

Risk Assessment for the

USE OF MALE *WOLBACHIA*-CARRYING *AEDES AEGYPTI* FOR SUPPRESSION

OF THE *AEDES AEGYPTI* MOSQUITO POPULATION



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1. Executive Summary

Low herd immunity, high human population density, the presence of *Aedes aegypti* in Singapore's highly urbanised environment and a continuous importation of multiple variants of dengue viruses have created a delicate landscape that is conducive for explosive transmission of the dengue. The challenge of dengue in Singapore is expected to escalate in the next decade, and the current strategy of preventive source reduction and elimination of mosquito adults in cluster management will have limited impact. A paradigm shift is needed, which includes the use of novel approaches to complement existing control methods to suppress the *Aedes aegypti* population.

The use of **male *Wolbachia*-carrying *Aedes* (*Wolbachia*-*Aedes*)** to suppress the vector population is a novel approach that has the potential to reduce the *Aedes aegypti* population to reduce the risk of dengue transmission. This approach, referred to as the **Incompatible Insect Technique (IIT)**, is a species-specific and benign approach for controlling vector populations. The release of male *Wolbachia*-*Aedes aegypti* mosquitoes will only impact the *Aedes aegypti* population, and not other insects. The approach exploits a biological phenomenon by which wild-type female *Aedes aegypti* (without *Wolbachia*) that successfully mate with male *Wolbachia*-carrying *Aedes aegypti* will produce non-viable eggs. A release of male *Wolbachia*-carrying *Aedes aegypti* to compete with wild-type males to mate with wild-type females is expected to result in a gradual reduction of the *Aedes aegypti* population in the field. The new proposed approach is consistent with Singapore's long term dengue control strategy of suppression of *Aedes aegypti* population

This strategy has been successfully used in an open release field trial to suppress the population of *Culex pipiens* in a village in Myanmar, and also for successful suppression of the Polynesian tiger mosquito, *Aedes polynesiensis*, in French Polynesia. More recently, Guangdong, China and California, United States have reported success of the approach in suppressing *Aedes albopictus* and *Aedes aegypti* populations respectively

Since 2012, the Environmental Health Institute of National Environment Agency has been studying the feasibility of using *Wolbachia*-*Aedes* for the suppression of *Aedes aegypti* in Singapore, and has conducted a comprehensive risk assessment of this approach. A local field strain of *Aedes aegypti*, made to carry a *Wolbachia* strain *wAlbB* from *Aedes albopictus*, has demonstrated stability of the infection with successful maternal transmission through six generations. They have been shown in the laboratory to be as competitive as wild-type males in mating with wild type females. Complete embryonic lethality was also observed when wild-type females mated with these males, demonstrating a high level of cytoplasmic incompatibility.

Concurrently, risk assessment of the *Wolbachia*-based IIT technology has been performed through critical review of literature, consultation with domain experts and laboratory experiments. The overall estimated risk to the environment and to human health is assessed to be very low or negligible. All low potential risks identified can be mitigated to negligible levels.

2. Objective and Scope

The main objective of this assessment is to identify any potential hazards in considering the use of *Wolbachia*-based IIT to suppress the *Aedes aegypti* population in Singapore, and to evaluate the possible

ecological and public health risks associated with such hazards. This risk assessment aims to answer the following questions:

What are the potential hazards associated with the release of *Wolbachia*-carrying *Aedes aegypti* males into our environment?

How severe are the potential hazards?

How likely are the potential hazards?

What are the possible consequences?

What are the levels of associated risk?

If the risks are high or severe, what additional measure(s) can be undertaken to reduce the levels of risk?

3. Background

3.1 Dengue and its vector

The global burden of dengue is estimated to be 50-100 million cases a year, and 40% of the world's population in more than 100 countries (**Error! Reference source not found.**) is at risk of dengue[1]. The infection is caused by the dengue virus (DENV), a single-stranded RNA virus with four immunologically related but distinct serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). Infection with one serotype confers lifelong immunity against that serotype, but only transient immunity to the other serotypes.

