Australia's National
Science Agency

Draft assessment report for *Aedes albopictus* (Asian Tiger Mosquito)

12th May 2020

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1. Taxonomy of species

a) Family Name: Culicidae

b) Genus Name: *Aedes**

c) Species: *albopictus*

d) Subspecies: No known subspecies of *Ae. albopictus* has been previously described

e) Common name: Asian tiger mosquito

f) This is not a genetically engineered organism (GMO)

* There have been recent suggested changes to the taxonomic classification of this mosquito, which are under scientific scrutiny and have not been fully accepted or implemented. The elevation of *Stegomyia* from sub-genus to genus level has led to some authors referring to this mosquito as *Stegomyia albopicta,* however this is controversial and is still most frequently referred to as *Aedes (Stegomyia) albopictus* [1-3]. This document will solely refer to the mosquito as *Aedes albopictus.*

http://mosquito-taxonomic-inventory.info/sites/mosquito-taxonomic-inventory.info/files/Valid%20Species%20%28composite%20Aedes%29_9.pdf
(Last updated 16th January 2020)

2. Status of species under CITES

Aedes albopictus is not listed either on Appendix I or II of the Convention of international trade in endangered species of wild fauna and flora (CITES).

3. Ecology of the Species

a) Longevity of species.

The *Ae. albopictus* (Skuse) lifespan in the wild is variable but studies estimate average lifespan as 1.5 to 24 and 8.5 to 24 days for males and females respectively [4]. In captivity, the maximum lifespan is 60 days for females and 48 days for males at 25°C and 70% relative humidity [5,6]

b) What is the maximum length and weight that the species attains?

Wing length is used as a proxy for body size of mosquitos with females being slightly larger at 2.61 mm than males at 2.1 mm wing length [7]. The dry mass of *Ae. albopictus* adults from Florida, USA are 0.22-0.26 and 0.35-0.38 grams for males and females, respectively [8].

c) Discuss the identification of the individuals in this species.

Aedes albopictus, also known as the Asian tiger mosquito, is an invasive and pestiferous species of both medical and veterinary significance [5]. The species originally described by Skuse in 1894 from specimens collected in Calcutta, India and is in a subgroup member of the *scutellaris* group of the *Stegomyia* subgenus [7]. The separation of *Ae. albopictus* from other twelve members of the *scutellaris* subgroup can be difficult, especially in areas of overlapping distribution like the Torres Strait [9]. It is a small to medium-sized mosquito with white bands on legs, a dark proboscis and wings dark scaled. Key anatomic features that differentiate adult *Ae. albopictus* from other *Stegomyia* can be carried using the keys of Huang [10] and include a white stripe of scales down the mid-line of the scutum (upper thorax, Figure 1), palpi with white scales, a patch of broad white scutum scales before the wing root, and the clypeus lacking white scales. Male and female mosquitoes are simple to differentiate, with male body size being smaller and displaying feathery antenna (Figure 2).

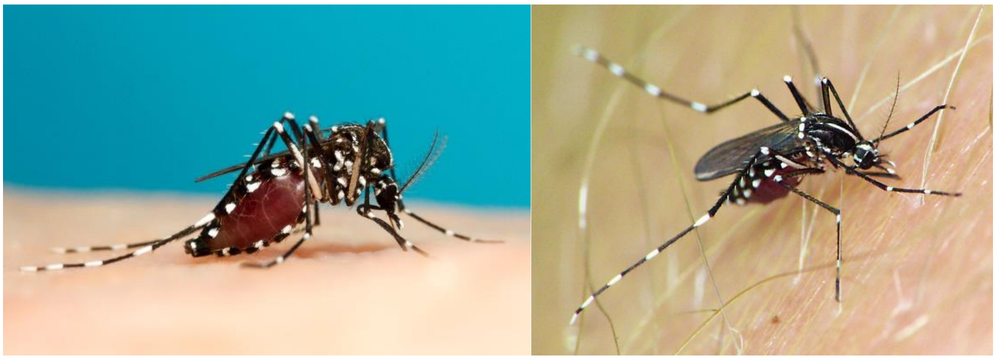

Figure 1. Adult female *Aedes albopictus* feeding showing the white stripe of scales down the mid-line of the scutum (upper thorax). Left image copyright Stephen Doggett (NSW Health, Australia) and right image used with permission from Sean McCann (Simon Fraser University, California, USA).

