community perspective due to prevention of the temporary increase in numbers of biting female mosquitoes. The mosquito- release suppression techniques (i.e., 1gSLT, SIT, Male *Wolbachia* release) may also lead to a temporary increase

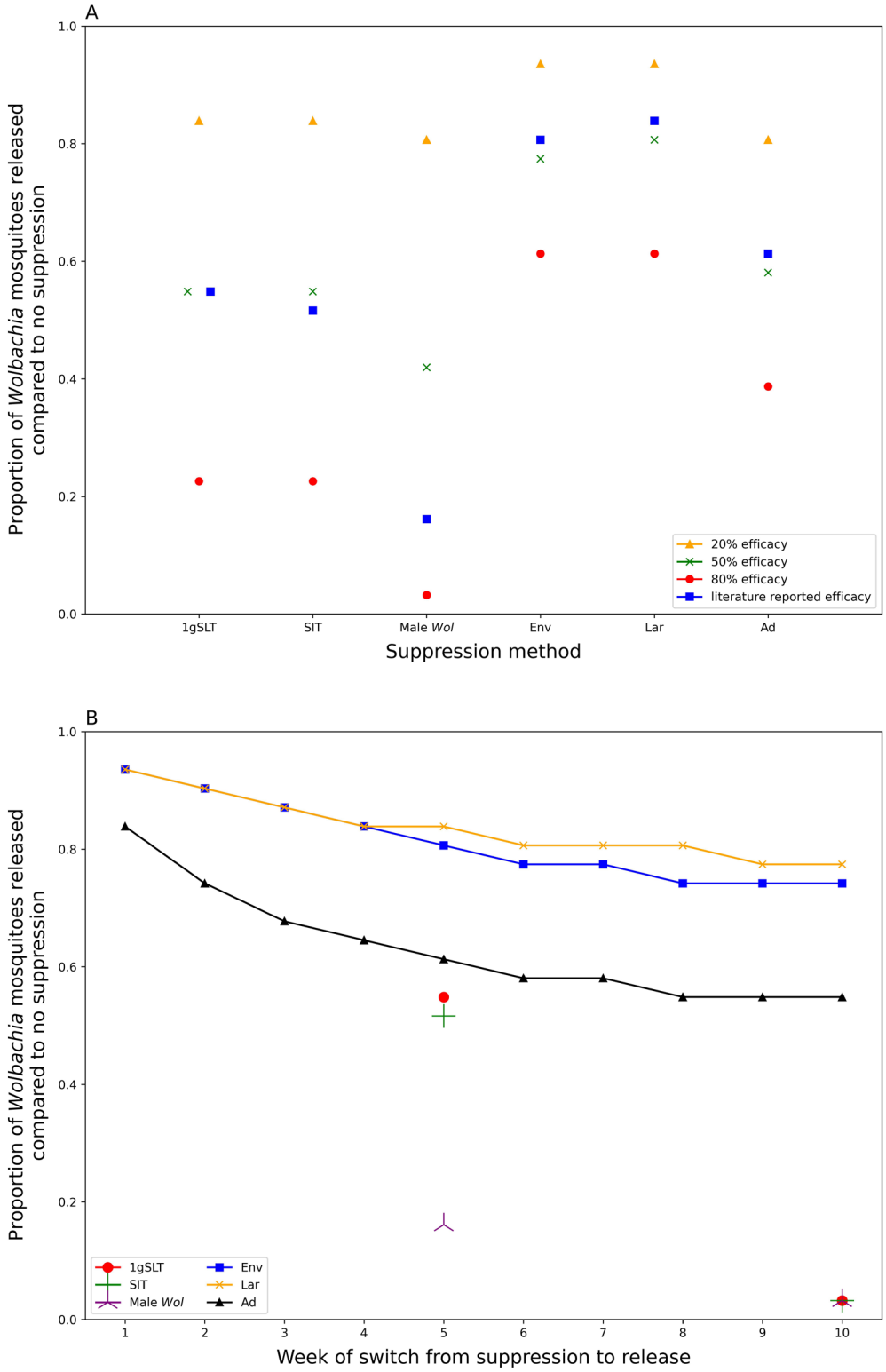

Figure 5. Proportional release ratios for *Wolbachia* replacement programmes post suppression. Replacement with no prior suppression requires a *Wolbachia* release ratio of 0.0775 to achieve target coverage (95%) within 6 months of first release. **A**) shows the proportional reduction in required *Wolbachia* release ratio following a 5-week suppression programme with different methods. **B**) shows how this proportional reduction in required *Wolbachia* release ratio declines with increasing rounds of suppression. All suppression methods use literature-derived efficacy; 1gSLT, SIT, and Male *Wolbachia* release only show two data points because the literature calculated efficacy at 5- and 10-weeks.

Table 3. Potential time reductions using the hybrid approach and maximum costs of suppression for comparable overall cost. Compared to a *Wolbachia* replacement-only programme with nine releases at a relative ratio of 0.09 achieving target coverage (95%) in 215 days.

Method of suppression	Maximum cost for suppression for the hybrid approach to be cost saving ($ per person covered)	Percentage reduction in days to achieve target coverage
1gSLT	$0.42 – 1.17	83.4
SIT	$0.41 – 1.13	82.5
Male *Wolbachia* release	$0.41 – 1.13	82.5
Environmental management	$0.09 – 0.25	0.0
Larvicide	$0.15 – 0.42	11.3
Adulticide	$0.18 – 0.50	18.5

in mosquito population and would require separate community engagement activities to emphasise that the released males do not bite or increase the risk of infection.

Longer suppression campaigns give diminishing returns when used as part of a hybrid approach (Figure 5B). The greatest benefits, in terms of reducing *Wolbachia* release requirements, are seen within the first few weeks of suppression with decreasing benefits beyond five weekly rounds of suppression. This effect is more pronounced for insect release suppression methods that see most of their benefits delivered from a one- to three-week suppression programme, while for conventional methods there may still be some benefit in continuing suppression for up to eight weeks (Figure 5B).

The primary role of these modelling results in developing the PPC was to clarify the potential benefits of the hybrid approach; namely that a hybrid approach could achieve *Wolbachia* coverage faster and with higher community acceptance. Citing this modelling input the TAG concluded in the PPC that a range of suppression methods can be considered for combination with *Wolbachia* replacement to achieve potential benefits of faster achievement of coverage by *Wolbachia* and higher community and programmatic acceptance. The draft PPC states that "trials of a hybrid approach would test the expected benefits of conducting suppression followed by replacement and that modelling thus far suggests that suppression methods involving insect releases will generally reduce the intensity of the *Wolbachia* replacement programme more than other methods". It was also agreed that modelling would be a useful tool for prioritising intervention combinations for field trials and could be used to directly inform trial design. Finally, the PPC recognises that hybrid approaches may require additional logistical and practical complexities over replacement alone, particularly for mosquito release suppression methods that may require additional regulatory approval. This may mean that rather than hybrid approaches superseding replacement-only approaches, their use may be restricted to areas where replacement-only cannot meet speed and acceptability goals.

Extension of this modelling work to account for seasonal variations in mosquito population size with the aim of optimising the seasonal timing of replacement-only and hybrid approaches was also requested. Since the original PPC meeting, our model has been expanded to include a typical seasonal profile. Our model assumes mosquito population sizes closely follow variation in precipitation with a 41-day lag and a peak population size ~ 3 times the dry season minimum, consistent with various field observations[50] (Supplementary Figure 4, seasonality function sensitivity analysis in Supplementary Table 2).

We predict that the optimal time to begin replacement-only or hybrid programmes is just before the seasonal lowest point in mosquito abundance (Supplementary Figure 7). The release ratio required for *Wolbachia* fixation in a replacement only programme mirrors precipitation (and thus wild type mosquito population) dynamics, with a short lag.

When mosquito populations fluctuate throughout the year, the timing of *Wolbachia* replacement has a large effect on the number of Wolbachia mosquitoes that need to be released to reach fixation. Starting the replacement programme at the optimum time could reduce the number of *Wolbachia* mosquitoes by 65.26% compared to the least optimal time. The seasonal scenario also follows the prioritisation of suppression methods observed in the non-seasonal analysis when using a hybrid suppression-then-release approach with male Wolbachia release most effective and larvicide least effective. Furthermore, at the optimal time, in the dry season, the hybrid approach could reduce the required *Wolbachia* release ratio by up to 93.94% compared to replacement alone, whereas at the least optimal time, in the wet season, the hybrid approach only reduces

the required *Wolbachia* release ratio by up to 64.21%. Finally, because of their delayed effects the insect-release suppression methods (1gSLT, SIT, Male *Wolbachia* release) allow a hybrid strategy to remain effective for longer in the early stages of the wet season, so may be a better choice for areas where the timing of mosquito seasonal cycles is less predictable.

Discussion

Mathematical and geostatistical models can make important quantitative and qualitative contributions when developing TPPs and PPCs. Here we show that models can: i) identify important trade-offs, such as the time taken for *Wolbachia* to reach target coverage and the temporary exacerbation in the mosquito population, ii) quantify threshold criteria, such as the $2.33 per person *Wolbachia* replacement cost target, iii) predict characteristics of a product in new areas and at broader scales than it is currently implemented, such as to meet the WHO 25% global burden reduction targets and iv) understand synergies and antagonisms between combinations of products that have not yet been tested, such as a hybrid suppress then replace approach. The population dynamics shown in our model are consistent with previous modelling and field studies showing temporary population exacerbation and successful fixation within one year[9,51,52]; this evidence supports the credibility of our findings quantifying the difference between scenarios with varying release ratio and release number.

Broad community acceptability of *Wolbachia* replacement will clearly be a critical aspect of achieving implementation at the scale envisioned by these TPP and PPC documents. The success of current replacement programmes has been underpinned by extensive community engagement activities[53,54] and other countries (Singapore[20] and China[19]) have chosen to use *Wolbachia* for suppression only, in part, due to concerns over any increases in mosquito abundance. Here we show temporary increases in mosquito abundance can be minimised or avoided entirely by using lower *Wolbachia* release ratios, timing releases to coincide with the dry season or conducting a prior suppression campaign in a hybrid approach. These steps, however, involve additional programmatic complexity and likely cost. More work is needed to better understand how mosquito abundance relates to community acceptability in different contexts and how such barriers can be overcome with different release intensities, timings, and hybrid approaches. One alternative use case, considered in the TPP, is to conduct longer lower intensity releases with larger distances between release sites. This would result in only around 50% of areas initially having a *Wolbachia* prevalence of over 90%, however over time mosquito diffusion would spread *Wolbachia* to all remaining areas to ensure all target areas had a *Wolbachia* prevalence over 90%. These lower density releases may have significant cost advantages and could be a more acceptable method of dissemination over broad areas where faster implementation is a lower priority. Such a strategy would, however, take longer, be dependent on patterns of mosquito movement and may be limited by environmental barriers to mosquito spread[55]. Development of spatial models of mosquito movement and dengue spread could help identify where additional release points may be necessary, target initial release points to high-risk areas and quantify the collateral benefit in disease reduction in neighbouring areas[56,57].

Cost continues to be a barrier to wider adoption of *Wolbachia* replacement when its high costs but long-term benefits are compared to lower cost but short acting suppression methods, despite differences in the evidence base underpinning these benefits[30]. A key strength of our analysis was to link TPP cost targets to conservative estimates of averted costs based on direct medical costs and emergency vector control expenditure over limited timeframes. This was critical to identify geographic differences in cost targets between, but also within countries. Pairing this analysis with high resolution global burden and cost maps identified cost targets that are compatible with wider international goals and equitable across a range of settings[25]. Work is currently underway to validate our approximation of willingness to pay for *Wolbachia* replacement through surveys targeted to key stakeholders in state and federal governments. The maps and models generated in this work could be adapted for planning national *Wolbachia* replacement campaigns and, in particular, could inform how re-use of release resources, variable pricing models, financing and slower release campaigns could be used to meet the TPP cost targets in even the most challenging countries[30]. Some of this functionality is already available in the freely available Wolbachia Decision-Support tool (https://wolbachia-tool.netlify.app/tool#map) which makes use of the outputs of this analysis along with other geospatial layers. It is important to clarify that these maps should not be used prescriptively, but rather give an indication of the kinds of areas that are likely to be most cost efficient to target, with the final decision on which areas are targeted for release subject to additional operational, entomological, financial and political considerations.

This analysis predicts that *Wolbachia* replacement releases over 924,557km^2 could lead to a 25% reduction in dengue burden globally, averting US$3.05 billion (2.62 – 3.96) worth of medical and outbreak response costs per annum. This may appear ambitious for a minimum product but can be understood in the following context. Although the lowest averted cost/person covered in this area is predicted to be US$2.33, all other release areas could support substantially higher costs (Figure 3). This means that a *Wolbachia* replacement product would still meet the TPP targets if initial programme costs were higher and if the product has the potential to reduce costs down to the $2.33 target. It is important to note that the TPP only requires that the product is suitable and available everywhere within the target areas, not that it is necessarily implemented in all suitable areas. In practice, commercial considerations, including the need to build capacity, to access funding that is incremental to the current routine control budgets, and to compete and combine with other methods, will limit the rate of uptake and the ultimate scale of deployment achieved. In areas where *Wolbachia* implementation may be challenging or less cost effective, other methods of dengue control, including vaccines, will likely be important in reaching the WHO 2030 goals.