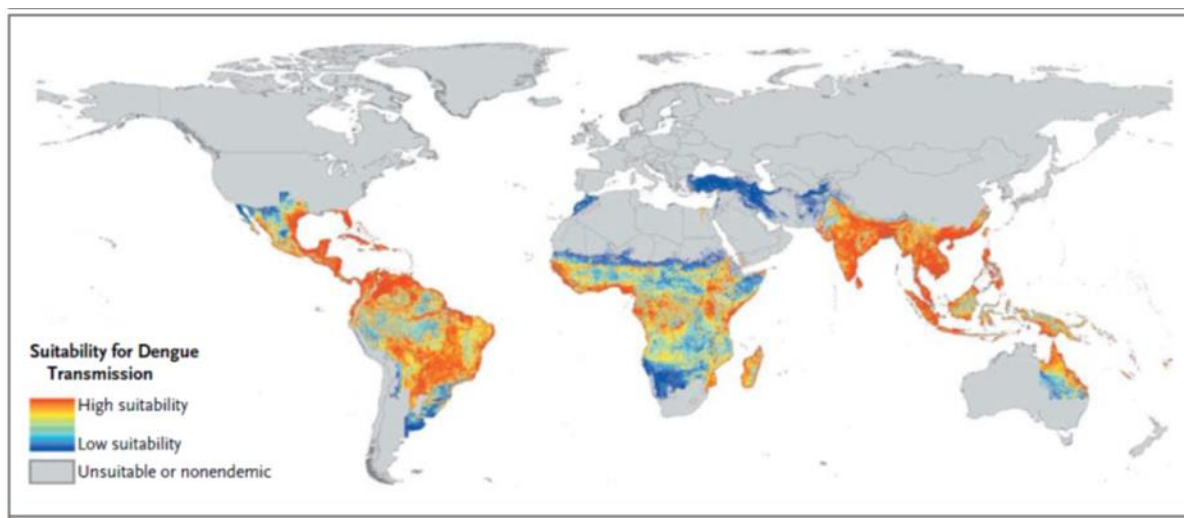


Figure 1. Global dengue risk map[2]

Dengue is endemic in Singapore, with regular outbreaks [3,4,5,6,7,8,9]. Not unlike most major cities along the tropical and subtropical belt, Singapore is a vibrant travel hub that receives a continual influx of genetically diverse dengue virus strains [10]. Singapore's vulnerability is reflected in the 2013 outbreak, which is the worst recorded dengue epidemic in its history, with 22,170 cases and 7 deaths [6].

2013

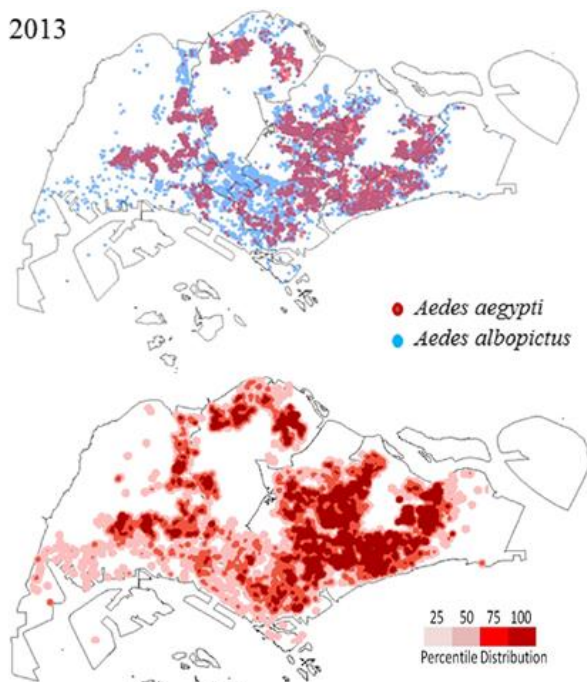


Figure 1. Colocation of dengue burden and *Aedes*

major vector role of *Aedes aegypti* in the transmission of dengue in Singapore and that *Aedes albopictus* likely plays a minor role. An approach that targets *Aedes aegypti* will likely make a significant impact on dengue transmission in Singapore.