Figure 2. *Aedes albopictus* female (left and male (right) showing the different physiology of antennae used to differentiate sexes. Female image copyright Stephen Doggett, NSW Health. Male image creative commons licence by Larah McElroy on Flickr.

In Australia *Ae. albopictus* can be misidentified as *Ae. scutellaris* from the Australasian region, particularly at the larval stage where they often share habitat (Figure 3). The key of Tanaka, *et al*. [11] can be used to separate the larval stage and in addition, assays have been developed to distinguish among *Ae. scutellaris, Ae. albopictus,* and *Ae. aegypti* (Linnaeus) [12,13].

Figure 3. Larval stage of *Aedes albopictus*.

In Australia, the larvae of *Ae. aegypti* can be separated from *Ae. albopictus* by the lateral comb scales. *Aedes aegypti* scales have a distinct middle denticle with lateral denticles, whereas *Ae. albopictus* scales have a single denticle (Figure 4).

Figure 4. Key defining larval features between *Aedes aegypti* (left) and *Aedes albopictus* (right). Lateral comb scales of *Ae. aegypti* have a distinct middle denticle with lateral denticles, whereas *Ae. albopictus* scales have a single denticle (right). Image taken with permission from *Teo, et al.* [14].

d) Natural geographic range.

Aedes albopictus has a wide natural geographic distribution across south-east Asia. The species has two phenotypes that are adapted to both temperate northern environments such as Beijing, China and Honshu, Japan and southern tropical conditions in Malaysia, India and Sri Lanka [5]. Only predation at the larval stage has been explored extensively. Invertebrates have been observed acting as predators of *Ae. albopictus* larvae including; *Culex tigripes*, *Cx. fuscanas*, *Cx. halifaxii*, Toxorhynchites rutilis Coq, *Tx. splendens*, *Corethrella appendiculata*, *Lacconectus punctipennis* and copepods [9,15].

e) Is the species migratory?

Aedes albopictus in a non-migratory species that rarely disperses more than one kilometre from larval habitat [16,17]. However, eggs have been suggested as the most likely life stage for long distance dispersal and recent introductions into Europe suggests adults travelling in cars as a likely alternative method of dispersal [18].

f) Does the species have the ability to hibernate in winter or aestivate in the summer months?

Certain temperate strains of this species can persist in the egg stage via diapause, in areas where temperatures drop below freezing (see Hibernation and Life Cycle sections below). This trait is not present in tropical populations, which show high levels of mortality when exposed to low temperatures [5]. Hawley, *et al*. [19] exposed eggs from tropical Asia to -10°C for 24 hours and recorded near 100% mortality, whereas eggs originating from a temperate region of Asia experienced considerably lower mortality. Survival differences can be explained by the process of diapause, where temporally adapted eggs enter a period of arrested growth during predictable adverse and unfavourable environmental conditions. Diapause in *Ae. albopictus* requires two to six months to complete and is influenced by photoperiod experienced by adult females [20].

g) Does the species have the ability to breathe atmospheric air?

Larval, pupal and adult stages of *Ae. albopictus* can breathe air.

h) Outline the habitat requirements for all life stages of the species.

Physical Parameters

Salinity: The aquatic larvae of *Ae. albopictus* have found to survive brackish water (2 to 15 ppt) in coastally adapted populations [21], but elsewhere they are most commonly found in natural and artificial containers filled with fresh water with low salinity.

Oxygen: As mosquito larvae and pupae can breathe air through siphons at the surface of the water, and do not require well-oxygenated water. Larvae can breathe air through gills when there is sufficient oxygen in water.

pH: The larvae of this species is capable of surviving in water greater than 4.8 pH but less than 7.1 pH [22].

Temperature of natural habitat: *Aedes albopictus* exhibits phenotypic plasticity, with strains that are temporally adapted thus there are a range of potential optimal temperatures for survival and development in the species. The lower temperature for development of the larval stage is between 8.8°C and 10.4°C with an optimal temperature of 29.7°C and an upper range of 35°C [23-25].

Climate: *Aedes albopictus* populations have adapted to a wide range of temperatures, from tropical to temperate climates.

What nest sites can the species use: Aedes albopictus is a container breeding species where it inhabits tree-hole habitats such as bamboo stumps in its natural distribution. The species has adapted to exploit urban environments in close association with humans and can be observed in natural and artificial water holding containers, rainwater tanks, bird baths, pot plants, guttering, drainage pipes, drums and tyres [5].

