

Australia’s National  
Science Agency

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

12th May 2020

### Table of Contents

[**Table of Contents 3**](#_Toc39221652)

[**1. Taxonomy of species 4**](#_Toc39221653)

[**2. Status of species under CITES 4**](#_Toc39221654)

[**3. Ecology of the Species 5**](#_Toc39221655)

[**4. Reproductive Biology of Species 11**](#_Toc39221656)

[**5. Feral Population establishment 13**](#_Toc39221657)

[**6. Environmental risk assessment for *Aedes albopictus* 14**](#_Toc39221658)

[**7. Likelihood of the species to establish a breeding population in Australia 15**](#_Toc39221659)

[**8. Potential impact of *Aedes albopictus* on Australia should it become established 17**](#_Toc39221660)

[**9. Reduction of potential negative environmental impacts to Australia 19**](#_Toc39221661)

[**10. Summary of proposed activities 20**](#_Toc39221662)

[**11. Guidelines on how species should be kept 21**](#_Toc39221663)

[**12. State/Territory controls 21**](#_Toc39221664)

[**13. References 24**](#_Toc39221665)

### Taxonomy of species

1. Family Name: Culicidae
2. Genus Name: *Aedes\**
3. Species: *albopictus*
4. Subspecies: No known subspecies of *Ae. albopictus* has been previously described
5. Common name: Asian tiger mosquito
6. 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)

### 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).

### Ecology of the Species

1. 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

1. 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.

1. 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).

*A insect on the ground

Description automatically generated*A picture containing animal, arthropod

Description automatically generated

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).

*A insect on the ground

Description automatically generatedA close up of a flower

Description automatically generated*

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.

A picture containing sky

Description automatically generated

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).

A picture containing object

Description automatically generated

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*.

1. 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.

1. 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.

1. 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.

1. Does the species have the ability to breathe atmospheric air?

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

1. 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.

1. Social behaviour or groupings.

*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 meal28. However, mosquitos are usually largely solitary throughout their life stages.

1. Is this species ever territorial or does it exhibit aggressive behaviour?

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

1. Characteristics that may cause harm to humans or any other species.

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.

### Reproductive Biology of Species

Reproductive characteristics

1. 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.

1. 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.

1. How frequently does breeding occur?

*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.

1. Can individuals of the species change sex?

Mosquitoes are not known to change sex.

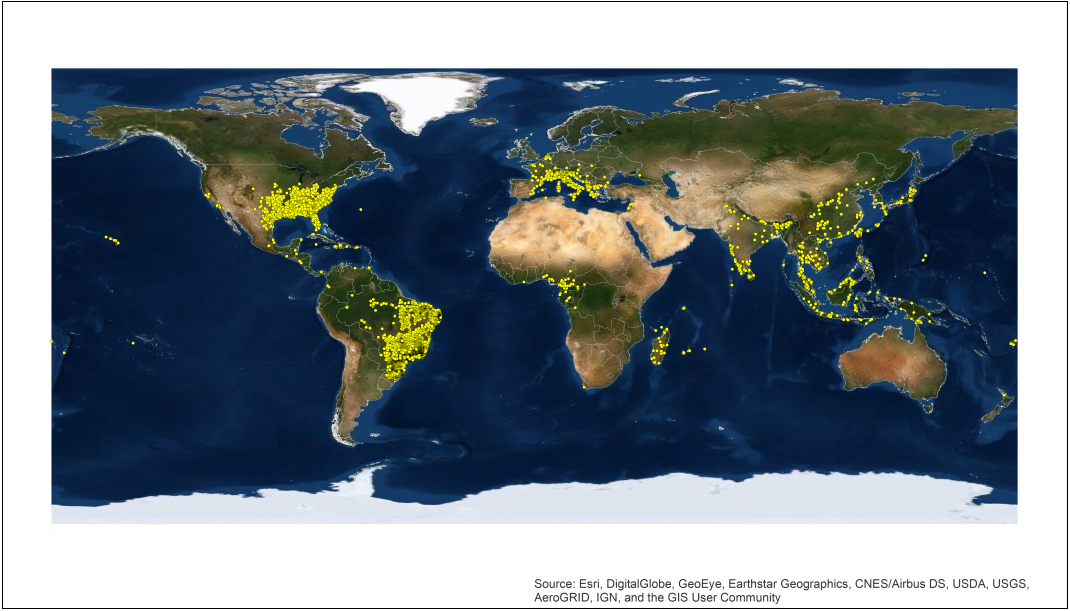
1. Ability of the species to hybridise. Describe any known crosses. Are progeny of such crosses fertile?

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.

1. 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.
2. Are individuals single sexed?  
   All mosquitoes are single-sexed but very rarely gynandromorphs have been observed in the laboratory 38,39.