3.2 *Aedes aegypti*

Aedes aegypti, commonly known as the 'Yellow Fever mosquito', is a small, brownish black mosquito with conspicuous white markings on its body and legs. It is morphologically similar to *Aedes albopictus*, commonly

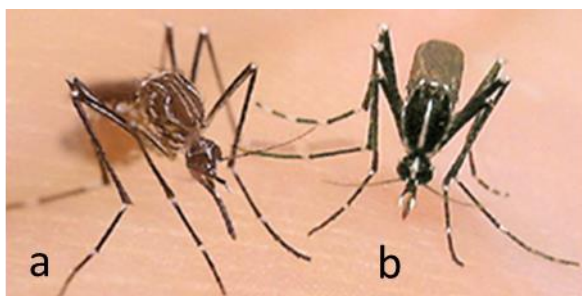


Figure 3. Comparing distinctive markings on the thorax between species: (a) *Aedes aegypti* with lyre-shape markings; (b) *Aedes albopictus* with median straight line

known as the Asian Tiger mosquito. The two can be differentiated from each other based on the ornamentation found on the back of their thorax (Figure 3). Female *Aedes aegypti* feeds almost exclusively on humans. The male does not bite and feeds only on plant juices for subsistence. This mosquito species bites predominantly during the day, with peak biting time at dawn and dusk. It is a highly domesticated mosquito that breeds primarily in artificial containers commonly found in and around residential premises. The eggs of this species can remain in a desiccated state and persist in the environment for up to 9 months. Once the eggs have

hatched, the larvae will undergo four stages of larval development, before metamorphosing into pupae, from which adults will emerge. The life-cycle duration is 7 to 10 days under optimal conditions in Singapore's climate.

The high dependence of *Aedes aegypti* on humans to provide it with shelter, a blood meal and suitable breeding habitats, means that source reduction is the primary means of controlling this vector. However, finding, treating and/or removing *Aedes aegypti* breeding is becoming increasingly challenging, particularly when there are cryptic or inaccessible breeding sites. Furthermore, the increased reliance on insecticides to

control dengue outbreaks, and the frequent use of household insecticides to control other household pests, have resulted in *Aedes aegypti* developing resistance to commonly used insecticides.

3.3 *Wolbachia*

Wolbachia is an obligate, intracellular, maternally-inherited, endosymbiotic bacterium that is commonly found in more than 60% of insect species, including mosquitoes, butterflies and dragonflies. It is also found in other arthropods such as spiders, mites, crustaceans. In insects, *Wolbachia* is transmitted vertically, from a female mosquito to her progenies. *Wolbachia* in insects render the insects resistant to viral infections, it has thus been postulated that *Wolbachia* confers fitness benefit to insects in nature[11,12]. When male *Wolbachia*-carrying mosquitoes mate with female mosquitoes that don't carry *Wolbachia* or carry different strains, all eggs laid by these females will not hatch due to incompatible mating. Together with the maternal transmission, this incompatible mating, termed "cytoplasmic incompatibility (CI)", gives an advantage to mosquitoes with *Wolbachia* and drives the spread of *Wolbachia* into the mosquito host population[13].

Though some mosquito species such as *Aedes albopictus* and *Culex* species, carry *Wolbachia*, *Aedes aegypti* does not. While the discrepancy is not understood, its absence in *Aedes aegypti* could partially explain the excellent vector competency of *Aedes aegypti* for many viruses.

3.4 *Wolbachia*-based Incompatible Insect Technique (IIT)

IIT takes advantage of the cytoplasmic incompatibility (CI) attribute of *Wolbachia* which causes a conditional

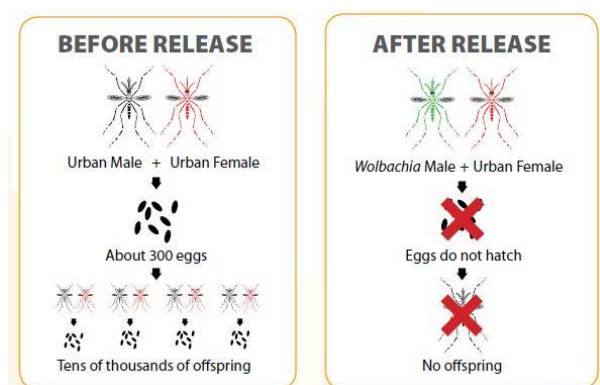


Figure 4. Mating between male *Aedes aegypti* with *Wolbachia* and urban females without *Wolbachia* results in non-viable eggs.