Does the species nest, shelter or feed in or around marshes or swamps; estuaries, lakes, ponds or dams, rivers, channels or streams, banks of water bodies; coastal beaches or sand dunes? Adult females require blood to reproduce, so distribution is highly correlated with a number of factors including access to blood, presence of larval habitat (containers) and rainfall. Unless containers like tree holes are present, *Aedes albopictus* will not lay eggs in swamps, marshes, lakes, ponds, dams, rivers, channels, streams, beaches or dunes. The species is capable of feeding of a wide range of animals for survival and adults will feed on humans, dogs, pigs, cats, deer, horses, rabbits, squirrels, raccoon and opossums [26,27]. It is often observed in highly vegetated environments such as parks and the boundaries of urbanized environments but rarely inside urban dwellings.

i) <u>Social behaviour or groupings.</u>

Aedes albopictus males have been observed swarming around the legs and feet of humans waiting for females (which are solitary) to approach for a blood meal[28]. However, mosquitos are usually largely solitary throughout their life stages.

j) <u>Is this species ever territorial or does it exhibit aggressive behaviour?</u>

This species is not territorial and besides biting behaviour, does not act in an aggressive manner towards humans.

k) <u>Characteristics that may cause harm to humans or any other species.</u>

In laboratory tests female *Ae. albopictus* is known to vector dengue, Zika, chikungunya, yellow fever, West Nile virus, eastern equine encephalitis and dog heartworm. Examination of the literature indicates *Ae. albopictus* is known as a major vector of chikungunya in Asia and likely serves as a maintenance (secondary) vector of dengue in rural areas of endemic countries of South America, south-east Asia and Pacific islands [29]. As with all blood feeding mosquitos, an allergic response can accompany a bite, however, this is usually mild and generally includes itchiness and minor inflammation.

4. Reproductive Biology of Species

Reproductive characteristics

a) At what age does this species reach sexual maturity (males and females)?

Male and female *Ae. albopictus* have reached sexual maturity once they have left the pupa and begin feeding and mating within two or three days [30].

b) Discuss the species' ability to reproduce; triggers for breeding; breeding site requirements?

Environmental conditions and the physiological state of the adult female are prerequisites for oviposition. Females search for a blood meal after mating and the protein acquired from this meal is used to produce eggs. *Aedes albopictus* females can lay up to 110 eggs three or four days after a blood meal and 300-345 eggs over a lifetime [7,9]. *Aedes albopictus* populations have been reported to exhibit low levels of autogeny, with an autogeny index of 0.3 to 3.0 being the number of eggs laid by wild females who have newly emerged, mated and fed on a carbohydrate source. This index value is also dependent upon larval nutrition which can provide adults with greater levels of protein and lipid reserves [31-33].

As mentioned above in 3.f. *Ae. albopictus* exhibits diapause, a process where desiccation resistant eggs enter a period of arrested growth during predictable adverse and unfavourable environmental conditions. Diapause in *Ae. albopictus* requires two to six months to complete and is influenced by photoperiod experienced by adult females [20]. Eggs that have entered diapause are less likely to hatch when immersed in water.

The species exhibits skip oviposition, a risk averse behaviour expressed as negative density dependent oviposition and a common trait in other container inhabiting species such as *Ae. aegypti*. In *Ae. albopictus* skip oviposition is modified by the onset of diapause; during summer when times are favourable eggs are spread across a range of different containers, however, when conditions become less favourable eggs are accumulated in more ideal container types [9,34].

Aedes albopictus eggs are preferentially laid in both artificial and natural water holding containers that exhibit dark colouring. The female deposits eggs individually on the sides of a container, slightly above the water line. The texture of containers influences oviposition, with rough surfaces preferred to smooth surfaces [35].