### Feral Population establishment

1. 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.

1. 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.

1. 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 interceptionsin the ports of Darwin, Townsville, Cairns, Brisbane, Sydney and Melbourne, Australia64,68,69, and Auckland, New Zealand 70.

### 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 [[1]](#footnote-2). 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.

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.

### Likelihood of the species to establish a breeding population in Australia

1. 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.
2. 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.

1. 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.
2. 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.*
3. 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.
4. 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.

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

1. 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.

1. 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.* overseas110,111*.*

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.

1. 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.
2. 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>

1. 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.*

1. 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.

1. 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.

1. 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.

1. 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.

### 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.

### 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.

### 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 cm3 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 1.8cm2 per mosquito 124, however for *Ae. albopictus* this can go as low as 0.9cm2/mosquito. The cages at the CSIRO ACDP have a DRS of 3600cm2, 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 (12cm2/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.

### 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.

### References

1 Huang, Y.-M. Neotype designation for Aedes (Stegomyia) albopictus (Skuse) (Diptera: Culicidae). *Proceedings of the Entomological Society of Washington* **70**, 297-302, doi:10.5281/zenodo.163519 (1968).

2 Reinert, J. F., Harbach, R. E. & Kitching, I. J. Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. *Zoological Journal of the Linnean Society* **142**, 289-368, doi:10.1111/j.1096-3642.2004.00144.x (2004).

3 Weaver, S. Journal policy on names of aedine mosquito genera and subgenera. *Am J Trop Med Hyg* **73**, 481, doi:10.4269/ajtmh.2005.73.481 (2005).

4 Bellini, R. *et al.* Dispersal and survival of Aedes albopictus (Diptera: Culicidae) males in Italian urban areas and significance for sterile insect technique application. *J Med Entomol* **47**, 1082-1091, doi:10.1603/me09154 (2010).

5 Hawley, W. A. The biology of Aedes albopictus. *J Am Mosq Control Assoc Suppl* **1**, 1-39 (1988).

6 Gubler, D. J. Competitive Displacement of Aedes (Stegomyia) Polynesiensis Marks by Aedes (Stegomyia) Albopictus Skuse in Laboratory Populations1. *Journal of Medical Entomology* **7**, 229-235, doi:10.1093/jmedent/7.2.229 %J Journal of Medical Entomology (1970).

7 Nicholson, J. *The potential colonisation and establishment of Aedes albopictus (Diptera: Culicidae) as an arbovirus vector in eastern Australia.* PhD thesis, The University of Queensland, (2012).

8 Lounibos, L. P. *et al.* Does temperature affect the outcome of larval competition between Aedes aegypti and Aedes albopictus? *J Vector Ecol* **27**, 86-95 (2002).

9 Davis, T. *Oviposition Strategies of Aedes Albopictus (Skuse) (Diptera Culicidae): Analyzing Behavioral Patterns for Surveillance Techniques and Control Tactics* PhD thesis, University of Florida, (2013).

10 Huang, Y. M. Contributions to the mosquito fauna of Southeast Asia. XIV. The subgenus Stegomyia of Aedes in Southeast Asia I-The scutellaris group of species. *Contributions of the American Entomological Institute* **9**, 1-110 (1972).

11 Tanaka, K., Mizusawa, K. & Saugstad, E. S. A revision of the adult and larval mosquitoes of Japan (Including the Ryukyn Archipelago and the Ogasawa islands) and Korea (Diptera: Culicidae). *Contributions of the American Entomological Institute* **16** (1979).

12 Beebe, N. W. *et al.* A Polymerase Chain Reaction-Based Diagnostic to Identify Larvae and Eggs of Container Mosquito Species from the Australian Region. *Journal of Medical Entomology* **44**, 376-380, doi:10.1093/jmedent/44.2.376 (2007).

13 Hill, L. A. *et al.* Rapid Identification of Aedes albopictus, Aedes scutellaris, and Aedes aegypti Life Stages Using Real-time Polymerase Chain Reaction Assays. **79**, 866-875, doi:10.4269/ajtmh.2008.79.866 (2008).

14 Teo, C. H. J., Lim, P. K. C., Voon, K. & Mak, J. W. Detection of dengue viruses and Wolbachia in Aedes aegypti and Aedes albopictus larvae from four urban localities in Kuala Lumpur, Malaysia. *Tropical Biomedicine* **34**, 583-597 (2017).

15 Marten, G. A survey of cyclopoid copepods for control of Aedes albopictus larvae. *Bulletin of the Society of Vector Entomology* **14** (1989).

16 Reiter, P. & Sprenger, D. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. *J Am Mosquito Contr* **3**, 494-501 (1987).