sterility of male-*Wolbachia* mosquitoes. The incompatible mating between male *Wolbachia*-*Aedes* with uninfected females, or between mosquitoes harbouring different strains of *Wolbachia* will lead to non-viable eggs, hence the term "conditional sterility" (Figure 4). Because there will be no offspring from such matings, a constant release of male *Wolbachia* *Aedes aegypti*, to compete with male urban wild type *Ae. aegypti* for the urban females, could lead to a gradual decline in the field population. The goal of IIT is to suppress the *Aedes aegypti* population to a level that cannot sustain dengue transmission (Figure 5).

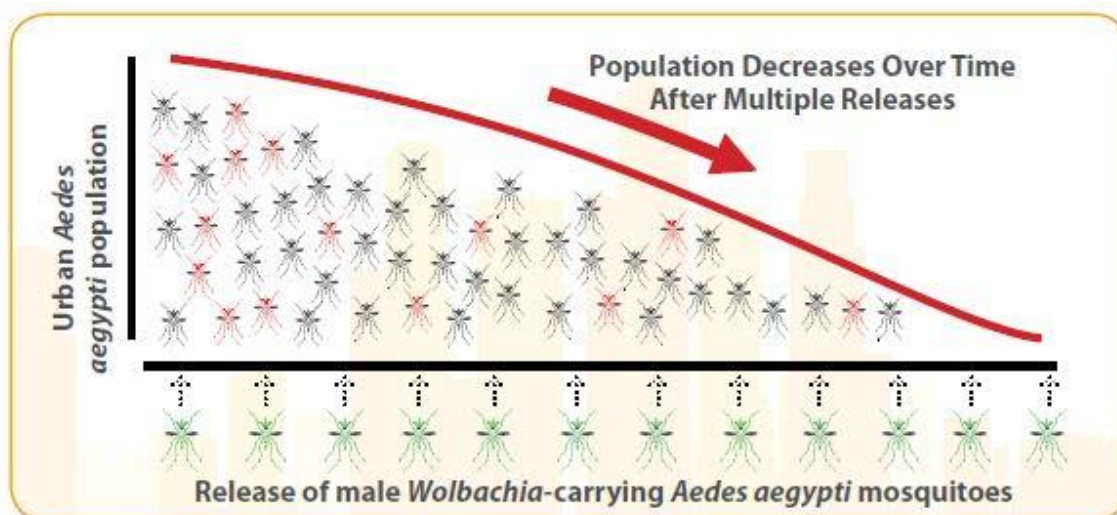


Figure 5. Regular release of *Wolbachia-Aedes aegypti* could theoretically reduce the population of urban *Aedes aegypti* in our environment.

3.5 Criteria for successful implementation of a IIT strategy

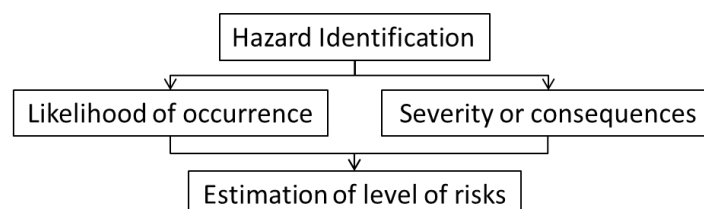
In order for the strategy to work, it needs to meet the following criteria: 1) the strain of *Wolbachia* used for IIT should display high level of Cytoplasmic Incompatibility; 2) it should show high rates of maternal transmission; 3) it should give minimal or no effect on the fitness and mating competitiveness of male mosquitoes; 4) there should be no risk of unwanted side-effects in the ecosystem caused by these releases; and 5) public consent/approval should be obtained prior to any release.