c) <u>How frequently does breeding occur?</u>

Life cycle times: The *Ae. albopictus* gonotrophic cycle (the time taken until first eggs are laid) is four-five days. *Aedes albopictus eggs* are capable of drying and surviving low temperatures for many weeks. Laboratories studies show that females produce an average of 42-110 eggs in the first cycle with an average of 300-345 over the lifetime [7,9]. Eggs can be resistant to desiccation, but this trait is dependent on strain, temperature, humidity and presence of diapause. Gubler [6] found nearly 50% survivorship after three months in eggs maintained at a relative humidity of 70-75% and a temperature of 25°C in the laboratory. In Japan, non-diapausing adults had a mean survival time (time to 50% mortality) of 101.6-125.1 at 90% relative humidity (RH), 77.7-80.5 at 73% RH and 30.3-31.1 at 44% RH at 25°C. However, in diapausing adults these increase to 191.4-212.6 at 90% RH, 155.9-174.4 at 73% RH and 63.5-67.4 at 44% RH at 25°C [36]. In Australia, research suggests the Torres Strait *Ae. albopictus* population lay eggs that are significantly impacted by temperature and relative humidity, with eggs unlikely to survive winter temperatures in temperate regions including Sydney and Melbourne [37].

d) <u>Can individuals of the species change sex?</u>

Mosquitoes are not known to change sex.

e) <u>Ability of the species to hybridise. Describe any known crosses. Are progeny of such crosses fertile?</u>

There has been no modern, high-quality scientific research to show *Ae. albopictus* reliably hybridizes with other members of the *Scutellaris* group, likely due to cytoplasmic incompatibility associated with *Wolbachia* symbionts.

f) Could the species hybridise with any Australian native species?

As stated above there is no quality evidence, genetically or experimentally to show that *Ae. albopictus* hybridises with any other mosquito species.

g) Are individuals single sexed?

All mosquitoes are single-sexed but very rarely gynandromorphs have been observed in the laboratory [38,39].

5. Feral Population establishment

a) Has this species ever established a breeding population outside of its native range?

The suitability of many climates for *Ae. albopictus* proliferation have been identified and the capability of mosquito spread is considered high, with it now having breeding populations in every continent except Antarctica [40-62] (Figure 5). Since 2005, *Ae. albopictus* have established a presence in the Torres Strait [63,64].

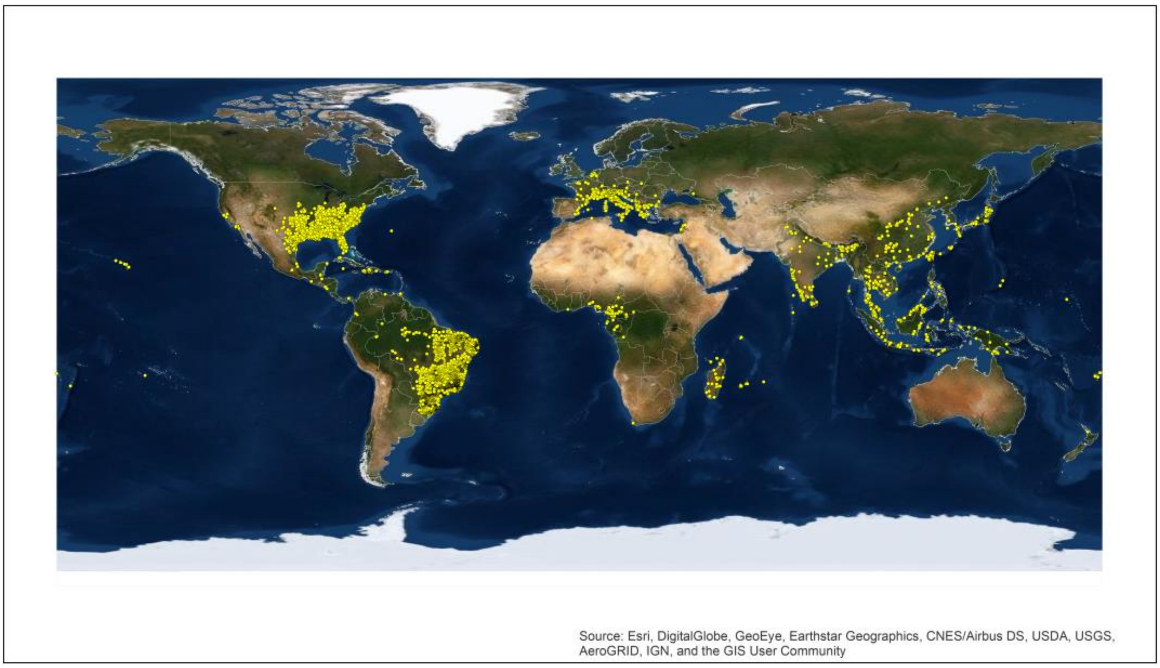

Figure 5. Global *Aedes Albopictus* occurrence from 1960-2014 [65].

b) Is the species considered a pest anywhere in its natural or introduced range?