17 Honório, N. A. *et al.* Dispersal of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil. *J Memórias do Instituto Oswaldo Cruz* **98**, 191-198, doi:10.1590/s0074-02762003000200005 (2003).

18 Eritja, R., Palmer, J. R. B., Roiz, D., Sanpera-Calbet, I. & Bartumeus, F. Direct Evidence of Adult Aedes albopictus Dispersal by Car. *Scientific Reports* **7**, 14399, doi:10.1038/s41598-017-12652-5 (2017).

19 Hawley, W. A., Pumpuni, C. B., Brady, R. H. & Craig, G. B., Jr. Overwintering survival of Aedes albopictus (Diptera: Culicidae) eggs in Indiana. *J Med Entomol* **26**, 122-129, doi:10.1093/jmedent/26.2.122 (1989).

20 Ho, B., Chan, K. & Chan, Y. in *Vector control in southeast Asia* 125-143 (University of Singapore Press, 1973).

21 Surendran, S. *et al.* Salinity tolerant Aedes aegypti and Ae. albopictus-Infection with dengue virus and contribution to dengue transmission in a coastal peninsula. *J Vector Borne Disease* **55**, 26-33, doi:10.4103/0972-9062.234623 (2018).

22 Medeiros-Sousa, A. *et al.* Influence of physical chemical water parameters on abundance and presence of the arbovirus vector Aedes Albopictus (Dipteria: Culicidae) in Larval habitats of forest fragments. *Revue d'Épidémiologie et de Santé Publique* **66**, S393, doi:10.1016/j.respe.2018.05.428 (2018).

23 Reinhold, J. M., Lazzari, C. R. & Lahondère, C. Effects of the Environmental Temperature on Aedes aegypti and Aedes albopictus Mosquitoes: A Review. *Insects* **9**, 158, doi:10.3390/insects9040158 (2018).

24 Delatte, H., Gimonneau, G., Triboire, A. & Fontenille, D. Influence of Temperature on Immature Development, Survival, Longevity, Fecundity, and Gonotrophic Cycles of Aedes albopictus, Vector of Chikungunya and Dengue in the Indian Ocean. *Journal of Medical Entomology* **46**, 33-41, doi:10.1603/033.046.0105 (2009).

25 Teng, H.-J. & Apperson, C. S. Development and Survival of Immature Aedes albopictus and Aedes triseriatus (Diptera: Culicidae) in the Laboratory: Effects of Density, Food, and Competition on Response to Temperature. *Journal of Medical Entomology* **37**, 40-52, doi:10.1603/0022-2585-37.1.40 (2000).

26 Ponlawat, A. & Harrington, L. C. Blood Feeding Patterns of Aedes aegypti and Aedes albopictus in Thailand. *Journal of Medical Entomology* **42**, 844-849, doi:10.1093/jmedent/42.5.844 (2005).

27 Richards, S. L., Ponnusamy, L., Unnasch, T. R., Hassan, H. K. & Apperson, C. S. Host-Feeding Patterns of Aedes albopictus (Diptera: Culicidae) in Relation to Availability of Human and Domestic Animals in Suburban Landscapes of Central North Carolina. *Journal of Medical Entomology* **43**, 543-551, doi:10.1093/jmedent/43.3.543 %J Journal of Medical Entomology (2006).

28 Oliva, C. F., Damiens, D. & Benedict, M. Q. Male reproductive biology of Aedes mosquitoes. *Acta Trop* **132** S12-19, doi:10.1016/j.actatropica.2013.11.021 (2014).

29 Gratz, N. G. Critical review of the vector status of Aedes albopictus. *Med Vet Entomol* **18**, 215-227, doi:10.1111/j.0269-283X.2004.00513.x (2004).

30 Pluskota, B., Storch, V., Braunbeck, T., Beck, M. & Becker, N. First record of Stegomyia albopicta (Skuse)(Diptera: Culicidae) in Germany. *European Mosquito Bulletin* **26**, 1-5 (2008).

31 Chambers, G. M. & Klowden, M. J. Nutritional reserves of autogenous and anautogenous selected strains of Aedes albopictus (Diptera: Culicidae). *J Med Entomol* **31**, 554-560, doi:10.1093/jmedent/31.4.554 (1994).

32 Sprenger, D. & Wuithiranyagool, T. The discovery and distribution of Aedes albopictus in Harris County, Texas. *J Am Mosq Control Assoc* **2**, 217-219 (1986).

33 Mori, A. Effects of larval density and nutrition on some attributes of immature and adult Aedes albopictus. *Tropical Medicine* **21**, 85-103 (1979).