Hybrid approaches offer one unproven but potential option for increasing speed or increasing the acceptability of *Wolbachia* replacement. The models presented here and the wider evidence provided to the PPC support field trials of hybrid approaches as a next logical step. These models can guide the

prioritisation of suppression methods, trial sample size calculations and suggest how effectiveness should be measured. We predict that insect-based suppression methods (1gSLT, SIT, Male *Wolbachia* release) will be more effective than conventional suppression tools for decreasing time to target coverage, but also outline a limited cost window which may be challenging for insect-based suppression methods to achieve. Investment in new infrastructure to conduct insect-based suppression may not be justified for a one-off suppression, but between overlapping resource requirements for suppression and replacement, ongoing use post-replacement (e.g., outbreak control or to achieve dengue elimination) and a continued drive to lower costs of mosquito suppression[58], this investment cost may be justified. Intervention developers and countries must ultimately decide how to balance cost and efficacy when considering hybrid approaches. Timing replacement to coincide with the seasonal low point of mosquito abundance is an alternative low-cost hybrid approach and would be a useful addition to trials of hybrid approaches. More generally, our results also suggest that suppression methods that have a longer residual effect are likely to be more beneficial in a hybrid approach. This would suggest some emerging vector control methods, including targeted indoor residual spraying (TIRS)[59,60] and Oxitec's second-generation Friendly™ mosquito technology[61] that allows male survival, would also be strong candidates for a hybrid approach and should also be considered for inclusion in modelling and potentially in hybrid field trials. The implementation of any suppression method should be accompanied by mosquito surveillance that confirms that the levels of suppression achieved are above and beyond what would have normally been achieved by the programme.

These models and the results they generate are not without their limitations and clear communication of these limitations was an important part of their use for the TPP and PPC. Our models of *Wolbachia* replacement do not include any spatial, temporal (beyond seasonal) or stochastic heterogeneities that may mean our model overestimates the speed to achieve fixation and target coverage, particularly in the latter stages (Supplementary Figure 2). While we simulate egg releases once every two weeks, we acknowledge that adult releases at weekly timescales may be possible and could accelerate the time to fixation. The fitness cost of *Wolbachia* infection selected in these models was a conservative estimate and, consequently, the decline of total mosquito population equilibrium from pre- to post-*Wolbachia* release (Figure 1A,B) is likely to be an over-estimation. We also do not account for a natural wild type egg bank emergence which can dilute *Wolbachia* release ratios, possibly accounting for the low RR values estimated in these analyses. The *Wolbachia* replacement system targets *Ae. aegypti*, which is the major vector of dengue, but *Ae. albopictus* can be locally important and the need for a Wolbachia replacement product to control both species is not considered in this modelling. *Ae. albopictus* and other mosquito species are assumed to be controlled by routine vector control programmes which would be ongoing in parallel with any *Wolbachia Ae. aegypti* replacement or hybrid control programmes. While this modelling was designed to be generalisable to successfully inform the TPP and PPC documents, Wolbachia replacement programmes should be context specific. Therefore, when considering extrapolation in new environments, successful long-term establishment will be context dependent as many variables may vary depending on environmental factors[11,12] which could not be simulated in this model, for example, mosquito entomological parameters and Wolbachia density is influenced by temperature[62], while physical barriers such as highways may affect mosquito dispersal[55]. There remain large gaps globally in data on the cost of dengue treatment and prevention and no comprehensive cost estimates for Zika, chikungunya or yellow fever, all of which *Wolbachia* will provide some efficacy against. Cost estimates are therefore generally conservative and should not replace primary data on willingness to pay or more detailed cost-benefit analyses when considering programmes in any one given country. We also recognise that only one modelling group was included for this TPP and PPC and that inclusion of multiple modelling teams can help better represent the structural uncertainty of models and their interpretation when deciding between policy options[63]. Fitting each suppression method to literature reported effectiveness estimates was challenging due to incomparable ways in which suppression was implemented and evaluated, and therefore the modelling outputs for each method may not be representative. In particular, our chosen source of evidence for adulticide suppression reported a 94% followed by a return to pre-suppression population within 7 days[46], a rate of rebound that our model was unable to replicate from newly emerging adult mosquitoes alone, thus we had to assume that the 94% effectiveness was only achieved in a fraction of the overall mosquito population. Moreover, this efficacy is higher than generally expected for outdoor space-spraying in urban environments in practice[64]. Additionally, suppression efficacy is highly context-dependent; to mitigate this we modelled an hypothetical 20%, 50%, and 80% suppression efficacy to allow clearer comparison between suppression techniques. Our hybrid approach results also assume that the suppression method acts independently of any other forms of vector control already in use in the area. Suppression will likely be reduced if the method of suppression (or similar methods that use the same modes of action or insecticides) is already routinely used.

In this paper we show the value dedicated modelling research can add to the development of TPPs and PPCs. By making the data, code and fitted models freely available to accompany the TPP for *Wolbachia* replacement and PPC for the hybrid approach, product developers are able to continue to use and adapt them to steer the development of a range of *Wolbachia* products to meet the rising challenge of global dengue control.

Ethics

No ethical approval was necessary as all data used was publicly available.

Data availability

GitHub. Using models and maps to inform Target Product Profiles and Preferred Product Characteristics: the example of Wolbachia replacement. DOI: https://github.com/katietiley/Wolbachia_TPP_PPC.git

This project contains the following data:

- All data and code used for each of the models used in this analysis and the predictions made by these models
- Supplementary file 1 and all supplementary figures referred to in the main text

Acknowledgements

We acknowledge Marjorie Opuni-Akuamoa for her contributions towards the cost of dengue vector control systematic review. We also acknowledge Raman Velayudhan for his guidance on experimental design, communication of results and for facilitating the modelling inputs to the TPP and PPC processes.

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Open Peer Review

Current Peer Review Status: ? ✓ ? ?

Version 3

Reviewer Report 08 November 2024

https://doi.org/10.21956/gatesopenres.17692.r38381

✓ **Perran Ross**
The University of Melbourne, Melbourne, Victoria, Australia

I thank the authors for addressing comments from myself and the other reviewers in Version 3. While the changes in Version 2 were relatively minor and did not satisfactorily address my key concerns, the authors have now expanded their discussion to highlight several assumptions and limitations. While I still disagree with the authors on some fundamental points (e.g. the value of direct comparisons between suppression techniques), my main concerns have been addressed (e.g. statements that hybrid suppress then replace releases constitute cost savings) and I would be happy to approve this version.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Wolbachia-based mosquito control

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 30 September 2024

https://doi.org/10.21956/gatesopenres.17127.r37731

? **Asha Mary Abraham**

Christian Medical College, Vellore, India

This paper describes the application of entomological and economic modelling for *Wolbachia* replacement in *Aedes egypti* mosquitoes as a method to decrease the burden of diseases spread by these mosquitoes (mainly dengue, Zika, yellow fever and chikungunya) in the absence of widely available vaccines (apart from that for yellow fever) and antiviral therapy. While the traditional methods of vector control (environmental management and application of larvicides and adulticides) do not control the mosquitoes effectively over the long term, novel technologies are currently under development of which *Wolbachia spp* have been used to replace, reduce and suppress these mosquitoes. Other technologies include first generation self-limiting (1gSLT) and sterile insect (SIT) technologies.

Aligning with The World Health Organisation's (WHO) goal to reduce dengue incidence by 25 % by 2030, the authors aimed to describe how these models can aid the development of Target Product Profiles (TPPs) for *Wolbachia* replacement and Preferred Product Characteristics (PPCs) documents for hybrid mosquito suppression followed by *Wolbachia* replacement.

The entomological model used data from the Yogyakarta RCT on *Wolbachia*-infected mosquito release, where the delivery mechanism was egg releases in release cups. The detailed statistical formulae used along with the description of the parameters involved are all given clearly for suppression of *Wolbachia*-infected mosquitoes as well as for initial intervention followed by *Wolbachia*-infected mosquito egg release. These initial interventions include release of *Wolbachia*-infected, 1gSLT- and SIT-derived adult males, as well as environmental management using larvicides and adulticides. The seasonality profile with distinct wet and dry seasons annually was produced with data from Rio de Janeiro, and this function was included in the model with respect to precipitation as well as temperature.

Various release scenarios were analysed at different suppression efficacies, week of switch from suppression to *Wolbachia* release with increments of release ratios needed to reach target coverage within 6 months of first release. The seasonality model was run for a year (the first 6 months of an 18-month run being discarded) with 5 weeks of suppression followed by 5 rounds of *Wolbachia* replacement release with minimum release rates simulated to reach target coverage.

The economic model was designed to give high spatial resolution estimates of costs of dengue that would be averted by *Wolbachia* replacement, comprising direct medical costs of treating dengue and outbreak vector control costs.

The authors estimate that the cost to reduce the global burden of dengue by 25% by the year 2030 is between US$ 7.63 to US$ 0.24 per person protected or less.

The specific clarifications I have relate to:
1. The economic model: the comment that cost savings from the hybrid programmes offer savings over replacement alone programmes is misleading since the cost of the additional suppressive methods has to be included in addition to replacement.
2. For large developing countries like
India, even the lowest cost per person protected in the predicted range works out to an enormous sum across the large population. It may actually prove more cost-effective to evaluate newer vaccines for dengue (with more specific neutralising antibody production and hence, less ADE

activity) as has currently begun.
3. Regarding the environmental model, can weather data from Rio de Janeiro be applicable to other countries in the tropics?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Dengue and other arbovirus diagnostics and research including cell culture, serology, molecular assays.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 14 Oct 2024

Katie Tiley

Thank you for reviewing this manuscript, we have addressed each of your comments.

1. *The economic model: the comment that cost savings from the hybrid programmes offer savings over replacement alone programmes is misleading since the cost of the additional suppressive methods has to be included in addition to replacement.*
Response: In response to another reviewer's suggestion, the manuscript has now been edited throughout to remove statements indicating that a combined suppress and then release programme presents cost savings.

2. *For large developing countries like India, even the lowest cost per person protected in the predicted range works out to an enormous sum across the large population. It may actually prove more cost-effective to evaluate newer vaccines for dengue (with more specific neutralising*

antibody production and hence, less ADE activity) as has currently begun.
Response: We agree that total programme costs can appear large when scaled up over populous countries. For clarity, our approach does not project scaling up *Wolbachia* to cover every area in India or elsewhere, but instead targets *Wolbachia* to a subset of high burden areas, which can help to decrease costs. We do acknowledge, however, that other methods of dengue control will likely be important in reaching the WHO 2030 goals, including vaccines, particularly in areas where *Wolbachia* implementation may be challenging or less cost effective. We have edited the discussion to clarify this:
"In practice, commercial considerations…will limit the rate of uptake and the ultimate scale of deployment achieved. In areas where *Wolbachia* implementation may be challenging or less cost effective, other methods of dengue control, including vaccines, will likely be important in reaching the WHO 2030 goals."

3. *Regarding the environmental model, can weather data from Rio de Janeiro be applicable to other countries in the tropics?*
Response: While the economic modelling components of our analysis were geographically explicit, the mosquito population dynamics model was intended to represent a more generalisable dengue-endemic setting with distinct wet and dry seasons that drive mosquito population dynamics. Rio de Janeiro has a typical meteorological profile of a dengue-endemic region with distinct wet and dry seasons. Basing our generic model on this typical seasonal profile allowed us to explore the effects of distinct seasonal changes on *Wolbachia* release ratio and other hybrid scenarios. The results of this analysis are now found in Supplementary Figure 7.

We hope you now find this manuscript acceptable for publication.

Competing Interests: No competing interests were disclosed.

Reviewer Report 16 September 2024

https://doi.org/10.21956/gatesopenres.17127.r37727

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Gabriela Paz-Bailey
Centers for Disease Control and Prevention, San Juan, Puerto Rico, USA

The paper reports on a mathematical model used to inform the WHO Target Product Profile (TPP) development for *Wolbachia* replacement. It contributes to the literature by discussing aspects of the TPP that influence *Wolbachia* implementation, using dengue cost data as a proxy for willingness to pay, and highlighting areas with the greatest benefit based on disease burden. However, there are several areas where the paper could be improved:

1. The components of the TPP need to be explained in the background section. Some elements are mentioned later in the paper without sufficient context. Adding a table with the TPP elements and their descriptions would be helpful.
2. Several parameters and assumptions in the model conflict with current replacement program practices:

a. Most programs release adult mosquitoes daily, rather than releasing eggs every two weeks. This discrepancy likely impacts costs and time to introgression.
b. Costs decrease when intervening in larger areas with high population density. Dividing endemic areas into 5x5 km pixels to prioritize regions seems unrealistic. For the model to be useful, it should reflect how countries are likely to implement the strategy, prioritizing cities based on population density and dengue incidence. What is the point of having a few pixels in one country and a few in another on the priority list if implementing the strategy based on these priorities is not feasible?