Aedes albopictus is considered a pest and disease vector in both its known and introduced habitats. Control measures are in place to limit populations across all its global range [66,67].

c) Has the species been introduced to other countries, even if it has not established feral populations?

As stated above, *Ae. albopictus* has been introduced and established in many countries. There have also been documented interceptions in the ports of Darwin, Townsville, Cairns, Brisbane, Sydney and Melbourne, Australia[64,68,69], and Auckland, New Zealand [70].

6. Environmental risk assessment for *Aedes albopictus*

As far as we are aware, no specific environmental risk assessments have been carried out in Australia nor overseas for *Ae. albopictus.* However, the European centre for disease prevention and control (ECDC) identified the need for *Ae. albopictus* risk mapping to be performed [71], and in 2013 completed a proof-of-concept environmental risk mapping technical report on the future potential for *Ae. albopictus* to spread within Europe [§]. Through the use of computational modelling, the ECDC suggested that major rivers and road networks may act as potential routes of vector dispersal from areas of current mosquito presence in southern Europe (primarily the Mediterranean basin) to areas of potential risk within Europe (such as parts of France, Spain, the northern Adriatic region, Greece, Switzerland and Germany). These predictions were based on the analysis of multiple datasets relating to; the environment, mosquito biology and mosquito hosts, human demographics, and transport network and travel networks.

Whilst not risk assessments per se, several other studies have focused on the potential distribution of *Ae. albopictus* using computational modelling – a technique often utilised to aid in developing risk assessments [72-78]. Across these studies it is important to note that differing parameters have been used to determine potential future mosquito spread; however they generally find that local humidity and annual minimum temperatures are good predictors for *Aedes* mosquito presence, yet there still can be a large range of local suitability within each area [79].

[§] www.ecdc.europa.eu/en/publications-data/environmental-risk-mapping-aedes-albopictus-europe-proof-concept-study-european

When assessing the risk of *Ae. albopictus* spread, it is important to consider both climatic and non-climatic events, as both can ultimately contribute to future distribution [76]. As temperatures are projected to increase over the next century, areas typically not associated with *Ae. albopictus* may become susceptible [40,72,77,79,80], and due to increased globalisation major transport/logistics pathways and international travel can inadvertently further aid in the spread of *Ae. albopictus* [79,81].

Whilst computational modelling and simulated population dispersals are an important tool in determining risk associated with mosquito spread, they also come with some caveats. The initial assumptions made may not indeed be correct and important variables could have been missed. Thus, it is imperative that models are revisited often and incorporate new data as it becomes available.

7. Likelihood of the species to establish a breeding population in Australia

a) Ability to find food sources.

The diet of adult and larval *Ae. albopictus* is similar to that of native Australian mosquitoes: aquatic detritus, algae and microorganisms for the larvae, nectar and mammalian blood for the adults [82]. *Ae. albopictus* has the potential to find food sources in Australia.

b) Ability to survive and adapt to climatic conditions.

Aedes albopictus mosquitoes have the ability to breed (and overwinter) in artificial containers such as plant pots, used tires and bird baths. Dependent on the mosquito strain (temperate or tropical), breeding could also continue in either drought or cold weather conditions [23,83-86]. Eggs of all *Aedes* mosquitoes can survive in a dry state for up to a few months until triggered to hatch by rain, however may also be able to survive low temperatures dependent on their origin and whether or not these eggs have been laid by an adult experiencing diapause [85,87,88]. It must be emphasised that *Ae. albopictus* reared in controlled insectary colonies are normally conditioned to tropical conditions (>27°C) and on a 12:12h light cycle. These conditions would limit the establishment of diapause in eggs which would be necessary for the species to survive during winter temperatures in temperate regions, especially those experienced in the southern areas of Australia. Due to the global distribution of the *Ae. albopictus* across varying conditions, the mosquito may be able to adapt itself to both temperate and tropical areas of Australia.