34 Fonseca, D. M., Kaplan, L. R., Heiry, R. A. & Strickman, D. Density-Dependent Oviposition by Female Aedes albopictus (Diptera: Culicidae) Spreads Eggs Among Containers During the Summer but Accumulates Them in the Fall. *Journal of Medical Entomology* **52**, 705-712, doi:10.1093/jme/tjv060 (2015).

35 Del Rosario, A. Studies on the biology of Philippine mosquitoes. II. Observations on the life and behaviour of Aedes albopictus (Skuse) in the laboratory. *Philippine Journal of Science* **92**, 80-103 (1963).

36 Sota, T. & Mogi, M. Interspecific variation in desiccation survival time of Aedes (Stegomyia) mosquito eggs is correlated with habitat and egg size. *Oecologia* **90**, 353-358, doi:10.1007/BF00317691 (1992).

37 Nicholson, J., Ritchie, S. A., Russell, R. C., Zalucki, M. P. & Van Den Hurk, A. F. Ability for Aedes albopictus (Diptera: Culicidae) to Survive at the Climatic Limits of Its Potential Range in Eastern Australia. *Journal of Medical Entomology* **51**, 948-957, doi:10.1603/ME14079 (2014).

38 Brust, R. A. Gynandromorphs and Intersexes in Mosquitoes (Diptera: Culicidae). *Canadian Journal of Zoology* **44**, 911-921, doi:10.1139/z66-093 (1966).

39 Jupp, P. G. Aberrant South African mosquitoes (Diptera: Culicidae): gynandromorphs and morphologic variants. *J Am Mosq Control Assoc* **14**, 470-471 (1998).

40 Caminade, C. *et al.* Suitability of European climate for the Asian tiger mosquito Aedes albopictus: Recent trends and future scenarios. *Journal of the Royal Society Interface* **9**, 2708-2717, doi:10.1098/rsif.2012.0138 (2012).

41 Ogata, K. & Samayoa, A. L. Discovery of Aedes albopictus in Guatemala. *J Am Mosquito Contr* **12**, 503-506 (1996).

42 Torres-Avendaño, J. I. *et al.* First Record of Aedes albopictus in Sinaloa, Mexico *J Am Mosquito Contr* **31**, 164-166, doi:10.2987/14-6461r (2015).

43 Worcester, D. J. & Bonnet, D. D. The Dispersal of Aedes Albopictus in the Territory of Hawaii. *The American Journal of Tropical Medicine and Hygiene* **s1-26**, 465-476, doi:10.4269/ajtmh.1946.s1-26.465 (1946).

44 Moore, C. G. & Mitchell, C. J. Aedes albopictus in the United States: Ten-Year Presence and Public Health Implications. *Emerging Infectious Diseases* **3**, 329-334, doi:10.3201/eid0303.970309 (1997).

45 Giordano, B. V. *et al.* Discovery of an Aedes (Stegomyia) albopictus population and first records of Aedes (Stegomyia) aegypti in Canada. *Med Vet Entomol* **34**, doi:10.1111/mve.12408 (2020).

46 Romi, R. Aedes albopictus in italy: Sanitary impact ten years after the first report. *Giornale Italiano di Medicina Tropicale* **4**, 69-73 (1999).

47 Schaffner, F. & Karch, S. First record of Aedes albopictus (Skuse, 1894) in metropolitan France. *Cr Acad Sci Iii-Vie* **323**, 373-375, doi:Doi 10.1016/S0764-4469(00)00143-8 (2000).

48 Girod, R. First record of Aedes Albopictus in Mayotte Island, comoros archipelago. *Parasite* **11**, 74-74 (2004).

49 Schaffner, F., Van Bortel, W. & Coosemans, M. First record of Aedes (Stegomyia) albopictus in Belgium. *J Am Mosquito Contr* **20**, 201-203 (2004).

50 Aranda, C., Eritja, R. & Roiz, D. First record and establishment of the mosquito Aedes albopictus in Spain. *Med Vet Entomol* **20**, 150-152, doi:DOI 10.1111/j.1365-2915.2006.00605.x (2006).

51 Klobucar, A., Merdic, E., Benic, N., Baklaic, Z. & Krcmar, S. First record of Aedes albopictus in Croatia. *J Am Mosquito Contr* **22**, 147-148, doi:10.2987/8756-971x(2006)22[147:Froaai]2.0.Co;2 (2006).

52 Coffinet, T. *et al.* First record of Aedes albopictus in Gabon. *J Am Mosquito Contr* **23**, 471-472, doi:10.2987/5636.1 (2007).

53 Ortega-Morales, A. I. *et al.* First Record of Stegomyia albopicta [Aedes albopictus] In Belize. *Southwest Entomol* **35**, 197-198, doi:10.3958/059.035.0208 (2010).