1. The focus on the combination of suppression followed by replacement is disconnected from practical realities. There are only two places in the world with *Wolbachia* suppression program (Singapore and China), and these do not represent the capabilities of endemic countries. This topic is prominently featured in the background, introduction, results, and discussion. I recommend moving it to the supplemental information and including a sentence in the results directing readers to it. Presenting it so prominently could distract from the main message and might influence policy and implementation discussions inappropriately. The main challenges with mosquito control include insecticide resistance, the limited impact of ULV spraying, and the difficulty of reaching cryptic breeding sites with larvicides. Mosquito control is largely ineffective and suggesting it as a viable strategy to accelerate replacement programs is dangerous. Effectiveness of SIT and *Wolbachia* methods have not been demonstrated outside a few small trials or in high-resource settings like Singapore and China. Wolbachia suppression is also potentially more expensive than the six-month replacement releases. Claiming that it may reduce costs without including the costs of various suppression methods is misleading. Table 3 should be eliminated.
2. Results:
    1. The results would benefit from examples to aid interpretation. For instance, figures 2c and 2d, and throughout the results section.
    2. Figure 1: The reduction in mosquito population has not been demonstrated in field trials. It would be important to show whether the model results can be corroborated by empirical data.
    3. Figure 2 highlights the challenges of the pixel approach. Covering only 12.5% of the burden means that smaller areas spread further apart would need to be covered, which may not be feasible with an intervention as logistically demanding as *Wolbachia* replacement. This should be noted somewhere in the paper.
    4. Page 10, third paragraph: Explain what is meant by "exacerbation issues."
    5. Table 2: The implications of the cost per person covered could be better explained in the results. Providing an example of a city with a certain population and comparing the costs of protection in areas with high versus low willingness to pay would be useful. The significance of the cost changes from 12.5% to 25% reduction is unclear.
3. Discussion: a. Page 17, first paragraph: In countries where Wolbachia has been implemented, releases stop when *Wolbachia* introgression reaches 50-60%, allowing it to continue to naturally progress to a higher prevalence. Proposing this as an alternative to current practice is wrong as it is already how it is done.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 14 Oct 2024

Katie Tiley

The paper reports on a mathematical model used to inform the WHO Target Product Profile (TPP) development for Wolbachia replacement. It contributes to the literature by discussing aspects of the TPP that influence Wolbachia implementation, using dengue cost data as a proxy for willingness to pay, and highlighting areas with the greatest benefit based on disease burden. However, there are several areas where the paper could be improved:

1. *The components of the TPP need to be explained in the background section. Some elements are mentioned later in the paper without sufficient context. Adding a table with the TPP elements and their descriptions would be helpful.*
1. Thank you for reviewing this manuscript. We agree that more context on the TPP would be helpful. This is boosted by the final approved version of the TPP now being publicly available which we now reference and should provide additional detail if readers are interested. We have expanded the relevant section of the introduction to summarise the main features of the TPP:

"In early 2022 the WHO convened a Technical Advisory Group (TAG) to develop a draft TPP

for Wolbachia replacement and a draft PPC for a hybrid mosquito suppression then Wolbachia replacement strategy. The TPP for Wolbachia replacement began with the development of a "use case characterisation", following which specific TPP criteria were established under the categories of product performance, product characteristics, production and delivery and intellectual property. For each of these, a minimum and preferred target was established with the former intended to inform a go / no go product development decision point. A combination of different types of evidence from the field, laboratory and modelling studies were used to inform these targets, with the modelling work focused on release and cost-related characteristics. The final WHO TPP was published February 2022 (World Health Organisation, 2022). TAG members decided that a core premise of the TPP and PPC was that they should closely align with the WHO's strategy and goals to control dengue globally. As such the WHO's goal to reduce dengue incidence by 25% by 2030 (2010 – 2020 baseline24) provided a basis to understand the scale and range of settings in which these TPPs, PPCs and the products they ultimately produce are relevant."

Several parameters and assumptions in the model conflict with current replacement program practices:
1a. *Most programs release adult mosquitoes daily, rather than releasing eggs every two weeks. This discrepancy likely impacts costs and time to introgression.*

1a. We acknowledge that the form and frequency of releases may have changed since the time this decision was made. Our focus on egg releases every two weeks was aligned with the major field evidence available at the time which was the Yogyakarta RCT (Utarini et al., 2021) which also informed other aspects of the TPP. Therefore, this choice was made to improve consistency with the available field data and consistency across different elements of the TPP. We have edited the text in the methods to emphasise this:

"An entomological Wolbachia replacement model was formulated and calibrated to the main evidence available at the time, namely the Yogyakarta RCT which comprised nine to 14 egg releases in release cups every two weeks. This approach was also chosen to maximize consistency with other elements of the TPP."

We have also added an additional limitation to the discussion:
"While we simulate egg releases once every two weeks, we acknowledge that adult releases at weekly timescales may be possible and could accelerate the time to fixation."

1b. *Costs decrease when intervening in larger areas with high population density. Dividing endemic areas into 5x5 km pixels to prioritize regions seems unrealistic. For the model to be useful, it should reflect how countries are likely to implement the strategy, prioritizing cities based on population density and dengue incidence. What is the point of having a few pixels in one country and a few in another on the priority list if implementing the strategy based on these priorities is not feasible?*

1b. We agree with the review that treating pixels in isolation is likely not realistic. The same point was raised by review #1 in their initial review (see 1.2.1b for comment and response). In this response we clarified that occurrence of isolated 5x5km pixels is very rare in our analysis. We summaries the treated area estimates for each 2nd administrative area and

provide this in the supplementary information.
Original response: “The administrative-unit summary provided in the supplement shows that 85.5% of 2nd administrative units (admin2s) have predicted release areas at or above 10km2. Among the remaining admin2s with smaller release areas all of them are in countries that also contain admin2s with larger (>10km2) release areas and 66% of smaller release admin2s occur in Vietnam, Colombia, Thailand, Brazil and Mexico which would all require large release areas across their respective countries. These smaller areas could thus be considered an extension of programmes in nearby areas. Introducing restrictions on contiguity or release area will, therefore, only affect a very small number of areas and have minimal impact on the overall findings.”

In this response we also acknowledge the gap between our predicted treated areas map and likely implementation area:
“We recognize and agree that many additional considerations will go into which areas will ultimately be targeted for Wolbachia implementation and the resulting map is not intended to be prescriptive, but rather give an indication of the kinds of areas that would be most sensible to priorities from a net cost efficiency perspective. Even if the decision to implement occurs at administrative unit-level, not everywhere within an administrative unit will see Wolbachia releases and the pixel-level maps can be useful for informing within-administrative unit targeting subject to a set of criteria that should be locally determined. It should also be emphasized that the TPP is, by definition, for a hypothetical Wolbachia replacement product, not the current most widely-used wMel programme. This means that such considerations may differ in their importance and, as such, are beyond the scope of the TPP and the accompanying modelling analysis.”

2. *The focus on the combination of suppression followed by replacement is disconnected from practical realities. There are only two places in the world with Wolbachia suppression program (Singapore and China), and these do not represent the capabilities of endemic countries. This topic is prominently featured in the background, introduction, results, and discussion. I recommend moving it to the supplemental information and including a sentence in the results directing readers to it. Presenting it so prominently could distract from the main message and might influence policy and implementation discussions inappropriately. The main challenges with mosquito control include insecticide resistance, the limited impact of ULV spraying, and the difficulty of reaching cryptic breeding sites with larvicides. Mosquito control is largely ineffective and suggesting it as a viable strategy to accelerate replacement programs is dangerous. Effectiveness of SIT and Wolbachia methods have not been demonstrated outside a few small trials or in high-resource settings like Singapore and China. Wolbachia suppression is also potentially more expensive than the six-month replacement releases. Claiming that it may reduce costs without including the costs of various suppression methods is misleading. Table 3 should be eliminated.*

2. The suppression then replacement strategies were developed for the PPC. We explicitly clarify in the Introduction that:
“PPCs identify broader areas of unmet need and aim to stimulate new products or product combinations that can address these needs.”
This work, therefore, is not intended to represent current practical realities, but rather understand under what circumstances such new strategy may be beneficial. As well as

having a clear theoretical basis, this intervention combination has been suggested by multiple previous modelling studies (Hu et al., 2021; Qu et al., 2018; Yakob et al., 2008) and is therefore a plausible potentially viable strategy that should be tested. We believe that the results presented in this paper are not particularly favorable for suggesting the hybrid approach over conventional replacement and list extensive barriers that will need to be overcome for it to become a viable alternative. We don't believe removing or relegating this work to the supplementary information would remove any perceived danger as implementers could still ask about these strategies based on work in prior publications. While we agree that the results text for the hybrid strategies is longer than for conventional replacement, this reflects the additional model development and complexity more than any perceived prominence or prioritization between these options.

We do, however, agree that we can do more to differentiate the work done for the TPP and PPC and as a result, we have made a number of modifications to sections to clarify that hybrid strategies are not currently used and the caveats that would need to be addressed for it to become a practical reality.

In the introduction:
"While not currently practiced, in theory, combining a prior programme of mosquito suppression followed by Wolbachia population replacement could offer community acceptance or dengue incidence reduction advantages."

In the discussion:
"Hybrid approaches offer one unproven but potential option for increasing speed or acceptability of Wolbachia replacement."
And
"The implementation of any suppression method should be accompanied by mosquito surveillance that confirms that the levels of suppression achieved are above and beyond what would have normally been achieved by the programme."

Furthermore, Figure 6 showing a seasonal analysis of hybrid suppress then release strategies has been moved to the Supplementary Materials.

Following the comments of reviewer #1 all reference to the cost savings of hybrid suppress then release programmes have been removed and we substantially modified Table 3 and the section of text where it is discussed:

"By reducing the number of Wolbachia mosquitoes released and duration of the replacement phase of the hybrid strategy, the costs of this phase could be reduced. If these levels of suppression can be achieved at a lower cost than the savings of the replacement phase, then a hybrid strategy could reduce costs overall. Here we use models to estimate these maximum costs for suppression, beyond which a replacement-only programme would be preferable. If the baseline replacement programme can meet the TPP minimum cost of $2.33 per person, then suppression would need to cost not more than $0.41 – $1.17 per person for mosquito-release suppression methods and $0.09 – 0.50 per person for conventional methods (Table 3)."

Results:
3a. *The results would benefit from examples to aid interpretation. For instance, figures 2c and 2d, and throughout the results section.*

3a. While we appreciate the suggestion, including these is challenging due to the fact that all well-known example cities are included in all of the targeting scenarios and our desire to keep the focus at a global level (see response 4e). We have slightly modified parts fo the results where figure 3 is described:
"This corresponds to 34.7% of the urban (> 300 people per km2) area at risk and all major dengue-endemic cities, and just 1.7% of the total area at risk of dengue."
And
"A full list of cities and 2nd administrative units included under each targeting scenario, the costs each will support and additional contextual information (population, density, etc) is included in the following repository: https://github.com/katietiley/Wolbachia_TPP_PPC.git."

3b. *Figure 1: The reduction in mosquito population has not been demonstrated in field trials. It would be important to show whether the model results can be corroborated by empirical data.*

3b. While we agree that measuring pre- and post-*Wolbachia* replacement mosquito population size in the field is important, we are currently not aware of any datasets where this has been robustly measured and reported. We believe this may be challenging to practically measure given the heterogeneity over time and space of mosquito populations and the variable measurement accuracy of different traps for estimating true mosquito abundance.
The modest (~ 30-35%) declines in mosquito abundance that we estimate are an implicit consequence of reduced *Wolbachia* mosquito fitness, a trait that is well documented in laboratory studies and a common assumption of many modelling studies (e.g., Joubert et al., 2016; Ross & Hoffmann, 2022). In comparison with these, the fitness cost we selected was a conservative estimate which, consequently, means our estimates of mosquito abundance reduction post-*Wolbachia* release are more likely to be an over-estimate. We have edited the discussion to acknowledge this:
"The fitness cost of *Wolbachia* infection selected in these models was a conservative estimate and, consequently, the decline of total mosquito population equilibrium from pre- to post-*Wolbachia* release (Figure 1A,B) is likely to be an over-estimation."

Figure 2 highlights the challenges of the pixel approach. Covering only 12.5% of the burden means that smaller areas spread further apart would need to be covered, which may not be feasible with an intervention as logistically demanding as Wolbachia replacement. This should be noted somewhere in the paper

In the discussion we mention:
"It is important to clarify that these maps should not be used prescriptively, but rather give an indication of the kinds of areas that are likely to be most cost efficient to target, with the final decision on which areas are targeted for release subject to additional operational, entomological, financial and political considerations."

3c. *Page 10, third paragraph: Explain what is meant by "exacerbation issues".*

3c. This has been amended:
"Higher RRs and higher release round numbers also lead to disproportionately undesirable temporary exacerbation of the mosquito population, particularly at higher values (Figure 1A,B,D)."

3d. *Table 2: The implications of the cost per person covered could be better explained in the results. Providing an example of a city with a certain population and comparing the costs of protection in areas with high versus low willingness to pay would be useful. The significance of the cost changes from 12.5% to 25% reduction is unclear.*

3d. We appreciate the review's implementer focussed perspective, but this was not the intended purpose of this model, or these results and we believe that discussing such exampled explicitly may lead to misinterpretation of this work. The cost targets presented in Table 2 are global targets that a Wolbachia replacement solution would need to meet to be compatible with the TPP. While it is true that for any one individual city, these targets could be higher (indicating higher willingness to pay), development of a Wolbachia replacement product at this cost point would not then achieve the TPP target of being affordable in enough areas to meet either the 12.5% or 25% global burden reduction targets. We therefore believe focussing the discussion of these results at the global level is a more appropriate summary of the model and its results.

Discussion:
4a. *Page 17, first paragraph: In countries where Wolbachia has been implemented, releases stop when Wolbachia introgression reaches 50-60%, allowing it to continue to naturally progress to a higher prevalence. Proposing this as an alternative to current practice is wrong as it is already how it is done.*

4a. The aim of this sentence is to indicate an alternative use case where Wolbachia is released in a checkerboard spatial pattern where the current standard release programme is completed in 50-60% of areas and, over time, mosquito movement allows Wolbachia to spread to non-release areas to gradually build up to 90% of areas covered. This is distinct from prevalence within a single area, where we agree that the current practice is to release Wolbachia mosquitoes until population prevalence is ~50-60% then relying on cytoplasmic incompatibility to further increase prevalence to 90% after releases stop.
Thank you for highlighting how the previous phrasing may be misleading. We have now edited to clarify how the alternative TPP use case differs from current practice and hope this improves interpretation:
"One alternative use case, considered in the TPP, is to conduct lower intensity releases with larger distances between release sites. This would result in only around 50% of areas initially having a Wolbachia prevalence of over 90%, however over time mosquito diffusion would spread Wolbachia to all remaining areas to ensure all target areas had a Wolbachia prevalence over 90%."

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Competing Interests: No competing interests were disclosed.

Reviewer Report 18 July 2024

https://doi.org/10.21956/gatesopenres.17127.r36946

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Scott O'neill
World Mosquito Program, Monash University, Clayton, Victoria, Australia

In general, the manuscript has been improved and is appropriately more qualified in this second version. I still have one problem though which I think is very important as I feel that as it currently stands the paper is quite misleading and this relates to the notion that hybrid release programs would be more cost effective than replacement alone.
My specific comments on this point are as follows:

In the introduction it is stated:
“Specifically, a programme of suppression followed by replacement has the potential to increase the likelihood of successful *Wolbachia* establishment and reduce the cost of, and risk of mosquito exacerbation associated with, achieving establishment22.”