Female *Aedes* mosquitoes, including *Aedes albopictus*, are known to store sperm for use in egg production. Two-to-three days after insemination, females become refractory to mating due to male accessory gland secretions, and sperm is stored in the female bursa inseminalis [89]. Although females usually mate only once in a typical lifetime, wild *Ae. albopictus* have been observed to produce ~26% of offspring to multiple parents [90]. Adult female *Ae. albopictus* are known to utilise skip-oviposition as a method for minimising risk for offspring. This strategy involves depositing eggs across multiple containers during ideal summer conditions, but accumulating eggs in certain containers in preparation for diapause and unfavourable winter conditions [34].

c) Ability to find shelter.

Aedes albopictus mosquitos have the ability to live in a wide variety of habitats both indoors and outdoors; including dense urban settings such as houses, gardens, drains, storage facilities, parklands and building sites [91,92].

d) Reproduction.

Aedes albopictus has been seen to be well adapted to human habitats, and their global spread has been greatly influenced by their breeding in used tyres [93-95]. The ability for *Ae. albopictus* to lay eggs in large numbers and in varying substrates are factors that could lead to the establishment of the species [96]. However, these characteristics are common to many other container breeding mosquito species and are not unique to *Ae. albopictus.*

e) Limiting influences on the species' natural range.

Aedes albopictus is native to tropical and sub-tropical regions in south east Asia where it is known to exhibit traits specific to the region of origin. As evidenced by its global spread, it is now prevalent across a range of climates, with some strains being able to persist in colder environments by egg overwintering [80,97]. However, evidence suggests that a tropical strain could persist in temperate regions without being adapted for diapause (see section 3-f). Arid zones across central and western Australia are not optimal for establishment of populations, with an annual precipitation of at least 500 mm proposed as a threshold for maintenance of breeding places [98]. An additional limiting factor in Australia that may influence *Ae. albopictus* establishment is competition from native mosquitoes. Other native container breeding mosquitos from the genus *Aedes* are present across Australia and would directly compete with any *Ae. albopictus* population establishment – with a similar phenomenon documented for the close relative *Ae. aegypti* [99].

f) Is there an increased potential for feral population establishment if more individuals were present in Australia?

Aedes albopictus is not present in mainland Australia, and the importation of eggs or live mosquitos should be strictly limited to high containment research facilities with appropriate arthropod quarantine accreditation and specialised trained staff to prevent any environmental escapes. With these control measures in place, the establishment of feral populations in Australia would be extremely unlikely.

8. Potential impact of *Aedes albopictus* on Australia should it become established

a) Does the species have similar niche/living requirements to native species?

Aedes albopictus fills similar niches to a number of Australian native container breeding mosquitos including *Ae. notoscriptus* & *Ae. scutellaris* - as well as the introduced *Ae. aegypti.* In the unlikely event of an environment release, *Ae. albopictus* would be expected to compete for habitat against native mosquitos.

b) Is the species susceptible to, or capable of transmitting any pests or diseases?

Aedes albopictus is known to be competent to vector several arboviruses of significant human medical concern (Including, but not limited to; chikungunya, Ross River, dengue, and Zika virus') [100-109]. There is also evidence to suggest that *Ae. albopictus* can transmit the nematode *Dirofilaria sp.* overseas [110,111].

c) Probable prey/food sources - Does the species attack or prey on domestic or commercial animals or plants?

The female *Ae. albopictus* is known to bite and imbibe blood meals from human and mammalian hosts (including rats, cats, dogs, livestock), as well as avian hosts such as chickens [82,112]. Studies on the diel activity patterns of *Ae. albopictus* have reported that biting generally occurs during daytime hours, with peaks in the early morning and late afternoon [113].

d) Impacts on habitat and local environments.

Aedes albopictus would have minimal impact on habitat and local environments in Australia. This mosquito does not reduce ground vegetation, nor does it construct burrows or dig around waterways. It has not been documented to cause damage to native animal or plant habitats and communities or have any effect on forestry or agriculture. *Ae. albopictus* mosquitos do not have any impact on tree seedling regenerations, and do not spread weeds.

e) Are there any control/eradication programs that have or could be used in Australia?

Australia has a successful control program against *Ae. albopictus* that has been well implemented on Australian islands in the Torres Strait, actioned through partnerships with the Australian Commonwealth Department of Health and Queensland Health [114]. Queensland Health, in collaboration with local governments in Queensland, have also developed the "Queensland dengue management plan" which stipulates mosquito monitoring and control programs that include *Ae. albopictus.*

https://www.health.qld.gov.au/__data/assets/pdf_file/0022/444433/dengue-mgt-plan.pdf

f) Behaviours that cause environmental degradation.