Using a *Wolbachia* strain from *Aedes albopictus*, *wAlbB*, controlled laboratory experiments at EHI have shown that *Wolbachia*-carrying *Aedes aegypti* males are equally competitive compared to wild-type males, and complete embryonic lethality was achieved when wild-type females mated with male *Wolbachia-Aedes aegypti*. Field studies are required to provide more information on the behaviour of male *Wolbachia-Aedes aegypti* in the field; mating competitiveness of male *Wolbachia-Aedes aegypti* compared to male wild-type (WT) *Aedes aegypti*; and the population suppression ability of male *Wolbachia-Aedes aegypti*.

4. Methods

4.1 Risk assessment process

The following steps were undertaken in conducting the risk assessment:



The hazard identification process involved 4 years of critical reviews of existing knowledge and research; and consultations with various overseas and local experts and stakeholders such as academic researchers, medical and healthcare professionals, government agencies and non-governmental organisations such as the Singapore Nature Society. More than 80 sessions of engagement workshops and sessions were held.

Feedback and concerns were gathered and evaluated, no matter how unlikely the risk appeared to be. Both the severity and the likelihood of occurrence are scored from 1 to 5. The eventual risk is determined as depicted in the matrix below (Figure 7).

| Likelihood | Frequent (5) | Likely (4) | Occasional (3) | Unlikely (2) | Remote (1) |
|------------|--|--|--|---|------------------------------------|
| Severity | | | | | |
| 5 | 25 HIGH RISK Operation not permissible | 20 HIGH RISK Operation not permissible | 15 SERIOUS RISK Mitigation needed | 10 LOW RISK Close monitoring needed | 5 NEGLIGIBLE RISK Acceptable |
| 4 | 20 HIGH RISK Operation not permissible | 16 HIGH RISK Operation not permissible | 12 SERIOUS RISK Mitigation needed | 8 LOW RISK Close monitoring needed | 4 NEGLIGIBLE RISK Acceptable |
| 3 | 15 SERIOUS RISK Mitigation needed | 12 SERIOUS RISK Mitigation needed | 9 LOW RISK Close monitoring needed | 6 LOW RISK Close monitoring needed | 3 NEGLIGIBLE RISK Acceptable |
| 2 | 10 LOW RISK Close monitoring needed | 8 LOW RISK Close monitoring needed | 6 LOW RISK Close monitoring needed | 4 NEGLIGIBLE RISK Acceptable | 2 NEGLIGIBLE RISK Acceptable |
| 1 | 5 NEGLIGIBLE RISK Acceptable | 4 NEGLIGIBLE RISK Acceptable | 3 NEGLIGIBLE RISK Acceptable | 2 NEGLIGIBLE RISK Acceptable | 1 NEGLIGIBLE RISK Acceptable |

Figure 7. Risk matrix illustrate how the Likelihood and Severity scores of each hazard is are integrated to derive a Risk Score

Risk Value of 1-5: Negligible Risk;

Risk Value 6-10: Low Risk

Risk Value 12-15: Serious Risk

Risk Value 16-25: High Risk

5. Results

5.1 Assessment of potential ecological and public health impact

The potential hazards identified are: 1) negative impact on the environment; 2) unintentional release of *Wolbachia*-carrying *Aedes aegypti* females into the environment; 3) niche replacement by other mosquitoes; and 4) ecological imbalance due to suppression of the *Aedes aegypti* population.

Four potential hazards have been identified, and the ecological and public health risks associated with each of these potential hazards have been evaluated. The scores for the likelihood, severity and risk levels of the potential ecological and public health hazards are presented in Table 1.

Table 1. Scores for likelihood, severity and risk level for ecological hazards.