54 Bockova, E., Kocisova, A. & Letkova, V. First record of Aedes albopictus in Slovakia. *Acta Parasitol* **58**, 603-606, doi:10.2478/s11686-013-0158-2 (2013).

55 Adeleke, M. A. *et al.* Twenty-three years after the first record of Aedes albopictus in Nigeria: its current distribution and potential epidemiological implications. *Afr Entomol* **23**, 348-355, doi:10.4001/003.023.0203 (2015).

56 Rubio-Palis, Y. *et al.* First record of Aedes (Stegomyia) albopictus (Skuse) (Diptera: Culicidae) in Bolivar State and epidemiological implications. *B Malariol Salud Amb* **55**, 110-112 (2015).

57 Doosti, S. *et al.* Mosquito Surveillance and the First Record of the Invasive Mosquito Species Aedes (Stegomyia) albopictus (Skuse) (Diptera: Culicidae) in Southern Iran. *Iran J Public Health* **45**, 1064-1073 (2016).

58 Ortega-Morales, A. I. & Rodriguez, Q. K. S. First record of Aedes albopictus (Diptera: Culicidae) in San Luis Potosi, Mexico. *J Vector Ecol* **41**, 314-315, doi:10.1111/jvec.12229 (2016).

59 Bennouna, A. *et al.* First record of Stegomyia albopicta (= Aedes albopictus) in Morocco: a major threat to public health in North Africa? *Med Vet Entomol* **31**, 102-106, doi:10.1111/mve.12194 (2017).

60 Goenaga, S. *et al.* Expansion of the Distribution of Aedes albopictus (Diptera: Culicidae): New Records in Northern Argentina and Their Implications From an Epidemiological Perspective. *J Med Entomol*, doi:10.1093/jme/tjaa009 (2020).

61 Giordano, B. V. *et al.* Discovery of an Aedes (Stegomyia) albopictus population and first records of Aedes (Stegomyia) aegypti in Canada. *Med Vet Entomol* **34**, 10-16, doi:10.1111/mve.12408 (2020).

62 Bonizzoni, M., Gasperi, G., Chen, X. & James, A. A. The invasive mosquito species Aedes albopictus: Current knowledge and future perspectives. *Trends in Parasitology* **29**, 460-468, doi:10.1016/j.pt.2013.07.003 (2013).

63 Ritchie, S. A. *et al.* Discovery of a Widespread Infestation of Aedes albopictus in the Torres Strait, Australia. *J Am Mosquito Contr* **22**, 358-365, doi:10.2987/8756-971x(2006)22[358:doawio]2.0.co;2 (2006).

64 van den Hurk, A. F. *et al.* Ten years of the Tiger: Aedes albopictus presence in Australia since its discovery in the Torres Strait in 2005. *One Health* **2**, 19-24, doi:10.1016/j.onehlt.2016.02.001 (2016).

65 Kraemer, M. U. G. *et al.* Data from: The global compendium of Aedes aegypti and Ae. albopictus occurance. . doi:10.5061/dryad.47v3c (2017).

66 Unlu, I. *et al.* Area-wide management of Aedes albopictus: choice of study sites based on geospatial characteristics, socioeconomic factors and mosquito populations. *Pest Manag Sci* **67**, 965-974, doi:10.1002/ps.2140 (2011).

67 Fonseca, D. M. *et al.* Area-wide management of Aedes albopictus. Part 2: gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. *Pest Manag Sci* **69**, 1351-1361, doi:10.1002/ps.3511 (2013).

68 Kay, B. H. *et al.* Is *Aedes albopictus* in Australia? *The Medical Journal of Australia* **153**, 31-34, doi:10.5694/j.1326-5377.1990.tb125460.x (1990).

69 Nguyen, H., Whelan, P., Finlay-Doney, M. & Soong, S. Y. Interceptions of Aedes aegypti and Aedes albopictus in the port of Darwin, NT, Australia, 25 January and 5 February 2010. *The Northern Territory Disease Control Bulletin* **17** (2010).

70 Holder, P. *et al.* A Biosecurity Response to Aedes albopictus (Diptera: Culicidae) in Auckland, New Zealand. *Journal of Medical Entomology* **47**, 600-609 (2010).

71 Straetemans, M. Vector-related risk mapping of the introduction and establishment of Aedes albopictus in Europe. *Euro Surveill* **13** (2008).

72 Kamal, M., Kenawy, M. A., Rady, M. H., Khaled, A. S. & Samy, A. M. Mapping the global potential distributions of two arboviral vectors Aedes aegypti and Ae. albopictus under changing climate. *Plos One* **13**, e0210122, doi:10.1371/journal.pone.0210122 (2018).

73 Ding, F., Fu, J., Jiang, D., Hao, M. & Lin, G. Mapping the spatial distribution of Aedes aegypti and Aedes albopictus. *Acta Trop* **178**, 155-162, doi:10.1016/j.actatropica.2017.11.020 (2018).