Aedes albopictus has not been shown to cause any physical disturbance to the environment. There is no evidence to indicate *Ae. albopictus* disturbs wetland or wetland vegetation, and it does not pollute bodies of water. As *Ae. albopictus* tends to typically proliferate in small (often artificial) bodies of water, there is no indication that any of these would be adversely affected by introduction of *Ae. albopictus.*

g) Impacts on primary industries.

Aedes albopictus is not known to cause any livestock or poultry damage, however if nearby to breeding sites they could be targeted for blood meals. Male *Ae. albopictus* feed on plant nectar, however not to an appreciable level that would be noticeable or considered detrimental. Wild populations of *Ae. albopictus* would not compete in any way with livestock. This species is not expected to eat or damage any trees, shrubs or seedlings.

h) Damage to property.

Aedes albopictus is not capable of physically damaging or defacing property. The species would not damage fences or equipment of any description.

i) Is the species a social nuisance or danger?

There is a potential for *Ae. albopictus* to be a social nuisance by biting if present in large numbers nearby to humans. However, this interaction is no different to that of other endemic Australian mosquitoes.

j) Describe any potentially harmful characteristics of the species.

Aedes albopictus is known to bite humans to imbibe blood meals. This can lead to allergic reactions that generally include itching and inflammation at the bite site. The mosquito is also a competent vector to several arboviruses of human medical concern including chikungunya, dengue and Zika virus'. Topical mosquito repellents that are available in Australia would be expected to be effective against *Ae. albopictus.*

Aedes albopictus is also a suspected vector for *Dirofilaria sp*. overseas that can affect dogs [110,111]. However, through a variety of veterinary interventions dogs can be adequately protected [115].

9. Reduction of potential negative environmental impacts to Australia

Recommended restrictions on the import of *Ae. albopictus* including the limitation of importation for research institutes only. Further restrictions should also include the requirement for an import permit, and for the mosquitoes to be contained within high containment quarantine accredited facilities. Imported mosquitos (live and eggs) should only be handled by experienced staff that have completed and maintained quarantine accreditation training.

The CSIRO Australian Centre for Disease Preparedness (ACDP) – [Previously the Australian Animal Health Laboratory (AAHL)] already holds a number of endemic and imported mosquitoes in colony and has substantial experience in their rearing and containment.

10. Summary of proposed activities

The zoonotic and arboviral pathogens group, as members of the CSIRO Health and Biosecurity business unit, based at the CSIRO ACDP has a long and comprehensive history of completing assessments in mosquito genetics [116,117], mosquito behaviour [118,119], vector competence [120], host pathogen interactions [121], and infection studies [122]. We have a long-standing collaboration with the University of California, San Diego (UCSD) to develop genomic resources that will facilitate an understanding of *Ae. albopictus* molecular biology, allowing us to develop mosquito population suppression methods to combat emerging arboviral infections. Our collaboration with UCSD will be greatly enhanced by having access to live material and aims to develop Australia's capability to combat arboviral disease outbreaks globally.

As *Ae. albopictus* eggs can be viably desiccated for extended periods of time, we intend to import viable eggs under an approved permit from UCSD to our high containment quarantine facility. Eggs will be hatched, and juvenile states will be reared to emerge as adult to initiate a laboratory-reared colony. Males and females will be allowed to mate, produce eggs, and continue their lifecycle according to our well-established laboratory protocols. As stated, mosquitos will be used for research purposes only, with experiments including genetic studies, protein studies, and laboratory based behavioural and physiological studies. Once initial experiments are performed, there is also potential to examine host-pathogen interactions with a number of medically significant arboviruses – another field of expertise the CSIRO ACDP strongly excels in.

CSIRO ACDP has extensive experience in dealing with quarantine mosquito species (including under infection settings) and currently maintains a number of exotic and local mosquito species in our state-of-the-art quarantine accredited biosecurity insectary containment level 3 (BIC3) invertebrate facility. Access to this area of the

CSIRO ACDP is only available to people who have passed appropriate vetting and are suitably trained, or to visitors under strict supervision.

11. Guidelines on how species should be kept

Mosquitoes not native to Australia will be contained within a quarantine approved insectary. Live mosquitos are never imported, and *Ae. albopictus* eggs will be shipped dry in approved IATA packaging by an approved courier. Permits and shipping declarations will accompany shipment and on arrival will be assessed, with important documentation checked by specifically trained personnel, and logged into a secure database for auditing and traceability purposes.