The paper does not provide any evidence that costs could be reduced by combining suppression and replacement – only if suppression is considered as being free, which in no context is that the case. This statement relating to cost should be removed. I have no problems with the other attributes of the potential benefits.

In the hybrid “suppress then replace” section it is stated that:
“This, however, does not take into account the costs of suppression. A different interpretation of these results would be: if suppression can be achieved for less than these costs, then a hybrid programme will cost less than replacement alone. This might refer to specific circumstances when suppression might be achieved at negligible cost, for example, if it’s already part of a vector control programme. These are ideal situations where the resources for suppression are already in place, however, in most cases the potential savings achieved by a hybrid suppress then replace programme won’t justify the additional cost of implementing a novel suppression programme.”

The notion that if suppression activities are already part of an existing vector control programme that they can be considered to have no cost is nonsensical. Of course they have cost to the government and the economics needs to support total costs of a dengue control program, not just a section of the cost. Finally, the authors are proposing that insect release technologies to undertake suppression would be most effective from a suppression perspective but there are no examples of these costs being less than the cost savings projected from a shortened replacement program, and indeed the evidence available from Singapore indicates the opposite. Thus the conclusions are unrealistically optimistic for the cost-effectiveness of a hybrid program and need to be more qualified.

The same criticism relating to cost applies to this sentence in the discussion:
“Hybrid approaches offer one promising option for increasing speed, reducing cost and increasing the acceptability of *Wolbachia* replacement. “
I think there is no justification for the reducing cost element of this statement. There may be a use case though and that is in contexts where mosquito populations are extremely large as occurs in some geographies, such as Kiribati for example.

In addition, and unrelated to the cost issue, the sentence in the introduction:
“To date, there have been no large-scale (national or regional), non-donor funded implementations of *Wolbachia* replacement or suppression approaches.” is factually incorrect. As of today there are large government funded replacement programs running across multiple cities in both Brazil and Indonesia that are not donor funded. This should be updated or deleted.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: I am the heavily involved in Wolbachia replacement technology implementation through my role in the World Mosquito Program.

Reviewer Expertise: Wolbachia biology and its use for disease control

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 14 Oct 2024

Katie Tiley

Thank you for reviewing this manuscript. Exactly as suggested, the manuscript has now been edited throughout to remove statements indicating that a combined suppress and then release programme presents cost savings. Additionally, the outdated statement about the lack of donor-funded implementations has now been removed. We hope you now find this manuscript acceptable for publication.

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 03 August 2023

https://doi.org/10.21956/gatesopenres.15611.r34325

Perran Ross
The University of Melbourne, Melbourne, Victoria, Australia

General comments

Mosquitoes infected with Wolbachia bacteria are being used to control the spread of arboviruses including dengue. These programmes involve the release of lab-reared mosquitoes at a large scale into the natural population. After reaching a high frequency, the Wolbachia infection can be self-sustaining in the mosquito population due to its maternal transmission and ability to induce cytoplasmic incompatibility, and this can provide ongoing protection against dengue. The World Health Organization (WHO) has recently drafted a Target Product Profile (TPP) which includes a set of desired characteristics for Wolbachia strains and release programmes that would be required to meet WHO targets of reducing the global burden of dengue by 25%

This manuscript addresses aspects of the TPP using models comparing variables that affect the success of Wolbachia population replacement and mapping of locations where Wolbachia releases are likely to provide the most benefit. While there is value in the latter, I have concerns with their Wolbachia replacement models and hybrid “suppress then replace” models. The Wolbachia replacement model does not provide information that isn’t already clear from previous modelling studies and analysis of the field trials, while the hybrid suppression then replace component makes inappropriate comparisons between different suppression interventions and does not consider the potentially substantial costs of these interventions.

In the first part of the paper, the authors model Wolbachia infection frequencies and mosquito population sizes when there are different numbers of releases. Unsurprisingly, more releases and higher release ratios equals faster population replacement and a temporarily higher number of adult mosquitoes. The importance of these variables has been taken into account when planning releases and they have also been addressed in other models (e.g. Hancock et al. 2011a and 2011b). Another outcome of the model is that complete Wolbachia coverage can be achieved in under a year, but this has already been demonstrated directly through several field trials, so it is unclear what value the model here adds.

The authors emphasize the importance of balancing the speed of Wolbachia replacement with the potentially negative outcomes of temporarily increasing the mosquito population. However, I am unsure if this is supported by the outcomes of their models, which show a maximum temporary increase in the population size of under 20%. Is this likely to be noticeable by the community? And even with this increase, won’t there be significantly more nuisance biting at other times of the year (assuming that Wolbachia releases take place when mosquito populations are low)? If this increase is likely to be a concern, then surely the use of a hybrid suppress then replace approach, which the authors discuss later in the paper, would also be a concern due to the much higher release ratios required for releases of incompatible or sterile males. While males don’t bite, they can also be regarded as a nuisance and many people will not be able to distinguish between the sexes.

The final set of results addresses the utility of a hybrid “suppress then replace” approach, where the mosquito population is suppressed through different tools (insecticides, sterile male releases etc.) prior to population replacement releases. This will make population replacement easier as there are fewer mosquitoes to replace. Previous studies have used this approach, for example, the

very first releases of wMel involved prior suppression of the population through the removal of larval habitats (Hoffmann et al. 2011). But a key question is cost effectiveness. The authors perform a cost analysis and conclude that a hybrid approach could be cost saving, but they don't include the cost of the suppression itself for any of the approaches being compared, making their conclusions baseless. Some of the approaches they compare can be quite expensive in their own right. The incompatible insect technique for instance requires sex sorting of mosquitoes which is very labour intensive if done mechanically or very expensive if using automated systems, and mosquitoes need to be reared at large scales to achieve suppression.

The authors then use models to test the effect of different suppression interventions on mosquito population sizes, and later, the effect on the release ratio of Wolbachia-infected mosquitoes required for successful Wolbachia establishment. These approaches have different effects (e.g. adulticides kill off adults quickly but the population bounces back quickly, while incompatible/sterile insect approaches have slower but longer term population suppression), which is a reasonable point to make. But the authors then compare these approaches directly using literature estimates of efficacy. Parameters were taken from a single study for each approach and these studies are in no way comparable to each other. They were in different environments with different populations of mosquitoes and were done at different geographic scales, time periods and intensities. There are also issues with using a single study to represent the expected efficacy for a typical suppression programme. I just don't see how it's reasonable to compare these approaches directly and conclude that one is more effective when the approaches have been applied at different intensities and durations.

In summary, while the paper addresses an important issue, in my view the entomological models are problematic and/or uninformative, and the paper is not suitable for indexing without substantial fundamental changes.

Specific comments

Abstract - results – These quantitative thresholds are adjusted to data from the wMel releases in Wolbachia and they should not be extrapolated to Wolbachia releases in other locations, given that Wolbachia releases can have vastly different outcomes depending on the environment

Abstract – results - Suppression interventions will reduce the number of mosquitoes required for replacement, but this statement ignores the very high numbers of mosquitoes that are needed for the suppression itself (if using incompatible males, which the authors conclude is the most effective approach).

Intro paragraph 2 – Not all mosquitoes infected with Wolbachia show reduced virus dissemination- be more specific about the species and Wolbachia strain.

Intro paragraph 3 – The paragraph is a bit of an oversimplification- Wolbachia doesn't always reach fixation even if very high frequencies are reached, for instance, due to maternal transmission failure. There is also now evidence from field trials showing that Wolbachia frequencies can fluctuate seasonally or even decline to zero even after reaching near-fixation depending on the environment.

Intro paragraph 4 – "widespread, long-term effectiveness" is true in some locations but there are

also cases where Wolbachia releases have failed (see above), meaning that there are likely to be environmental constraints on where Wolbachia infections can successfully establish

Methods – entomological model paragraph 3 – this seems to only cover a scenario where Wolbachia-infected mosquitoes are released as eggs. Please provide some context in the introduction as egg releases are not mentioned before here. The models are built on the assumption of egg releases, and the fact that Wolbachia-infected larvae are initially separate from wild larvae is an important component, but there is no justification for this or acknowledgement of other types of release. The authors discuss population suppression through male releases, but this will require adult releases.

Methods- suppression – "The efficacy of each method was based on evidence sourced from the literature" - this section is quite subjective and there is no information about how the authors searched for studies or selected them aside from having a preference for large randomised controlled studies.

Methods – suppression – I would prefer if the authors didn't use "larvicides" here- guppies are not larvicides – they are predators. "Larvicides" typically refers to chemical insecticides or bacterial pathogens like Bt.

Figure 1 – The model shows that the mosquito population size permanently decreases after Wolbachia establishment, but I'm not aware of any evidence for this from field release data. For instance, data from the releases in Yogyakarta shows that Wolbachia releases had minimal impact on the population size: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0010284

Supp figure 1 – I'm not sure why the authors only considered costs up to 20% - data from release programs have estimated costs of around 30% for wMel (Hoffmann et al. 2011) and this is likely to vary substantially depending on the environment.

Table S1 – Why is SIT included here if the authors found no studies to base its efficacy on? It is true that few SIT release programmes in Ae. aegypti have been published, but there is at least one (e.g. de Castro Poncio et al. 2021)

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2. Hancock PA, Sinkins SP, Godfray HC: Population dynamic models of the spread of Wolbachia.*Am Nat*. 2011; **177** (3): 323-33 PubMed Abstract | Publisher Full Text
3. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, et al.: Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission.*Nature*. 2011; **476** (7361): 454-7 PubMed Abstract | Publisher Full Text
4. de Castro Poncio L, Dos Anjos FA, de Oliveira DA, Rebechi D, et al.: Novel Sterile Insect Technology Program Results in Suppression of a Field Mosquito Population and Subsequently to Reduced Incidence of Dengue.*J Infect Dis*. 2021; **224** (6): 1005-1014 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am actively involved in research on Wolbachia population replacement programmes in Aedes mosquitoes, including lab experiments and analysis of field data. My comments focus on Wolbachia and mosquito biology and the outcomes of release programmes. I am not a statistician and am not qualified to comment on the equations behind the models, only their biological relevance.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 18 Apr 2024

Katie Tiley

2.1. General comments:
2.1.1. *Mosquitoes infected with Wolbachia bacteria are being used to control the spread of arboviruses including dengue. These programmes involve the release of lab-reared mosquitoes at a large scale into the natural population. After reaching a high frequency, the Wolbachia infection can be self-sustaining in the mosquito population due to its maternal transmission and ability to induce cytoplasmic incompatibility, and this can provide ongoing protection against dengue. The World Health Organization (WHO) has recently drafted a Target Product Profile (TPP) which includes a set of desired characteristics for Wolbachia strains and release programmes that would be required to meet WHO targets of reducing the global burden of dengue by 25%.*
2.1.1. We thank the reviewer for the time taken to read and thoroughly respond to this work which informed the World Health Organisation (WHO) Target Product Profile (TPP) and Preferred Product Characteristics (PPC) documents for *Wolbachia* replacement technology contributing to the goal of reaching the WHO targets of reducing global burden of dengue by 25%.

2.1.2. *This manuscript addresses aspects of the TPP using models comparing variables that affect the success of Wolbachia population replacement and mapping of locations where Wolbachia releases are likely to provide the most benefit. While there is value in the latter, I have concerns with their Wolbachia replacement models and hybrid "suppress then replace" models. The Wolbachia replacement model does not provide information that isn't already clear from previous modelling studies and analysis of the field trials, while the hybrid suppression then replace component makes inappropriate comparisons between different suppression interventions and does not consider the potentially substantial costs of these interventions.*
2.1.2. We agree with the reviewer that previous modelling studies and field trials have reported similar findings to our *Wolbachia* replacement model. We consider our model outcomes being consistent with these previous published findings to be a strength, not a weakness, as this provides supporting evidence for the appropriateness of our methodology, and this supported the decision of the Technical Advisory Group (TAG) to include these specific criteria in the TPP. Specifically, the value of our *Wolbachia* replacement model and hybrid suppress-then-replace model is in quantifying the differences in mosquito population dynamics compared across different variables, such as different levels of release ratio or release number. Quantifying these measures is important because it allows us to link different scenarios to cost estimates and enables more informed decisions when addressing trade-offs between different scenarios, both essential for producing reliable values for the TPP and PPC documents.
Furthermore, we would like to make clear that the hybrid suppress-then-replace scenarios are presented to enable public health departments and organisations to better integrate their current vector control efforts with a novel *Wolbachia* replacement programme. These scenarios demonstrate the potential benefits of leveraging vector control interventions that are already in use (or could be with minor adaptations) during the transition to *Wolbachia* replacement and we acknowledge that the additional cost of building the capabilities to implement new methods of suppression solely for the purpose of reducing the cost of *Wolbachia* replacement is unlikely to reduce overall cost. Since current vector control practices are already budgeted for in routine vector control programmes, we assume no additional cost for using conventional vector control methods prior to implementing *Wolbachia* replacement. That the suppression scenarios outlined in our study are most applicable to circumstances where suppression is already in place has been further clarified in the fifth paragraph of the results section under the subheading "Exploring the dynamics of a hybrid 'suppress then replace' approach":
"…than a hybrid programme will cost less than replacement alone. This might refer to specific circumstances when suppression might be achieved at negligible cost, for example, if it's already part of a vector control programme. These are ideal situations where the resources for suppression are already in place, however, in most cases the potential savings achieved by a hybrid suppress then replace programme won't justify the additional cost of implementing a novel suppression programme."

2.1.3. *In the first part of the paper, the authors model Wolbachia infection frequencies and mosquito population sizes when there are different numbers of releases. Unsurprisingly, more releases and higher release ratios equals faster population replacement and a temporarily higher number of adult mosquitoes. The importance of these variables has been taken into account when planning releases and they have also been addressed in other models (e.g. Hancock et al.*

2011a and 2011b). Another outcome of the model is that complete Wolbachia coverage can be achieved in under a year, but this has already been demonstrated directly through several field trials, so it is unclear what value the model here adds.