74 Leta, S. *et al.* Global risk mapping for major diseases transmitted by Aedes aegypti and Aedes albopictus. *Int J Infect Dis* **67**, 25-35, doi:10.1016/j.ijid.2017.11.026 (2018).

75 Dickens, B. L., Sun, H. Y., Jit, M., Cook, A. R. & Carrasco, L. R. Determining environmental and anthropogenic factors which explain the global distribution of Aedes aegypti and Ae. albopictus. *Bmj Glob Health* **3**, doi:10.1136/bmjgh-2018-000801 (2018).

76 Shabani, F., Shafapour Tehrany, M., Solhjouy-Fard, S. & Kumar, L. A comparative modeling study on non-climatic and climatic risk assessment on Asian Tiger Mosquito (Aedes albopictus). *PeerJ* **6**, e4474, doi:10.7717/peerj.4474 (2018).

77 Medlock, J. M., Avenell, D., Barrass, I. & Leach, S. Analysis of the potential for survival and seasonal activity of Aedes albopictus (Diptera: Culicidae) in the United Kingdom. *J Vector Ecol* **31**, 292-304, doi:10.3376/1081-1710(2006)31[292:aotpfs]2.0.co;2 (2006).

78 Benedict, M. Q., Levine, R. S., Hawley, W. A. & Lounibos, L. P. Spread of the tiger: global risk of invasion by the mosquito Aedes albopictus. *Vector Borne Zoonotic Dis* **7**, 76-85, doi:10.1089/vbz.2006.0562 (2007).

79 Thomas, S. M., Tjaden, N. B., van den Bos, S. & Beierkuhnlein, C. Implementing Cargo Movement into Climate Based Risk Assessment of Vector-Borne Diseases. *Int J Env Res Pub He* **11**, 3360-3374, doi:10.3390/ijerph110303360 (2014).

80 Cunze, S., Kochmann, J., Koch, L. K. & Klimpel, S. Aedes albopictus and Its Environmental Limits in Europe. *Plos One* **11**, doi:10.1371/journal.pone.0162116 (2016).

81 Caminade, C. *et al.* Suitability of European climate for the Asian tiger mosquito Aedes albopictus: recent trends and future scenarios. *J R Soc Interface* **9**, 2708-2717, doi:10.1098/rsif.2012.0138 (2012).

82 Richards, S. L., Ponnusamy, L., Unnasch, T. R., Hassan, H. K. & Apperson, C. S. Host-feeding patterns of Aedes albopictus (Diptera: Culicidae) in relation to availability of human and domestic animals in suburban landscapes of central North Carolina. *J Med Entomol* **43**, 543-551, doi:10.1603/0022-2585(2006)43[543:hpoaad]2.0.co;2 (2006).

83 Tran, A. *et al.* A Rainfall- and Temperature-Driven Abundance Model for Aedes albopictus Populations. *Int J Env Res Pub He* **10**, 1698-1719, doi:10.3390/ijerph10051698 (2013).

84 Vitek, C. J. & Livdahl, T. Hatch plasticity in response to varied inundation frequency in Aedes albopictus. *J Med Entomol* **46**, 766-771, doi:10.1603/033.046.0406 (2009).

85 Romi, R., Severini, F. & Toma, L. Cold acclimation and overwintering of female Aedes albopictus in Roma. *J Am Mosquito Contr* **22**, 149-151, doi:Doi 10.2987/8756-971x(2006)22[149:Caaoof]2.0.Co;2 (2006).

86 Tsuda, Y. & Takagi, M. Survival and development of Aedes aegypti and Aedes albopictus (Diptera : Culicidae) larvae under a seasonally changing environment in Nagasaki, Japan. *Environ Entomol* **30**, 855-860, doi:10.1603/0046-225x-30.5.855 (2001).

87 Sota, T. & Mogi, M. Survival-Time and Resistance to Desiccation of Diapause and Nondiapause Eggs of Temperature Aedes (Stegomyia) Mosquitos. *Entomol Exp Appl* **63**, 155-161, doi:10.1111/j.1570-7458.1992.tb01570.x (1992).

88 Thomas, S. M., Obermayr, U., Fischer, D., Kreyling, J. & Beierkuhnlein, C. Low-temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, Aedes albopictus (Diptera: Culicidae). *Parasit Vectors* **5**, 100, doi:10.1186/1756-3305-5-100 (2012).

89 Oliva, C. F., Damiens, D., Vreysen, M. J. B., Lemperière, G. & Gilles, J. Reproductive Strategies of Aedes albopictus (Diptera: Culicidae) and Implications for the Sterile Insect Technique. *PLOS ONE* **8**, e78884, doi:10.1371/journal.pone.0078884 (2013).