Studies have shown that *Ae. albopictus* stick to short range flight, with one study reporting that released mosquitos dispersed less than 100 m [123]. Mosquitoes are typically kept in secure 30 cm^3 cages with a density of several hundred individuals per cage, which is adequate for them to fly and find resting surfaces. Cages of mosquitos will be manipulated secondarily contained in a cabinet enclosed with escape proof mesh within specific isolation rooms in quarantine approved insectary and are inspected frequently for damage. Selection of mosquitos should be performed with vacuum aspirators within these secondary containers to avoid escape. Mosquitoes cage density is measured using Density-Resting Surface (DRS) and the standard for most mosquitoes is 1.8cm^2 per mosquito [124], however for *Ae. albopictus* this can go as low as 0.9cm^2/mosquito. The cages at the CSIRO ACDP have a DRS of 3600cm^2, which could provide housing for 2000 mosquitoes, but on average, mosquito density does not exceed a maximum of 200-300 mosquitoes per cage. *Ae. albopictus* have a poor active dispersal ability by flight, often less than 300m [125]. As such our cages will still enable them room to fly. They also have a greater DSR per mosquito (12cm^2/mosquito) than the average required amount. The ideal ratio of males to females is 1:3. Reducing this ratio would limit breeding in females [124]. Female *Ae. albopictus* mosquitoes require a blood meal to produce eggs, by withholding blood meals (and only supplying a diet of 10% sugar solution) or oviposition sites within the cage, egg production will cease.

Aedes albopictus will be kept in specifically built facilities complying with OGTR, the Department of Agriculture, Water and the Environment (DAWE), and the Australian Standard 2243.3. The DAWE sets out requirements for how activities can be performed under an Approved Arrangement (AA). The facilities at the CSIRO ACDP contain AA sites used for research, analysis, and/or testing of imported biological material including micro-organisms, animal and human products. These facilities are inspected annually. The OGTR provide guidelines for certification of a PC2, PC3 and PC4 facilities issued pursuant to section 90 of the Gene Technology Act 2000 (the Act). Once a facility is certified, the certification instrument imposes conditions on the facility pursuant to section 86 of the Act. All certified facilities must be inspected before certification and annually. The arthropod facility where *Ae. albopictus* will be contained is classified as a PC3, class. 7.3 BIC3 facility (AA: V0275, OGTR Cert: 3570/2012(Var 10155)), and is located at the CSIRO ACDP, Geelong, Victoria. Mosquitoes and their eggs will be used for research only and destroyed at the completion of the project. Recovery of escaped specimens is primarily facilitated through the use of vacuum aspirators, light traps and air curtains; and a log of any escapee mosquito is kept. Sink traps fitted with 100-micron escape proof mesh are installed on all sinks and are frequently inspected. To further prevent escape, access to and from the laboratory is through designated antechambers, where mirrors and additional light traps are installed to ensure no escaped mosquitoes are transported. Prior to leaving the Insectary, personal protective equipment is removed, and each person inspected for mosquitoes. Vacuum aspirators are available to collect any escaped specimens. Further to this, the insectary maintains negative air pressure gradient with directional airflow into the laboratory to prevent the egress of mosquitoes. Air is exhausted from the Insectary through HEPA filters that are inspected and tested annually. Access to the Insectary is limited to authorised persons only and monitored via electronic access card. Any waste material generated throughout will be destroyed as per DAWE, OGTR and CSIRO ACDP guidelines, typically by two of the following methods: chemical treatment, freezing at -20°C for 48 hours, autoclaving at 121°C, filtering through 100-micron mesh, or high temperature incineration. Gaseous decontamination of entire laboratory space is completed through the use of formaldehyde or chlorine dioxide gas. All containment procedures at CSIRO ACDP are heavily audited annually and controlled through both internal and external sources.

12. State/Territory controls

There are no Commonwealth, state or territory legislative controls on *Ae. albopictus* other than quarantine legislation. *Ae. albopictus* must be added to the Department of Agriculture, Water and the Environment's "List of Specimens Suitable for Live Import (requiring an Import Permit)" before an appropriate import permit can be applied for. Import and containment conditions are stipulated on the import permit.

13. References

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