2.1.3. Again, we consider the consistency of our findings with the existent literature to be an advantage, especially when considering fundamental dynamics mentioned by the reviewer; specifically, that more releases and higher release ratios leading to faster population replacement and that population replacement is achievable in under a year (e.g. Hancock et al, 2011a, 2011b). This is also the case with our finding that *Wolbachia* replacement can cause mosquito population exacerbation, previously modelled by Yakob et al (2017). The strength of this evidence from a variety of methods has been further clarified in the manuscript discussion:

"The population dynamics shown in our model are consistent with previous modelling and field studies showing temporary population exacerbation and successful fixation within one year (44-46); this evidence supports the credibility of our findings quantifying the difference between scenarios with varying release ratio and release number."

This manuscript aims to highlight the value modelling specifically adapted to the questions raised by the TAG in the TPP process can bring. These questions inevitably span a range of field, laboratory and data analysis studies which are also cited as evidence for the criteria that are set. The additional value of the bespoke modelling we did for this showed the trade-offs between criteria, e.g. yes replacement is achievable within 6 months, but what release ratios are necessary to achieve this and what consequences do they have for cases and community acceptability?

2.1.4. *The authors emphasise the importance of balancing the speed of Wolbachia replacement with the potentially negative outcomes of temporarily increasing the mosquito population. However, I am unsure if this is supported by the outcomes of their models, which show a maximum temporary increase in the population size of under 20%. Is this likely to be noticeable by the community? And even with this increase, won't there be significantly more nuisance biting at other times of the year (assuming that Wolbachia releases take place when mosquito populations are low)? If this increase is likely to be a concern, then surely the use of a hybrid suppress then replace approach, which the authors discuss later in the paper, would also be a concern due to the much higher release ratios required for releases of incompatible or sterile males. While males don't bite, they can also be regarded as a nuisance and many people will not be able to distinguish between the sexes.*

2.1.4. Any temporary population increase has the potential to decrease rates of community acceptance and undermine community engagement. This was an issue specifically raised as a concern by the TAG with our models quantifying the trade-offs between faster time to fixation but with higher temporary exacerbation when release ratios are increased. The temporary population increase shown in our model could be considered small (maximum exacerbation 20%) but it is important to quantify these levels for obtaining informed consent from communities. Furthermore, despite not being modelled here, even temporary minor increases could be epidemiologically important if *Wolbachia* is implemented at times of high transmission intensity, e.g. as an outbreak response. We agree with the reviewer that some suppression techniques would require additional mosquito releases, such as the male *Wolbachia*release or sterile insect technique, however, these would be accompanied by substantial separate community engagement activities and, since we recommend the hybrid suppress-then-release scenarios to complement existing vector control activities,

likely already have taken place ahead of implementing *Wolbachia* replacement. Specifically, building community support through education campaigns would involve emphasising that males do not bite, as the reviewer comments, and that the exacerbation will not increase risk of infection. This has been further clarified in the manuscript:
"The mosquito-release suppression techniques (i.e., 1gSLT, SIT, Male Wolbachia release) may also lead to a temporary increase in mosquito population and would require separate community engagement activities to emphasise that the released males do not bite or increase the risk of infection."

2.1.5. *The final set of results addresses the utility of a hybrid "suppress then replace" approach, where the mosquito population is suppressed through different tools (insecticides, sterile male releases etc.) prior to population replacement releases. This will make population replacement easier as there are fewer mosquitoes to replace. Previous studies have used this approach, for example, the very first releases of wMel involved prior suppression of the population through the removal of larval habitats (Hoffmann et al. 2011). But a key question is cost effectiveness. The authors perform a cost analysis and conclude that a hybrid approach could be cost saving, but they don't include the cost of the suppression itself for any of the approaches being compared, making their conclusions baseless. Some of the approaches they compare can be quite expensive in their own right. The incompatible insect technique for instance requires sex sorting of mosquitoes which is very labour intensive if done mechanically or very expensive if using automated systems, and mosquitoes need to be reared at large scales to achieve suppression.*
2.1.5. We acknowledge the reviewers concern regarding the cost of suppression, and our conclusions around cost savings previously recognise this depends on the cost of suppression, for example in the results:
"All hybrid programmes had the potential to offer cost savings over replacement alone, depending on the cost of suppression… This, however, does not take into account the costs of suppression. A different interpretation of these results would be: if suppression can be achieved for less than these costs, then a hybrid programme will cost less than replacement alone."
And in the discussion:
"We predict that insect-based suppression methods (1gSLT, SIT, Male Wolbachia release) will be more effective than conventional suppression tools, but also outline a limited cost window which may be challenging for insect-based suppression methods to achieve. Investment in new infrastructure to conduct insect-based suppression may not be justified for a one-off suppression, but between overlapping resource requirements for suppression and replacement, ongoing use post-replacement (e.g., outbreak control or to achieve dengue elimination) and a continued drive to lower costs of mosquito suppression (52), this investment cost may be justified."
However, we understand that expressing the hybrid approach as cost saving has the potential to be misinterpreted and therefore, as mentioned in point 2.1.2, have further edited the results to better clarify our conclusions.

2.1.6. *The authors then use models to test the effect of different suppression interventions on mosquito population sizes, and later, the effect on the release ratio of Wolbachia-infected mosquitoes required for successful Wolbachia establishment. These approaches have different effects (e.g. adulticides kill off adults quickly but the population bounces back quickly, while incompatible/sterile insect approaches have slower but longer term population suppression), which is a reasonable point to make. But the authors then compare these approaches directly using literature estimates of efficacy. Parameters were taken from a single study for each*

approach and these studies are in no way comparable to each other. They were in different environments with different populations of mosquitoes and were done at different geographic scales, time periods and intensities. There are also issues with using a single study to represent the expected efficacy for a typical suppression programme. I just don't see how it's reasonable to compare these approaches directly and conclude that one is more effective when the approaches have been applied at different intensities and durations.

2.1.6. We appreciate the reviewer's comments regarding the difficulties encountered when comparing different suppression interventions. A WHO Evidence Review Group is currently conducting a rigorous systematic review of *Aedes* mosquito control effectiveness that was unfortunately not available at the time of analysis and remains in development. In the absence of this we aimed to derive evidence-based scenarios for suppression effectiveness which allowed approximation of the differences between methods of suppression at 5- and 10-week periods. We acknowledge that building the evidence base for these scenarios is not always straightforward to extract from the literature due to different study designs and contexts. To mitigate this complexity in our models we also include a range of hypothetical fixed effectiveness values for direct comparison between different suppression methods, this has been clarified in the discussion of limitations:

"Additionally, suppression efficacy is highly context-dependent; to mitigate this we modelled an hypothetical 20%, 50%, and 80% suppression efficacy to allow clearer comparison between suppression techniques."

Our main findings (ie that insect release strategies offer advantages over conventional suppression strategies) are the same whether using the literature-derived efficacy estimates or the fixed efficacies. Another advantage of using a range of fixed efficacies is that it allows readers or implementers to interpret these results in different contexts where higher or lower suppression effectiveness may be feasible.

2.1.7. *In summary, while the paper addresses an important issue, in my view the entomological models are problematic and/or uninformative, and the paper is not suitable for indexing without substantial fundamental changes.*

2.1.7. We are glad the reviewer agrees that this modelling work informs decisions in an important area of research. We hope that the above general responses and below specific responses clarify the value of the entomological models and their importance for this specific application of informing the *Wolbachia* replacement TPP and hybrid suppress then replace PPC.

2.2. Specific comments:

2.2.1. *Abstract - results – These quantitative thresholds are adjusted to data from the wMel releases in Wolbachia and they should not be extrapolated to Wolbachia releases in other locations, given that Wolbachia releases can have vastly different outcomes depending on the environment*

2.2.1. The statement:

"We estimate that for Wolbachia replacement to be deployable in enough areas to make major contributions to reducing global dengue burden by 25% (in line with 2030 WHO targets), it must have the potential for cost be reduced to between $7.63 and $0.24 (USD) per person protected or less"

Does not depend on any data from wMel releases. These figures come directly from table 2 with a legend:

"The predicted target cost per person for Wolbachia replacement based on different assumptions about desired global impact (rows) and averted medical and outbreak control costs (assumed proxy of willingness to pay, columns)."
These figures are based on the cost of illness and conventional control alone, while effectiveness was set by the TAG for the TPP at an assumed 70%, neither of which depend on data from the *wMel* releases.

2.2.2. *Abstract – results - Suppression interventions will reduce the number of mosquitoes required for replacement, but this statement ignores the very high numbers of mosquitoes that are needed for the suppression itself (if using incompatible males, which the authors conclude is the most effective approach).*
2.2.2. This statement refers to how suppression reduces the number of mosquitoes required during *Wolbachia*replacement. This was identified as a key parameter by the TAG because it requires release of biting, potentially dengue-transmitting, mosquitoes and therefore has a different set of community concerns, engagement strategies, costs and regulatory implications to the release of male mosquitoes. We also clarify in various sections of the manuscript that the suppression in hybrid suppress-then-replace strategies is recommended to complement existing vector control strategies, therefore, releasing mosquitoes for insect-release suppression technologies (i.e., Male *Wol*release, SIT, and 1gSLT) would already be in effect, meaning that there would be a net reduction in released mosquitoes (both male and female) in such a hybrid strategy.

2.2.3. *Intro paragraph 2 – Not all mosquitoes infected with Wolbachia show reduced virus dissemination- be more specific about the species and Wolbachia strain.*
2.2.3. We agree it was not clear from the text that not all *Wolbachia* strains reduce virus dissemination, therefore this has been clarified in text:
"Ae. aegypti mosquitoes infected with certain strains of the bacterium Wolbachia, such as wMel, wMelPop, and wAlbB (7) show reduced rates of virus dissemination, making them less capable of transmitting arboviruses (8)."

2.2.4. *Intro paragraph 3 – The paragraph is a bit of an oversimplification- Wolbachia doesn't always reach fixation even if very high frequencies are reached, for instance, due to maternal transmission failure. There is also now evidence from field trials showing that Wolbachia frequencies can fluctuate seasonally or even decline to zero even after reaching near-fixation depending on the environment.*
2.2.4. We agree that *Wolbachia* is not always successful reaching fixation and has been known to fluctuate or decline. That environmental variation affects *Wolbachia* replacement dynamics has been clarified in the text:
"Modelling has shown that once a critical proportion of mosquitoes in the population have Wolbachia, coverage should continue to increase to fixation without further releases, but below this threshold Wolbachia coverage may decline (possibly to zero) once releases stop due to fitness costs associated with released mosquito strains[8] … It should be noted that in practice, Wolbachia frequencies may fluctuate seasonally and still decline to zero after reaching fixation depending on environmental variables such as temperature, rainfall, and physical barriers (12, 13)."

2.2.5. *Intro paragraph 4 – "widespread, long-term effectiveness" is true in some locations but there are also cases where Wolbachia releases have failed (see above), meaning that there are*

likely to be environmental constraints on where Wolbachia infections can successfully establish
2.2.5. We further acknowledge the need to emphasise there is heterogeneity in the success of *Wolbachia*replacement, and have edited the text to underscore these considerations:
"A growing range of entomological, epidemiological and modelling evidence supports the widespread, long-term effectiveness of Wolbachia replacement (14-16), and research continues to identify environmental conditions associated with spatially and temporally heterogeneous Wolbachia establishment (13)."

2.2.6. *Methods – entomological model paragraph 3 – this seems to only cover a scenario where Wolbachia-infected mosquitoes are released as eggs. Please provide some context in the introduction as egg releases are not mentioned before here. The models are built on the assumption of egg releases, and the fact that Wolbachia-infected larvae are initially separate from wild larvae is an important component, but there is no justification for this or acknowledgement of other types of release. The authors discuss population suppression through male releases, but this will require adult releases.*
2.2.6. We appreciate the reviewers feedback that the text was unclear about the different methods of mosquito release during *Wolbachia* replacement. Our work modelled egg releases in release cups only; text has been added to the entomological model section of the methods for additional context:
"Since this model was calibrated using data of Wolbachia fixation dynamics from the Yogyakarta RCT (16), any features of this RCT influenced the methodology, such as delivery mode (egg releases in release cups) and the number of releases (ranging from nine to 14), therefore, other delivery modes such as releasing Wolbachia-infected adult Ae. aegypti were not simulated."
Each of the insect-release suppression technologies (Male *Wol* release, SIT, and 1gSLT) were simulated with adult releases.

2.2.7. *Methods- suppression – "The efficacy of each method was based on evidence sourced from the literature" - this section is quite subjective and there is no information about how the authors searched for studies or selected them aside from having a preference for large randomised controlled studies.*
2.2.7. We acknowledge the literature-derived efficacies have limitations, such as estimates of effectiveness being context specific, variations in implementation and ambiguous reporting of effectiveness values. It is precisely because of this that we did not pursue a systematic search for studies with specific inclusion or exclusion criteria. Instead, we chose studies that we judged best evaluated the effectiveness of short-term pulses of the intervention. We clearly cite the sources of these assumed literature-derived effectiveness values and acknowledge that readers may expect alternative values in different settings. To address this, we chose to also include a range of hypothetical values (20%, 50%, and 80%) for suppression effectiveness which allow a more standardised comparison of the dynamics of different methods of suppression and how such dynamics affect requirements for successful replacement programmes. As mentioned earlier, this has been further emphasised in the discussion of limitations.
"Additionally, suppression efficacy is highly context-dependent; to mitigate this we modelled an hypothetical 20%, 50%, and 80% suppression efficacy to allow clearer comparison between suppression techniques."