| No. | Potential Hazard | Risk | Impact | Risk Evaluation | | |
|-----|---|--|---------------|-----------------|----------|-------------------------------------|
| | | | | Likelihood | Severity | Risk Level |
| i | Release of Male <i>Wolbachia Aedes aegypti</i> having an impact on the environment | <i>Wolbachia</i> (wAlbB strain) becomes established in the environment, outside its intentional host | Ecological | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Animals become infected with <i>Wolbachia</i> (wAlbB strain) | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Predators become infected with <i>Wolbachia</i> (wAlbB strain) | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | No potential public health risk identified | | | | |
| | | | | | | |
| ii | Unintentional release of <i>Wolbachia</i> -carrying <i>Aedes aegypti</i> females into the environment | WT <i>Aedes aegypti</i> replaced by <i>Wolbachia-Aedes aegypti</i> | Ecological | 3 | 1 | 3 NEGLECTIBLE RISK Acceptable |
| | | <i>Wolbachia</i> (wAlbB strain) becomes established in the environment, outside its intentional host | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Animals become infected with <i>Wolbachia</i> (wAlbB strain) | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Predators become infected with <i>Wolbachia</i> (wAlbB strain) | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Increase in mosquito population, resulting in increase in biting pressure | Public Health | 2 | 1 | 2 NEGLECTIBLE RISK Acceptable |
| | | Contribute to increase in vector-borne disease cases (relative to current level), due to unintentional release in an area where vector-borne disease transmission is ongoing | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Humans are infected with insect <i>Wolbachia</i> | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |

| No. | Potential Hazard | Risk | Impact | Risk Evaluation | | |
|-----|---|--|---------------|-----------------|----------|--|
| | | | | Likelihood | Severity | Risk Level |
| iii | Niche replacement by other mosquito species when <i>Aedes aegypti</i> is eliminated | <i>Aedes aegypti</i> replaced by <i>Aedes albopictus</i> | Ecological | 2 | 1 | 2 NEGLECTIBLE RISK Acceptable |
| | | <i>Aedes aegypti</i> replaced by <i>Culex quinquefasciatus</i> | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Contribute to increase in dengue incidence (relative to current level) | Public Health | 2 | 1 | 2 NEGLECTIBLE RISK Acceptable |
| | | Contribute to increase in chikungunya incidence | | 2 | 3 | 6 LOW RISK Close monitoring needed |
| iv | Ecological imbalance due to suppression of the <i>Aedes aegypti</i> population | Density of the predator affected | Ecological | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Density of other insects | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | Flowers that rely on mosquitoes for pollination are affected | | 1 | 1 | 1 NEGLECTIBLE RISK Acceptable |
| | | No potential public health risk identified | | | | |

5.2 Reasoning of Assessment

Based on the risk matrix, the potential ecological and public health risks associated with the release of male *Wolbachia*-carrying *Aedes* into the environment, is negligible or low. The reasoning is as follows.

i) Release of Male *Wolbachia Aedes aegypti* having an impact on the environment

Can *Wolbachia* (wAlbB strain) become established in the environment, outside its intention host? The transfer of *Wolbachia* (wAlbB strain) into the environment (outside its intentional host) is unlikely to occur. *Wolbachia* is a fastidious, obligate, endosymbiotic bacterium, which means that it can only survive only inside a host's cells. *In vitro* studies have shown that *Wolbachia* is only able to survive outside a host's cell if in a medium containing high amounts of amino acids. Thus, it is not expected to persist in the environment outside the host carrying it. *Wolbachia* will degrade together with the insect host's body when the latter dies,

and the residue will not be different from that of natural organic detritus found in the environment[14,15,16].

Can animals become infected with insect *Wolbachia*? Nature has been continuously exposed to *Wolbachia* for millions of years. To date, there is no scientific evidence to show that *Wolbachia*-carrying mosquitoes are able to transfer the bacterium to vertebrate hosts during blood feeding. A recent study in Australia showed that human volunteers exposed to bites from *Wolbachia*-*Aedes aegypti* did not elicit an immune response – suggesting that *Wolbachia* or its parts are not transferred to humans through the bites of mosquitoes.