90 Boyer, S., Toty, C., Jacquet, M., Lempérière, G. & Fontenille, D. Evidence of Multiple Inseminations in the Field in Aedes albopictus. *PLOS ONE* **7**, e42040, doi:10.1371/journal.pone.0042040 (2012).

91 Kraemer, M. U. G. *et al.* The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. *Elife* **4**, doi:10.7554/eLife.08347 (2015).

92 Dieng, H. *et al.* Indoor-Breeding of Aedes albopictus in Northern Peninsular Malaysia and Its Potential Epidemiological Implications. *Plos One* **5**, doi:10.1371/journal.pone.0011790 (2010).

93 Seng, C. M. & Jute, N. Breeding of Aedes aegypti (L.) and Aedes albopictus (Skuse) in urban housing of Sibu town, Sarawak. *Southeast Asian J Trop Med Public Health* **25**, 543-548 (1994).

94 Ramasamy, R., Surendran, S. N., Jude, P. J., Dharshini, S. & Vinobaba, M. Larval development of Aedes aegypti and Aedes albopictus in peri-urban brackish water and its implications for transmission of arboviral diseases. *PLoS Negl Trop Dis* **5**, e1369, doi:10.1371/journal.pntd.0001369 (2011).

95 Grist, N. R. Aedes-Albopictus - the Tyre-Traveling Tiger. *J Infection* **27**, 1-4, doi:Doi 10.1016/0163-4453(93)93418-4 (1993).

96 Day, J. F. Mosquito Oviposition Behavior and Vector Control. *Insects* **7**, doi:10.3390/insects7040065 (2016).

97 Medlock, J. M. *et al.* An entomological review of invasive mosquitoes in Europe. *B Entomol Res* **105**, 637-663, doi:10.1017/S0007485315000103 (2015).

98 Roiz, D., Neteler, M., Castellani, C., Arnoldi, D. & Rizzoli, A. Climatic Factors Driving Invasion of the Tiger Mosquito (Aedes albopictus) into New Areas of Trentino, Northern Italy. *Plos One* **6**, doi:10.1371/journal.pone.0014800 (2011).

99 Russell, R. C. Larval Competition between the Introduced Vector of Dengue Fever in Australia, Aedes-Aegypti (L), and a Native Container-Breeding Mosquito, Aedes-Notoscriptus (Skuse) (Diptera, Culicidae). *Aust J Zool* **34**, 527-534, doi:10.1071/Zo9860527 (1986).

100 Bowers, D. F., Abell, B. A. & Brown, D. T. Replication and Tissue Tropism of the Alphavirus Sindbis in the Mosquito Aedes albopictus. *Virology* **212**, 1-12, doi:10.1006/viro.1995.1447 (1995).

101 De Castro, M. G., Nogueira, R. M. R., Schatzmayr, H. G., Miagostovich, M. P. & Lourenço-de-Oliveira, R. Dengue virus detection by using reverse transcription-polymerase chain reaction in Saliva and progeny of experimentally infected Aedes albopictus from Brazil. *Memorias do Instituto Oswaldo Cruz* **99**, 809-814, doi:10.1590/s0074-02762004000800005 (2004).

102 Gerhardt, R. R. *et al.* First isolation of La Crosse virus from naturally infected Aedes albopictus. *Emerging Infectious Diseases* **7**, 807-811, doi:10.3201/eid0705.017506 (2001).

103 Gutiérrez-López, R. *et al.* Vector competence of aedes caspius and ae. Albopictus mosquitoes for zika virus, Spain. *Emerging Infectious Diseases* **25**, 346-348, doi:10.3201/eid2502.171123 (2019).

104 Holick, J., Kyle, A., Ferraro, W., Delaney, R. R. & Iwaseczko, M. Discovery of Aedes albopictus infected with west nile virus in southeastern Pennsylvania. *J Am Mosquito Contr* **18**, 131 (2002).

105 Turell, M. J. & Beaman, J. R. Experimental transmission of Venezuelan equine encephalomyelitis virus by a strain of Aedes albopictus (Diptera: Culicidae) from New Orleans, Louisiana. *Journal of medical entomology* **29**, 802-805, doi:10.1093/jmedent/29.5.802 (1992).

106 Turell, M. J., Beaman, J. R. & Tammariello, R. F. Susceptibility of selected strains of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) to chikungunya virus. *Journal of medical entomology* **29**, 49-53, doi:10.1093/jmedent/29.1.49 (1992).

107 Mitchell, C. J., Miller, B. R. & Gubler, D. J. Vector competence of Aedes albopictus from Houston, Texas, for dengue serotypes 1 to 4, yellow fever and Ross River viruses. *J Am Mosquito Contr* **3**, 460-465 (1987).