2.2.8. *Methods – suppression – I would prefer if the authors didn't use "larvicides" here- guppies*

are not larvicides – they are predators. "Larvicides" typically refers to chemical insecticides or bacterial pathogens like Bt.
2.2.8. Since both chemical larvicides and guppies primary mode of action is to kill eggs, larvae and pupae, their effects on the model are identical, therefore we believe the hypothetical range of values (20%, 50%, and 80%) can refer to either larvicides, which are more widely used, or guppies. However, in response to this comment the text has been edited to clarify this labelling specifically:
"Larvicides were simulated by equally reducing the number of eggs, larvae, and pupae. Because the effect is similar and because the best measurement of effectiveness of an intervention that targets the aquatic stages of the mosquito came from a trial of predatory guppies, this effectiveness as measured by Hustedt et al (37) was chosen to represent the effectiveness of larvicides."

2.2.9. *Figure 1 – The model shows that the mosquito population size permanently decreases after Wolbachia establishment, but I'm not aware of any evidence for this from field release data. For instance, data from the releases in Yogyakarta shows that Wolbachia releases had minimal impact on the population size:*
https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0010284
2.2.9. We assumed that *Wolbachia* exerts a fitness cost, following the reported evidence (e.g., Joubert et al, 2016; Ross & Hoffmann, 2022), and as a consequence of this, the total population size was reduced following replacement with *Wolbachia*-infected mosquitoes. The fitness cost we selected was intended as a conservative estimate to reduce risk of under-estimation in field deployments, so the projected reduction in total mosquito numbers is thus likely a concomitant over-estimation.

2.2.10. *Supp figure 1 – I'm not sure why the authors only considered costs up to 20% - data from release programs have estimated costs of around 30% for wMel (Hoffmann et al. 2011) and this is likely to vary substantially depending on the environment.*
2.2.10. We agree that the fitness cost of *Wolbachia* infection is very dependent on the environment. We edited the manuscript to emphasise this:
"Due to the fitness cost of Wolbachia (conservatively modelled to be 20% (39), but highly variable depending on environment (41))…"
Furthermore, we have extended the range of values included in the sensitivity analysis in Supplementary Figure 1 to encompass the 30% value highlighted by the reviewer.

2.2.11. *Table S1 – Why is SIT included here if the authors found no studies to base its efficacy on? It is true that few SIT release programmes in Ae. aegypti have been published, but there is at least one (e.g. de Castro Poncio et al. 2021)*
2.2.11. The lack of a literature-derived efficacy value for SIT suppression technique was a genuine oversight and we're grateful for the reviewer's feedback; the manuscript has been amended to include a literature-derived efficacy for SIT. A literature-derived efficacy of approximately 49% at 5-weeks and 77% at 10-weeks has been extracted from the field trial by De Castro Poncio et al (2021) Figure 2b. The analyses which previously used an hypothetical 50% efficacy for this suppression technique have been updated to use the literature-derived 49% efficacy, correspondingly there has been only marginal changes in resulting population dynamics and no change in the overall trends or findings discussed. The methods section for SIT suppression have been updated to include this development:

"Parameters for the hypothetical fixed rate efficacy of 20%, 50%, and 80% were calculated by comparing the total adult population at model equilibrium with the minimum adult population reached after five weeks of application. The literature-derived efficacy values were 49% five weeks after the last suppression period and 77% ten weeks after the last suppression period (33), calculated by comparing the total adult population at model equilibrium to the total adult population after five- or ten-weeks of suppression which achieved the desired efficacy (summarised in Supplementary Table 1). A caveat of this approach is that the resulting minimum adult population is reached later than five- or ten-weeks, therefore, the maximum efficacy calculated in these scenarios is marginally greater than the literature value stated (shown in Supplementary Figure 2)."
In addition, SIT values have been updated in Figures 4, 5, and 6, added to Supplementary Figure 2, and the SIT literature source has been added to Supplementary Table 1.

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Competing Interests: No competing interests were disclosed.

Reviewer Report 03 August 2023

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Scott O'neill
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This paper uses entomological and economic modeling approaches to derive programmatic cost targets to support a WHO defined TPP for *Wolbachia* replacement. The starting premise for the modeling approach is that *Wolbachia* replacement needs to be deployable on a global scale at a sufficiently low cost to achieve the WHO's goal of a 25% reduction in global dengue incidence by 25% (relative to a 2010-2020 baseline). This premise, and the consequent epidemiologic and economic assumptions for how *Wolbachia* could be most cost-effectively deployed at a global scale, lead to overly simplistic conclusions around a target global cost of deployment of *Wolbachia* replacement. Although the authors have qualified this by stating that "TPP targets represent the lowest averted medical and outbreak control costs per person among all areas where releases are required, there are many areas that could support higher programme costs", the unrealistic target of reducing 25% of national dengue burden and averting these costs against the lowest medical and outbreak control costs over three years provides an overly optimistic global PCC of $0.24 per person. The linkage in this instance has a substantial impact on the total cost of coverage. The current modeling approach does not adequately address this issue. The entomological modelling of the hybrid "suppress then replace" is based largely on theoretical estimates of suppression efficacy (Figure 4) or extrapolation of data from small scale pilot studies. The assumptions behind some of these approaches are flawed, and present an unrealistic scenario around the feasibility of undertaking hybrid suppress and then replace methods. This section is highly speculative, adds little to the paper and should be removed.

Specific comments:

1. In order to frame the impact targets in the TPP in terms of the WHO NTDs roadmap target of a 25% reduction in global dengue burden by 2030, the authors identify the priority settings for achievement of that target by ranking 5x5km pixels globally (Page 4). There are several issues with this approach to ranking target geographies:

1a. The authors state on page 4 that they "identified which areas (5km x 5km pixels) would need to be targeted to reach the WHO goal of reducing global dengue burden by 25% in the most net cost-efficient manner. To do this, each 5km x 5km pixel was ranked from highest to lowest based on a benefit (averted medical and outbreak costs) to cost (approximate *Wolbachia* program cost estimate based on population density from Brady et al 2020) ratio." A critical input into this calculation of the pixel-level benefit-to-cost ratio, which forms the basis for identifying the target geographies considered in the rest of the modeling, is the assumed pixel-level cost of *Wolbachia* deployment, yet insufficient detail is provided about that assumed intervention cost. The paper cited reports an economic analysis of *Wolbachia* deployment in Indonesia, in which the predicted

Wolbachia program cost was modeled as a cost per km2, as a function of human population density. Presumably the current paper also used cost per km2 as the input for *Wolbachia* program cost in calculating pixel-level benefit-cost ratios, and this should be described in more detail. More fundamentally, it seems circular to identify target geographies based on a benefit-to-cost ratio that requires an assumption of programmatic cost per km2 / per person as an input, and then use the predicted avertable cost of dengue in those target geographies to define a target programmatic cost of *Wolbachia* per person. Please clarify the logic of this approach, and why the inclusion of a *Wolbachia* cost assumption in the initial step of ranking the target geographies doesn't compromise the approach.

1b. The ranking of 5x5 km pixels to identify target geographies for *Wolbachia* deployment produces unrealistic scenarios of fragmented small release areas (totalling 924,557 km2 in size) that are distributed across 73 different countries. This undermines the cost-per-person assumptions, as it would clearly be more costly to deploy across these fragmented areas than to cover large contiguous urban populations.To achieve the most net cost-efficient implementation of *Wolbachia* at a global scale, ranking would be better done at e.g. administrative 2 level to identify the contiguous highest-burden areas in which the global reduction targets could be achieved or, if the pixel-based approach is to be used, at least apply an additional criterion that filters out identified target release areas below a minimum km2 (e.g. 10 km2) or population size (e.g. 50,000 population). It is evident from the datasets of target release area km2 and population at 2nd level administrative units, provided in the supplementary materials, that many of the target geographies are far too small to support *Wolbachia* replacement deployments in reality.

Despite this being one of the major limitations of the modeling approach, the authors give no consideration at all in the discussion to the fact that in real-world programmatic implementation of *Wolbachia* replacement, it will always be most efficient from operational, entomological, and cost-efficiency perspectives to deploy across a large contiguous urban area rather than small fragmented release areas. This needs to be addressed in the discussion.

2. The 'Required cost per person covered' thresholds in Table 2 are presented at a global level, based on the projected costs averted in the least cost effective setting in any given scenario. Although regional and national cost targets are included in the supplementary materials, and the authors acknowledge in the discussion that many countries could support higher costs and 'that a *Wolbachia* replacement product would still meet the TPP targets if initial program costs were higher and if the product has the potential to reduce costs down to the $2.33 target, this should be presented more explicitly within the primary results. It is not meaningful to report the primary cost targets at a global level without including also the range of national- and regional-level target costs, as that's not the spatial scale at which economies of scale in production and implementation costs are achieved, or at which customers' willingness-to-pay or funding decisions operate.

3. The cost targets are very conservative in requiring cost-neutrality from a health system perspective (i.e. implementation cost $\leq$ direct medical costs + outbreak vector control costs averted) and assuming only 3-5 years of benefits for the preferred TPP. Although a scenario of 10 years of benefits is also included in Table 2, the authors present the cost target of $2.33 as the minimum TPP, based on a scenario of achieving a 25% global burden reduction and only 5 years of benefit, with a PPC cost target of $0.24 (25% national burden reduction in all countries and only 3 years of benefit). These durations of benefit are too pessimistic given the evidence from field studies of *Wolbachia* durability in *Ae. aegypti* populations for at least 10 years.

4. The entomological modelling of the hybrid "suppress then replace" is based largely on theoretical estimates of suppression efficacy (Figure 4), or extrapolation of population suppression results from small scale pilot interventions. This reviewer questions whether the efficacy of these suppression methods can ever be achieved at the scale required to avert 25% of the global dengue burden. The assumptions around the suppressive effects of a single round of intervention seem overly simplistic, and are generally not consistent with field implementations that take many rounds of intervention to produce consistent and reproducible suppression (see Supplementary Table 1 references below). "With reductions in adult mosquito population size in the range of 8.18 – 43.51%, from a single application all methods of suppression were predicted to remove the ~1–10% mosquito population exacerbation seen in replacement only programmes." (Page 12) Further, "Our model predicts that a prior suppression programme of five weekly rounds could reduce the number of *Wolbachia* mosquitoes required to reach target coverage within 6 months by 16-81% depending on suppression method used." (Pages 12-13)

Based on empirical data from a Male *Wolbachia* release program that targeted *Ae. aegypti*, there is questionable evidence that a 5-week suppression program would substantially impact mosquito population abundance. In the Supplementary Table 1, it indicates a 5-week suppression efficacy of 60%, and 10-week suppression efficacy of 90% (sourced from Ching et al. 2021). This is incorrect. Figure 2, Panel G indicates <10% suppression out to week 7 in the Yishun Core Area where releases were concentrated. In the Tampines Core area, suppression efficacy varied significantly over the first 12-13 weeks, ranging from 0-40%. Ching et al. (2021) presented a statistical analysis of the suppression effect (Figure 3, Panels A and C) and found no significant suppression in Yishun until the fourth month of releases, and variable results in Tampines (statistically significant suppression in first month, but not significant in the second month of releases).

Similarly for the "1gSLT" suppression rates of 45% and 75% at weeks 5 and 10, respectively (Supplementary Table 1. Suppression Efficacy Carvalho et al. 2015). These data were extracted from Figure 2, panel D, from the 5- and 10-week timepoints in release area A, the authors have failed to state that there were 7-months of releases undertaken across these combined Areas A and B prior to the 5- and 10-week suppression period over which the above rates were calculated (for Area A). Also of note was the very small size of the suppression area in this trial (Area A = 0.055km2). Again, the assumptions drawn from these studies are inaccurate and should be withdrawn.

How have the SIT suppression efficacy estimates been validated? In Supplementary Table 1, it states there is no currently published trials for suppression using SIT, and also in the Methods it states "There are currently no published randomised controlled trials for suppression of *Aedes* mosquitoes using SIT, therefore 50% efficacy is shown for SIT in analyses where only literature-derived efficacy is used. What is the "literature derived efficacy"?

The assumptions underpinning the modelling of the suppress then replace section of the paper appear to be erroneous, or at least very optimistic of their effectiveness in terms of suppression of the mosquito populations. This section is highly speculative, adds little to the paper and should be removed.

5. "All hybrid programmes had the potential to offer cost savings over replacement alone, depending on the cost of suppression" (Page 14). This statement is not supported by presentation

of any costing data for suppression. From an economic perspective, there is no evidence that a hybrid approach of suppression followed by replacement would be more cost effective than *Wolbachia* replacement alone, and a recent study based on data from Singapore's *Wolbachia* suppression program suggests that a hybrid suppression-followed-by-replacement program could be significantly more expensive (Soh *et al*. 2021[1]). The current annual per capita cost of suppression in Singapore is approximately USD $5 per person reached. This cost is roughly equivalent to the one-time, all-inclusive cost required to achieve *Wolbachia* replacement across some target settings (Brady et al BMC Med 2020; Turner et al PLoS Negl Trop Dis 2023). The 'potential cost savings enabled by a hybrid approach' presented in Table 3 "assumes suppression has no cost or is an in-kind contribution", which is entirely implausible in any setting, let alone in a scenario of large-scale global implementation across the 73 countries (>900,000 km2), which would be required to achieve the TPP minimum targets. Without a more extensive analysis of the efficacy of suppression programs and their cost, the current analyses are highly speculative and add little to the paper. This reviewer feels the whole section on hybrid "suppress then replace" should be removed.

Minor comments:

1. Page 3, Introduction, second paragraph: The virus-blocking phenotype is specific to the species of *Wolbachia,* mosquito, and virus - the authors should use more specific language here.

2. Page 3, Introduction, second paragraph: There is no empirical evidence that temporary increases in the *Ae. aegypti* mosquito population have been a key barrier to community acceptability of *Wolbachia* releases; this should be expressed as theoretical consideration.

3. Page 6, Results, paragraph 1 (and throughout): the terminology 'coverage' is misleading as it implies the % coverage of a geographic area, whereas the authors are referring to % *Wolbachia* prevalence in the local *Ae. aegypti* population. The proportion of *Wolbachia*-infected mosquitoes in the population should be considered the "percentage prevalence," not the target coverage.