Can predators become infected with insect *Wolbachia*? To date, there are no reports of mosquito predators (e.g. fish, lizards, frogs, spiders) becoming infected with *Wolbachia*, after ingesting insects that naturally carry *Wolbachia*. A recent study conducted in Australia showed that spiders could not become infected by insect *Wolbachia*, despite being continually fed with *Wolbachia*-carrying *Aedes aegypti* [14].

ii) Unintentional release of *Wolbachia*-*Aedes aegypti* females into the environment

Laboratory security, and thorough sorting of males and females have been put in place to prevent unintentional release of a large number of females. Male mosquito pupae are smaller than female pupae, thus male and female mosquitoes can be sorted by size in the laboratory, at the pupae stage. After sorting, the male *Wolbachia*-*Aedes aegypti* pupae are allowed to emerge as adult mosquitoes. Further screening by skilled entomologists will reduce the chance of females among the male population. A very small number of female *Wolbachia*-*Aedes* may be released along with the males. Current methodology results in 99.9% of purity of male *Wolbachia*-*Aedes*, thus the number of females released will be very small.

Will the urban wild type *Aedes aegypti* be replaced by *Wolbachia*-carrying *Aedes aegypti*? The urban *Aedes aegypti* population may be replaced by *Wolbachia*-*Aedes aegypti* if a large number of female *Wolbachia*-*Aedes* is unintentionally and continuously released. In our strategy, the number of females that could be released along with the males would be very small in comparison to the population in the environment, thus the female *Wolbachia*-*Aedes* mosquitoes will have no impact. The number would be too small to achieve any spread of the *Wolbachia* bacterium. It is highly unlikely that *Wolbachia*-*Aedes aegypti* will displace the WT *Aedes aegypti* population. Trials in Australia, Vietnam, Indonesia and other countries, that aim to replace WT population with *Wolbachia*-*Aedes* has shown that regular large scale release of females are required to achieve replacement. However, should this happen, any possible damage to the ecosystem is considered negligible. *Wolbachia* is a naturally occurring in more than 60% of insects.

Will *Wolbachia* becomes established in the environment (outside its intentional host): refer to (i) above

Will animals become infected with insect *Wolbachia*: refer to (i) above

Will predators become infected with insect *Wolbachia*: refer to (i) above

Will the increase in mosquito population result in an increase in biting pressure? A very small number of female *Wolbachia*-*Aedes* may be released along with the males. The number would be very small in comparison to the population in the environment, thus increased biting would be negligible when compared to the original situation.

Will it contribute to increase in vector-borne disease cases (relative to current level) due to unintentional release in an area where vector-borne disease transmission is ongoing? A very small number of female

Wolbachia-Aedes may be released along with the males. The number would be very small in comparison to the population in the environment, thus the female *Wolbachia-Aedes* mosquitoes will have no impact. Moreover, virus transmission (e.g. Dengue, Chikungunya and Zika) will be largely blocked by *Wolbachia*, as reported by many laboratories, including studies performed at the Environmental Health Institute of NEA.

Can humans be infected with insect *Wolbachia* through mosquito bites? Insect *Wolbachia* is not known to infect humans. A recent study has demonstrated that human volunteers exposed to periodic bites of *Wolbachia*-carrying *Aedes aegypti* do not show any immune response against *Wolbachia*[14]. In addition, humans are already regularly exposed to mosquitoes, such as *Aedes albopictus* and *Culex quinquefasciatus*, which naturally-carry *Wolbachia*. Despite this, there have been no reports of humans being infected with insect *Wolbachia*.

iii) Niche replacement by other mosquito species when *Aedes aegypti* is eliminated

In Singapore, despite the presence of around 140 species of mosquitoes recorded in Singapore to-date, we have not observed other mosquito species taking over areas with low *Aedes aegypti* population.

Will *Aedes albopictus* take over the urban niche vacated by *Aedes aegypti* ? Though *Aedes aegypti* prefers urban spaces, and *Aedes albopictus* prefers greeneries, *Aedes aegypti* and *Aedes albopictus* are known to share some common habitats. E.g. they may breed in the same containers. Such replacement has been seen previously in Hawaii decades ago, when *Aedes aegypti* was eliminated in some of the islands [17,18,19,20] . However, in the local context, our experience and mosquito population data collected from our Gravitrap surveillance have suggested that the risk of *Aedes albopictus* taking over the vacant niche of *Aedes aegypti* is low, especially if the community continues to remove breeding habitats in our surroundings. Firstly, the population dynamic of the two *Aedes* population showed that a decrease in *Aedes aegypti* population does not coincide or lead to an increase in *Aedes albopictus* population. Secondly, the *Aedes aegypti* population in Singapore has already been reduced to relatively low numbers in the past few decades, with breeding found in less than 1 in 100 premise inspected. Despite this, we have not observed *Aedes albopictus* moving into indoor spaces and taking over the niche of *Aedes aegypti*. The ecological risk is low. Nevertheless, NEA's Gravitrap surveillance system will detect any unusual increase in the *Aedes albopictus* population.