108 Vega-Rua, A., Zouache, K., Girod, R., Failloux, A. B. & Lourenco-de-Oliveira, R. High Level of Vector Competence of Aedes aegypti and Aedes albopictus from Ten American Countries as a Crucial Factor in the Spread of Chikungunya Virus. *J Virol* **88**, 6294-6306, doi:10.1128/Jvi.00370-14 (2014).

109 Mitchell, C. J. *et al.* Isolation of eastern equine encephalitis virus from Aedes albopictus in Florida. *Science* **257**, 526-527, doi:10.1126/science.1321985 (1992).

110 Cancrini, G. *et al.* First finding of Dirofilaria repens in a natural population of Aedes albopictus. *Med Vet Entomol* **17**, 448-451, doi:10.1111/j.1365-2915.2003.00463.x (2003).

111 Cancrini, G. *et al.* *Aedes albopictus* is a natural vector of *Dirofilaria immitis* in Italy. *Veterinary Parasitology* **118**, 195-202, doi:doi.org/10.1016/j.vetpar.2003.10.011 (2003).

112 Niebylski, M. L., Savage, H. M., Nasci, R. S. & Craig, G. B., Jr. Blood hosts of Aedes albopictus in the United States. *J Am Mosq Control Assoc* **10**, 447-450 (1994).

113 Nunes de Lima-Camara, T. Activity patterns of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) under natural and artificial conditions. *Oecological Australia* **14**, 737-744, doi:10.4257/oeco.2010.1403.09 (2010).

114 Muzari, M. O. *et al.* Holding back the tiger: Successful control program protects Australia from Aedes albopictus expansion. *PLoS Negl Trop Dis* **11**, e0005286, doi:10.1371/journal.pntd.0005286 (2017).

115 Hoch, H. & Strickland, K. Canine and feline dirofilariasis: prophylaxis, treatment, and complications of treatment. *Compend Contin Educ Vet* **30**, 146-151; quiz 151-142 (2008).

116 Buchman, A. *et al.* Engineered resistance to Zika virus in transgenic Aedes aegypti expressing a polycistronic cluster of synthetic small RNAs. *Proc Natl Acad Sci U S A* **116**, 3656-3661, doi:10.1073/pnas.1810771116 (2019).

117 Buchman, A. *et al.* Broad dengue neutralization in mosquitoes expressing an engineered antibody. *PLoS Pathog* **16**, e1008103, doi:10.1371/journal.ppat.1008103 (2020).

118 Gaburro, J. *et al.* Neurotropism and behavioral changes associated with Zika infection in the vector Aedes aegypti. *Emerg Microbes Infect* **7**, 68, doi:10.1038/s41426-018-0069-2 (2018).

119 Gaburro, J. *et al.* Dengue virus infection changes Aedes aegypti oviposition olfactory preferences. *Sci Rep* **8**, 13179, doi:10.1038/s41598-018-31608-x (2018).

120 Duchemin, J. B. *et al.* Zika vector transmission risk in temperate Australia: a vector competence study. *Virol J* **14**, 108, doi:10.1186/s12985-017-0772-y (2017).

121 Haqshenas, G. *et al.* A Role for the Insulin Receptor in the Suppression of Dengue Virus and Zika Virus in Wolbachia-Infected Mosquito Cells. *Cell Rep* **26**, 529-535 e523, doi:10.1016/j.celrep.2018.12.068 (2019).

122 Vedururu, R. K. *et al.* RNASeq Analysis of Aedes albopictus Mosquito Midguts after Chikungunya Virus Infection. *Viruses* **11**, doi:10.3390/v11060513 (2019).

123 Estrada-Franco, J. G. & George B. Craig, J. Biology, Disease Relationships, and Control of Aedes albopictus. *Pan American Sanitary Bureau, Regional office of the World Health Organisation* (1995).

124 Zhang, D. J. *et al.* Establishment of a medium-scale mosquito facility: tests on mass production cages for Aedes albopictus (Diptera: Culicidae). *Parasite Vector* **11**, doi:10.1186/s13071-018-2750-7 (2018).

125 Minard, G. *et al.* Shared larval rearing environment, sex, female size and genetic diversity shape Ae. albopictus bacterial microbiota. *Plos One* **13**, doi:10.1371/journal.pone.0194521 (2018).

1. [www.ecdc.europa.eu/en/publications-data/environmental-risk-mapping-aedes-albopictus-europe-proof-concept-study-european](http://www.ecdc.europa.eu/en/publications-data/environmental-risk-mapping-aedes-albopictus-europe-proof-concept-study-european) [↑](#footnote-ref-2)