4. Page 11, Figure 3: The axes titles and figure legend needs to make explicit what time period this data relates to, in terms of the cumulative cases and costs averted. Presumably 5 years of benefit, since the $2.33 cost threshold is shown, however this is inconsistent with the $0.24 preferred criteria referred to in the figure legend, which relates to 3 years of benefit. And the lower horizontal dotted line appears to be at $0.39, not $0.24.

5. Page 16, Discussion: "Cost continues to be a barrier to wider adoption of *Wolbachia* replacement when its high costs but long-term benefits are compared to lower cost but short acting suppression methods". This statement needs to reflect the fact that *Wolbachia* replacement has an evidence base for epidemiological impact while the short acting suppression methods do not.

References

1. Soh S, Ho SH, Seah A, Ong J, et al.: Economic impact of dengue in Singapore from 2010 to 2020 and the cost-effectiveness of Wolbachia interventions.*PLOS Glob Public Health*. 2021; **1** (10):

e0000024 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: I am the CEO of the World Mosquito Program, a not-for-profit that has developed and implements Wolbachia replacement technologies. My conflict is non-financial but relevant given that the paper describes the use of our technology

Reviewer Expertise: Wolbachia biology and its use for disease control

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 18 Apr 2024

Katie Tiley

1.1 Summary comments:
1.1.1. *This paper uses entomological and economic modeling approaches to derive programmatic cost targets to support a WHO defined TPP for Wolbachia replacement. The starting premise for the modeling approach is that Wolbachia replacement needs to be deployable on a global scale at a sufficiently low cost to achieve the WHO's goal of a 25% reduction in global dengue incidence by 25% (relative to a 2010-2020 baseline). This premise, and the consequent epidemiologic and economic assumptions for how Wolbachia could be most cost-effectively deployed at a global scale, lead to overly simplistic conclusions around a target global cost of deployment of Wolbachia replacement. Although the authors have qualified this by stating that "TPP targets represent the lowest averted medical and outbreak control costs per person among all areas where releases are required, there are many areas that could support higher programme costs", the unrealistic target of reducing 25% of national dengue burden and averting these costs against the lowest medical and outbreak control costs over three years*

provides an overly optimistic global PCC of $0.24 per person. The linkage in this instance has a substantial impact on the total cost of coverage. The current modeling approach does not adequately address this issue.
1.1.1. We are grateful that the reviewer read and thoroughly responded to this work which informed the World Health Organisation (WHO) Target Product Profile (TPP) and Preferred Product Characteristics (PPC) documents for *Wolbachia* replacement technology contributing to the goal of reaching the WHO targets of reducing global burden of dengue by 25%. We have carefully considered the reviewers feedback and responded point-by-point in detail below.

1.1.2. *The entomological modelling of the hybrid "suppress then replace" is based largely on theoretical estimates of suppression efficacy (Figure 4) or extrapolation of data from small scale pilot studies. The assumptions behind some of these approaches are flawed, and present an unrealistic scenario around the feasibility of undertaking hybrid suppress and then replace methods. This section is highly speculative, adds little to the paper and should be removed.*
1.1.2. The criticism of the hybrid suppress-then-replace modelling justifiably covers some specific areas where the evidence supporting the effectiveness of several suppression tools could have been improved (which has now been implemented, see points 1.2.4a – e), but also covers wider conceptual issues of the value of the hybrid approach. One key aspect here is to clarify that modelling the hybrid approach was for the PPC process and not the TPP. This PPC process is designed to guide the development of novel products or strategies and are both more forward looking and less prescriptive than the TPP. The results from the modelling for these two sections should, therefore, also be interpreted within these different use contexts. We agree that the current evidence for hybrid suppress then replace strategies is limited, the modelling is intended to guide the (limited) use cases in which future improvements to suppression interventions may enable hybrid strategies.

1.2 Specific comments:
1.2.1. *In order to frame the impact targets in the TPP in terms of the WHO NTDs roadmap target of a 25% reduction in global dengue burden by 2030, the authors identify the priority settings for achievement of that target by ranking 5x5km pixels globally (Page 4). There are several issues with this approach to ranking target geographies:*
1.2.1a. The authors state on page 4 that they "identified which areas (5km x 5km pixels) would need to be targeted to reach the WHO goal of reducing global dengue burden by 25% in the most net cost-efficient manner. To do this, each 5km x 5km pixel was ranked from highest to lowest based on a benefit (averted medical and outbreak costs) to cost (approximate Wolbachia program cost estimate based on population density from Brady et al 2020) ratio." A critical input into this calculation of the pixel-level benefit-to-cost ratio, which forms the basis for identifying the target geographies considered in the rest of the modeling, is the assumed pixel-level cost of Wolbachia deployment, yet insufficient detail is provided about that assumed intervention cost. The paper cited reports an economic analysis of Wolbachia deployment in Indonesia, in which the predicted Wolbachia program cost was modeled as a cost per km2, as a function of human population density. Presumably the current paper also used cost per km2 as the input for Wolbachia program cost in calculating pixel-level benefit-cost ratios, and this should be described in more detail. More fundamentally, it seems circular to identify target geographies based on a benefit-to-cost ratio that requires an assumption of programmatic cost per km2 / per person as an input, and then use the predicted avertable cost of dengue in those target geographies to

define a target programmatic cost of Wolbachia per person. Please clarify the logic of this approach, and why the inclusion of a Wolbachia cost assumption in the initial step of ranking the target geographies doesn't compromise the approach.
1.2.1a. We thank the reviewer for raising this lack of clarity and concur the current description may be misinterpreted to be circular. This section has been edited to address this issue:
"Next we identified which areas (5km x 5km pixels) would need to be targeted to reach the WHO goal of reducing global dengue burden by 25% in the most net cost efficient manner. For a generic environmental intervention, where cost of the intervention only depends on area covered, this would involve targeting areas with the highest density of dengue costs. However, because the cost of Wolbachia programmes have been shown to depend on the human population density and per capita GDP in the release area (27), this can change which areas are most important to prioritise from an optimal net cost perspective. To account for these variable implementation cost factors Wolbachia replacement is most cost effective if targeted to higher density, high dengue burden areas (27). We therefore identified which areas (5km x 5km pixels) would need to be targeted to reach the WHO goal of reducing global dengue burden by 25% in the most net cost-efficient manner. To do this, each 5km x 5km pixel was ranked from highest to lowest based on a benefit (averted medical and outbreak costs) to cost (approximate Wolbachia programme cost estimate based on population density and per capita GDP from Brady et al. (27)) ratio. For clarity, the approximate Wolbachia programme cost from Brady et al. (27) only affects the ranking of pixels (i.e. targeting), not the TPP target cost estimates. Cumulative averted cases were then calculated and pixel selection ended when averted cases first exceeded 25% of the global total…."

1.2.1b. *The ranking of 5x5 km pixels to identify target geographies for Wolbachia deployment produces unrealistic scenarios of fragmented small release areas (totalling 924,557 km2 in size) that are distributed across 73 different countries. This undermines the cost-per-person assumptions, as it would clearly be more costly to deploy across these fragmented areas than to cover large contiguous urban populations. To achieve the most net cost-efficient implementation of Wolbachia at a global scale, ranking would be better done at e.g. administrative 2 level to identify the contiguous highest-burden areas in which the global reduction targets could be achieved or, if the pixel-based approach is to be used, at least apply an additional criterion that filters out identified target release areas below a minimum km2 (e.g. 10 km2) or population size (e.g. 50,000 population). It is evident from the datasets of target release area km2 and population at 2nd level administrative units, provided in the supplementary materials, that many of the target geographies are far too small to support Wolbachia replacement deployments in reality. Despite this being one of the major limitations of the modeling approach, the authors give no consideration at all in the discussion to the fact that in real-world programmatic implementation of Wolbachia replacement, it will always be most efficient from operational, entomological, and cost-efficiency perspectives to deploy across a large contiguous urban area rather than small fragmented release areas. This needs to be addressed in the discussion.*
1.2.1b. We recognise and agree that many additional considerations will go into which areas will ultimately be targeted for *Wolbachia* implementation and the resulting map is not intended to be prescriptive, but rather give an indication of the kinds of areas that would be most sensible to prioritise from a net cost efficiency perspective. Even if the decision to implement occurs at administrative unit-level, not everywhere within an administrative unit

will see *Wolbachia* releases and the pixel-level maps can be useful for informing within-administrative unit targeting subject to a set of criteria that should be locally determined. It should also be emphasised that the TPP is, by definition, for a hypothetical *Wolbachia* replacement product, not the current most widely-used *wMel* programme. This means that such considerations may differ in their importance and, as such, are beyond the scope of the TPP and the accompanying modelling analysis. We have added the following sentence to third paragraph of the discussion to convey this:
"It is important to clarify that these maps should not be used prescriptively, but rather give an indication of the kinds of areas that are likely to be most cost efficient to target, with the final decision on which areas are targeted for release subject to additional operational, entomological, financial and political considerations."
The administrative-unit summary provided in the supplement shows that 85.5% of 2nd administrative units (admin2s) have predicted release areas at or above 10km^2. Among the remaining admin2s with smaller release areas all of them are in countries that also contain admin2s with larger (>10km^2) release areas and 66% of smaller release admin2s occur in Vietnam, Colombia, Thailand, Brazil and Mexico which would all require large release areas across their respective countries. These smaller areas could thus be considered an extension of programmes in nearby areas. Introducing restrictions on contiguity or release area will, therefore, only affect a very small number of areas and have minimal impact on the overall findings.

1.2.2. *The 'Required cost per person covered' thresholds in Table 2 are presented at a global level, based on the projected costs averted in the least cost effective setting in any given scenario. Although regional and national cost targets are included in the supplementary materials, and the authors acknowledge in the discussion that many countries could support higher costs and 'that a Wolbachia replacement product would still meet the TPP targets if initial program costs were higher and if the product has the potential to reduce costs down to the $2.33 target, this should be presented more explicitly within the primary results. It is not meaningful to report the primary cost targets at a global level without including also the range of national- and regional-level target costs, as that's not the spatial scale at which economies of scale in production and implementation costs are achieved, or at which customers' willingness-to-pay or funding decisions operate.*
1.2.2. The primary purpose of this work was to support the TPP. TPPs are used to decide if particular products should or should not be developed. For this TPP the Technical Advisory Group (TAG) decided that these criteria should be set to be globally relevant – i.e. the product should only be developed if it can be globally relevant – for equity reasons. The TPP uses the specific wording that a product "must have the potential to reach" these thresholds. We have now updated the abstract and the results sections to use this specific phrase for consistency. The pathway to achieving this with initially higher costs is mentioned repeatedly in the results and discussion sections with Figure 3 and the detailed municipality-level summaries in the supplement giving precise figures.

1.2.3. *The cost targets are very conservative in requiring cost-neutrality from a health system perspective (i.e. implementation cost* $\leq$ direct medical costs + outbreak vector control costs averted) and assuming only 3-5 years of benefits for the preferred TPP. Although a scenario of 10 years of benefits is also included in Table 2, the authors present the cost target of $2.33 as the minimum TPP, based on a scenario of achieving a 25% global burden reduction and

only 5 years of benefit, with a PPC cost target of $0.24 (25% national burden reduction in all countries and only 3 years of benefit). These durations of benefit are too pessimistic given the evidence from field studies of Wolbachia durability in Ae. aegypti populations for at least 10 years.

1.2.3. It is worth reiterating that these estimates should not be interpreted as predictions of the likely costs and benefits of *Wolbachia* programmes, but as a proxy for willingness to pay for *Wolbachia* implementation. We agree that there is good evidence that *Wolbachia* persists for at least 10 years and specifically included a scenario with 10 years of benefits in our analysis because of this (Table 2). However, these longer duration benefits were judged by members of the TAG (which included programme managers) as being less relevant when estimating willingness to pay for a new programme and hence why the 5-year option was chosen for the minimum criteria.

1.2.4a. *The entomological modelling of the hybrid "suppress then replace" is based largely on theoretical estimates of suppression efficacy (Figure 4), or extrapolation of population suppression results from small scale pilot interventions. This reviewer questions whether the efficacy of these suppression methods can ever be achieved at the scale required to avert 25% of the global dengue burden. The assumptions around the suppressive effects of a single round of intervention seem overly simplistic, and are generally not consistent with field implementations that take many rounds of intervention to produce consistent and reproducible suppression (see Supplementary Table 1 references below). "With reductions in adult mosquito population size in the range of 8.18 – 43.51%, from a single application all methods of suppression were predicted to remove the ~1–10% mosquito population exacerbation seen in replacement only programmes." (Page 12) Further, "Our model predicts that a prior suppression programme of five weekly rounds could reduce the number of Wolbachia mosquitoes required to reach target coverage within 6 months by 16-81% depending on suppression method used." (Pages 12-13)*

1.2.4a. We agree that the evidence base for hybrid suppress and replace strategies is not as developed as that for *Wolbachia* replacement alone. It is this very fact that led WHO to develop a TPP for replacement but only a PPC for hybrid strategies. PPCs are not subject to the same specific criteria (like the 25% mentioned by the reviewer) as TPPs and come with clear guidelines that they should be tested using a range of methods and experimental designs before a TPP can be developed. We believe our modelling work is proportionate to the differing needs of these two distinct policy objectives.

We also agree that achieving the efficacy of the hybrid suppress-then-release scenarios at scale would be challenging and generally only an option that might be considered if cost, community acceptability or speed considerations could not be met with a replacement-only approach. We state this explicitly in the relevant section of the results:

"This may mean that rather than hybrid approaches superseding replacement-only approaches, their use may be restricted to areas where replacement-only cannot meet speed, acceptability, and cost goals."