Will *Culex quinquefasciatus* take over the urban niche vacated by *Aedes aegypti*? There are occasional instances where both *Aedes aegypti* and *Culex quinquefasciatus* breeding have been found in the same habitat, but it is highly unlikely that *Culex quinquefasciatus* will occupy the niche of *Aedes aegypti*, as the requirements of these two species are quite distinct. *Culex quinquefasciatus* prefers to breed in more polluted and resource-rich habitats, such as in drains and larger water bodies, rather than in resource-poor breeding habitats such as household containers. The ecological risk is negligible.

Will other mosquitoes contribute to increase in dengue incidence? The likelihood of niche replacement by *Aedes albopictus*, in Singapore's context, has been assessed to be low. Furthermore, *Aedes albopictus* is not as competent a vector as *Aedes aegypti*. This is evident internationally and locally, where places with presence of *Aedes albopictus* and absence of *Aedes aegypti* do not experience dengue outbreaks. Studies conducted at EHI have also shown that *Aedes albopictus* is less efficient at transmitting dengue compared to *Aedes aegypti*. Thus, even if *Aedes albopictus* takes over the niche vacated by *Aedes aegypti*, the dengue situation is still expected to improve.

Will other mosquitoes contribute to an increase in chikungunya incidence? The likelihood of niche replacement in Singapore's context has been assessed to be low. However, in the unlikely event of *Aedes albopictus* taking over the niche and becoming the predominant *Aedes* vector in Singapore, there could be a slight increase in risk of chikungunya transmission. Some strains of Chikungunya virus are known to be more transmissible by *Aedes albopictus*. Close monitoring of the situation is needed. The existing NEA vector surveillance and community-based control strategies, which have been successful in tackling chikungunya outbreaks, will be able to address the low risk.

iv) Ecological imbalance due to suppression of the *Aedes aegypti* population

Will the density of predators be affected with the removal of the *Aedes aegypti* population? Reducing the *Aedes aegypti* population will not affect animals that feed on mosquitoes, because *Aedes aegypti* is an urban mosquito found in the built environment and typically not in natural settings (such as forests and parks). *Aedes aegypti* dwells in built up areas and breeds primarily in artificial containers in and around human habitats. The species thus has limited interaction with nature and does not make any significant contribution to the diet of animals that feed on insects. It also has low biomass[21]. There are insectivores (e.g. lizards, small animals) that feed on mosquitoes in the urban environment, but there is also an abundance of other mosquito species (e.g. *Aedes albopictus* and *Culex quinquefasciatus*) and other insect species, which they can feed on. There are around 140 species of mosquitoes in Singapore, found mainly in the forests. Together with other insects, they contribute to the diets of insectivores in nature.

Will the density of other insects be affected? The approach is species-specific. Thus, the release of male *Wolbachia*-*Aedes aegypti* mosquitoes will only impact the *Aedes aegypti* population, and not other insects.

Will flowers that rely on mosquitoes for pollination be affected? Though male mosquitoes feed on nectar, they are not known to play a role in the pollination of flowers[22]. Reduction in the mosquito population, especially in the urban setting, will not have an impact on the flowering of plants.

6. Summary

Overall, the potential ecological and public health impact for the release of male *Wolbachia*-*Aedes aegypti* males to suppress the *Aedes aegypti* population in Singapore are considered to be negligible. The only risk above negligible is the low risk of increased chikungunya, due to *Aedes albopictus* potentially taking over the urban niche should *Aedes aegypti* be eliminated. Our continued source reduction programme and close monitoring with Gravitrapp surveillance system will be able to reduce the risk to negligible. When compared with the potential benefit of reducing the burden of dengue in Singapore, the associated low risk is deemed as very acceptable.

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