We also agree that it takes time and multiple rounds of application to consistently achieve suppression. Areas most likely to consider hybrid programmes will likely already have a large suppression component to their vector control programmes, so a hybrid programme could be considered more of an extension (e.g. continuing suppression for longer or at times of the year when suppression is limited) than a new application. Under such circumstances experienced personnel with a high degree of familiarity with the local vector ecology, supported by existing programme infrastructure would be more likely to achieve

the levels of vector control suggested by these studies than if starting from scratch. Because we agree that it is difficult to make comparisons between different study designs for suppression effectiveness we also include fixed efficacies that readers can use instead that give a more standardised comparison between different suppression methods and can be used to explore lower effectiveness suppression options if they believe these are more realistic in their own contexts:
"Additionally, suppression efficacy is highly context-dependent; to mitigate this we modelled an hypothetical 20%, 50%, and 80% suppression efficacy to allow clearer comparison between suppression techniques."

1.2.4b. *Based on empirical data from a Male Wolbachia release program that targeted Ae. aegypti, there is questionable evidence that a 5-week suppression program would substantially impact mosquito population abundance. In the Supplementary Table 1, it indicates a 5-week suppression efficacy of 60%, and 10-week suppression efficacy of 90% (sourced from Ching et al. 2021). This is incorrect. Figure 2, Panel G indicates <10% suppression out to week 7 in the Yishun Core Area where releases were concentrated. In the Tampines Core area, suppression efficacy varied significantly over the first 12-13 weeks, ranging from 0-40%. Ching et al. (2021) presented a statistical analysis of the suppression effect (Figure 3, Panels A and C) and found no significant suppression in Yishun until the fourth month of releases, and variable results in Tampines (statistically significant suppression in first month, but not significant in the second month of releases).*
1.2.4b. We acknowledge the reviewers concerns regarding the source for the literature-derived efficacy values for Male *Wolbachia* release, which we cite as Ching (2021). Having revisited the paper and our extraction process we agree that the time interval over which these levels of suppression can be achieved is overoptimistic in this example. We have since searched for alternative studies that give more appropriate estimates of short-term effectiveness. The analyses have been updated to include literature-derived efficacy values for Male *Wolbachia* release sourced from Crawford et al (2020), a field trial which reported suppression efficacy values reaching approximately 65% and 92% at 5- and 10-weeks, respectively; values extracted using WebPlotDigitizer (Rohatgi, 2021) from Figure 6d. The previous analyses used 60% at 5-weeks and 95% 10-weeks and this update has only caused small changes in the population dynamics and have not altered the overall trends or our findings. In addition, Male *Wolbachia* values have been updated in Figures 4, 5, and 6, added to Supplementary Figure 2, and the literature source for Male *Wolbachia*release has been updated in Supplementary Table 1.

1.2.4c. *Similarly for the "1gSLT" suppression rates of 45% and 75% at weeks 5 and 10, respectively (Supplementary Table 1. Suppression Efficacy Carvalho et al. 2015). These data were extracted from Figure 2, panel D, from the 5- and 10-week timepoints in release area A, the authors have failed to state that there were 7-months of releases undertaken across these combined Areas A and B prior to the 5- and 10-week suppression period over which the above rates were calculated (for Area A). Also of note was the very small size of the suppression area in this trial (Area A = 0.055km2). Again, the assumptions drawn from these studies are inaccurate and should be withdrawn.*
1.2.4c. We acknowledge the reviewers concerns that the literature-derived value extracted for 1gSLT was from the period of releases in Area A only (Carvalho et al, 2015), while previously there were seven months of releases across the combined Areas A and B.

However, the prior suppression efforts concerned the refinement and scale up of technology and did not consistently impact vector populations until after the effort to area ratio was increased. This is confirmed within the paper: "Up to 11th February 2012 we released into areas A and B (Fig 1), comprising 11 ha in total. However, despite improvements in rearing over the period, in this highly infested area we were unable to produce enough OX513A males with the available resources to consistently maintain a mating fraction of 50%, as judged by the percentage of fluorescent larvae. We therefore reduced the release area to an area of 5.5 ha (Fig 1A). As expected, the fluorescence ratio increased correspondingly" and further supported by the observations from Figure 2, panel B, that the suppression effort was increased approximately 3-fold from levels prior to release in Area A only. Since these hybrid scenarios are most informative to circumstances where suppression technology is already in use as part of the vector control programme, it is appropriate for us to assume the region already has the necessary resources for suppression and therefore use values from more mature examples where initial training and testing phases are omitted. This makes our assumptions about using such methods for hybrid programmes more comparable with our assumptions about replacement, for which we also assume relatively rapid implementations can be achieved (< 6 months) when compared to the currently published literature based on the same argument of product maturity and familiarity. Furthermore, the extracted values for this technology used in the analysis are conservative in the short-term when compared with another field trial reported by Harris *et al* (2012), which shows an estimated 82% and 68% efficacy after 5- and 10-weeks of intervention, respectively (extracted from Figure 2c). Finally, we agree that the size of the suppression area is not extensive, and this detail has been added to the Supplementary Table 1:
"Extracted from Figure 2, panel D, from the 5- and 10-week timepoints of release in area A only, a concentrated area of 0.055km^2."

1.2.4d. *How have the SIT suppression efficacy estimates been validated? In Supplementary Table 1, it states there is no currently published trials for suppression using SIT, and also in the Methods it states "There are currently no published randomised controlled trials for suppression of Aedes mosquitoes using SIT, therefore 50% efficacy is shown for SIT in analyses where only literature-derived efficacy is used. What is the "literature derived efficacy"?*
1.2.4d. Previously, we had no literature reference for SIT and therefore this statement from the manuscript refers to the fact that an hypothetical 50% efficacy value was used instead of a literature-derived efficacy in figures which otherwise presented only literature-derived efficacy values; this was stated in the legend. However, the lack of a SIT literature source has now been highlighted as an oversight by the other reviewer and a literature-derived efficacy of approximately 49% at 5-weeks and 77% at 10-weeks has been extracted from the field trial by De Castro Poncio et al (2021), Figure 2b. All analyses which previously used an hypothetical 50% efficacy for this suppression technique have been updated to use the literature-derived 49% efficacy, correspondingly there has been only marginal changes in resulting population dynamics and no change in the overall trends or findings discussed. The methods section for SIT suppression have been updated to include this development:
"Parameters for the hypothetical fixed rate efficacy of 20%, 50%, and 80% were calculated by comparing the total adult population at model equilibrium with the minimum adult population reached after five weeks of application. The literature-derived efficacy values were 49% five weeks after the last suppression period and 77% ten weeks after the last suppression period (33),

calculated by comparing the total adult population at model equilibrium to the total adult population after five- or ten-weeks of suppression which achieved the desired efficacy (summarised in Supplementary Table 1). A caveat of this approach is that the resulting minimum adult population is reached later than five- or ten-weeks, therefore, the maximum efficacy calculated in these scenarios is marginally greater than the literature value stated (shown in Supplementary Figure 2).”
In addition, SIT values have been updated in Figures 4, 5, and 6, added to Supplementary Figure 2, and the SIT literature source has been added to Supplementary Table 1.

1.2.4e. *The assumptions underpinning the modelling of the suppress then replace section of the paper appear to be erroneous, or at least very optimistic of their effectiveness in terms of suppression of the mosquito populations. This section is highly speculative, adds little to the paper and should be removed.*
1.2.4e. We acknowledge the reviewer’s concerns about the measurement and comparability of effectiveness of suppression-based vector control for Aedes mosquitoes and agree that the values we use from the cited studies are context and application specific (and state so in the discussion). WHO is currently conducting a Cochrane review of *Aedes* mosquito control effectiveness that originally planned to inform these parameters with a more rigorous evidence base, however delays meant that we instead chose values based on a single well-designed study. Acknowledging this approach may introduce bias, we also simulate a range of fixed values for suppression effectiveness (20%, 50%, and 80%) which allowed us to better compare different suppression methods for the purpose of hybrid programmes and set expectations for areas where suppression efficacy is likely to be lower. We hope that the above improvements to the evidence we cite and further explanation of the differing objective of the modelling for the TPP and PPC more clearly clarifies the value of this section of the work.

1.2.5. *“All hybrid programmes had the potential to offer cost savings over replacement alone, depending on the cost of suppression” (Page 14). This statement is not supported by presentation of any costing data for suppression. From an economic perspective, there is no evidence that a hybrid approach of suppression followed by replacement would be more cost effective than Wolbachia replacement alone, and a recent study based on data from Singapore’s Wolbachia suppression program suggests that a hybrid suppression-followed-by-replacement program could be significantly more expensive (Soh et al. 20211). The current annual per capita cost of suppression in Singapore is approximately USD $5 per person reached. This cost is roughly equivalent to the one-time, all-inclusive cost required to achieve Wolbachia replacement across some target settings (Brady et al BMC Med 2020; Turner et al PLoS Negl Trop Dis 2023). The ‘potential cost savings enabled by a hybrid approach’ presented in Table 3 “assumes suppression has no cost or is an in-kind contribution”, which is entirely implausible in any setting, let alone in a scenario of large-scale global implementation across the 73 countries (>900,000 km2), which would be required to achieve the TPP minimum targets. Without a more extensive analysis of the efficacy of suppression programs and their cost, the current analyses are highly speculative and add little to the paper. This reviewer feels the whole section on hybrid “suppress then replace” should be removed.*
1.2.5. As mentioned above, the hybrid suppress-then-replace scenarios are specifically for circumstances where suppression is already part of an existing vector control programme. Since areas which would benefit from *Wolbachia* replacement are likely to already engage in

vector control we included this model to quantify the potential gains from utilising existing suppression resources when transitioning to novel *Wolbachia* technology. In these specific contexts, suppression resources are likely to already have been purchased therefore the cost of suppression is assumed to be zero, however if this were not the case then the additional cost savings from implementing a new suppression technique are likely to be marginal. This has been further emphasised in the manuscript:
"...then a hybrid programme will cost less than replacement alone. This might refer to specific circumstances when suppression might be achieved at negligible cost, for example, if it's already part of a vector control programme. These are ideal situations where the resources for suppression are already in place, however, in most cases the potential savings achieved by a hybrid suppress then replace programme won't justify the additional cost of implementing a novel suppression programme."
We acknowledge the reviewers concern regarding the cost of suppression, and our conclusions around cost savings previously recognise this depends on the cost of suppression, for example in the results:
"All hybrid programmes had the potential to offer cost savings over replacement alone, depending on the cost of suppression... This, however, does not take into account the costs of suppression. A different interpretation of these results would be: if suppression can be achieved for less than these costs, then a hybrid programme will cost less than replacement alone."
And in the discussion:
"We predict that insect-based suppression methods (1gSLT, SIT, Male Wolbachia release) will be more effective than conventional suppression tools, but also outline a limited cost window which may be challenging for insect-based suppression methods to achieve. Investment in new infrastructure to conduct insect-based suppression may not be justified for a one-off suppression, but between overlapping resource requirements for suppression and replacement, ongoing use post-replacement (e.g., outbreak control or to achieve dengue elimination) and a continued drive to lower costs of mosquito suppression (51), this investment cost may be justified."
Finally, it is worth repeating that the aim of the PPC is to motivate the development of new products and strategies. It is precisely because one-off suppression campaigns can't meet these cost objectives that a PPC is needed, and these results give approximate targets that novel products or strategies could aim for to be useful for a hybrid suppress and replace approach.

1.3. Minor comments:
1.3.1. *Page 3, Introduction, second paragraph: The virus-blocking phenotype is specific to the species of Wolbachia, mosquito, and virus - the authors should use more specific language here.*
1.3.1. We agree that more specific language should be used when referring to the virus-blocking potential of *wMel*, therefore this has been clarified in text:
"Ae. aegypti mosquitoes infected with certain strains of the bacterium Wolbachia, such as wMel, wMelPop, and wAlbB (7) show reduced rates of virus dissemination, making them less capable of transmitting arboviruses (8)."

1.3.2. *Page 3, Introduction, second paragraph: There is no empirical evidence that temporary increases in the Ae. aegypti mosquito population have been a key barrier to community acceptability of Wolbachia releases; this should be expressed as theoretical consideration.*
1.3.2. We agree that temporary increases in *Ae. aegypti* mosquito population have not been a barrier to community acceptability in the past, therefore we have edited the text to reflect

this nuance:
"All of these options increase cost and can also lead to undesirable temporary increases in the Ae. aegypti mosquito population which should be addressed during community engagement to avoid it becoming a barrier to community acceptability (9, 10)."

1.3.3. *Page 6, Results, paragraph 1 (and throughout): the terminology 'coverage' is misleading as it implies the % coverage of a geographic area, whereas the authors are referring to % Wolbachia prevalence in the local Ae. aegypti population. The proportion of Wolbachia-infected mosquitoes in the population should be considered the "percentage prevalence," not the target coverage.*
1.3.3. We agree the terminology "coverage" could be a potential point of confusion, therefore we have clarified the phrasing of coverage in the introduction so that the usage is clear throughout:
"...self-sustaining coverage, defined as the percentage of Ae. aegypti population infected with Wolbachia, can be achieved by: increasing the number of releases..."
Our definition of coverage must be consistent with that used by the TAG for the TPP and was hence a decision outside of our control.

1.3.4. *Page 11, Figure 3: The axes titles and figure legend needs to make explicit what time period this data relates to, in terms of the cumulative cases and costs averted. Presumably 5 years of benefit, since the $2.33 cost threshold is shown, however this is inconsistent with the $0.24 preferred criteria referred to in the figure legend, which relates to 3 years of benefit. And the lower horizontal dotted line appears to be at $0.39, not $0.24.*
1.3.4. We thank the reviewer for their feedback and have changed the axis labels and figure legend to make it clear what the temporal denominator is for each measure.

1.3.5. *Page 16, Discussion: "Cost continues to be a barrier to wider adoption of Wolbachia replacement when its high costs but long-term benefits are compared to lower cost but short acting suppression methods". This statement needs to reflect the fact that Wolbachia replacement has an evidence base for epidemiological impact while the short acting suppression methods do not.*
1.3.5. Agreed, this sentence now reads:
"Cost continues to be a barrier to wider adoption of Wolbachia replacement when its high costs but long-term benefits are compared to lower cost but short acting suppression methods, despite differences in the evidence base underpinning these benefits"

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