Australian Government
Department of Agriculture, Water and the Environment

Department Risk Analysis

Application to add *Aedes albopictus* (Asian Tiger Mosquito) to the Environment Protection and Biodiversity Conservation Act 1999 *List of Specimens taken to be Suitable for Live Import*

August 2021

Introduction

Purpose of the proposed import

The purpose of the application is to allow the importation of Asian Tiger Mosquitoes (*Aedes albopictus)* to the Commonwealth Scientific and Industrial Research Organisation's (CSIRO) Australian Centre for Disease Preparedness (ACDP), formerly the Australian Animal Health Laboratories. The CSIRO ACDP has a long comprehensive record of completing assessments in mosquito genetics, mosquito behaviour, vector competence, host pathogen interactions, and infection studies.

The Asian Tiger Mosquito 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). There is also evidence overseas to suggest that the Asian Tiger Mosquito can transmit the dog nematode *Dirofilaria* sp.

The mosquito will be used for research purposes and will be held in a Quarantine Accredited Biosecurity Insectary Containment level 3 (BIC3) invertebrate facility. CSIRO ACDP has a long-lasting collaboration with the University of California, San Diego (UCSD) to develop genomic resources that will facilitate an understanding of the Asian Tiger Mosquito's molecular biology, allowing the research partners to develop mosquito population suppression methods to combat emerging arboviral infections. The collaboration between the CSIRO ACDP and the UCSD will be greatly enhanced by having access to live material and aims to develop Australia's capability to combat arboviral disease outbreaks globally.

Background

Under s.303EC of the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act), the responsible Minister may amend the *List of Specimens taken to be suitable for live import* (Live Import List) by including a specimen on the list. There are two parts to the list:

- Part 1 comprises specimens that can be imported without a permit under the EPBC Act and
- Part 2 comprises specimens that require a permit under the EPBC Act to be imported. Import restrictions generally apply to the species listed on Part 2, such as 'for research only' and 'high security facilities only'. Additional conditions may also be applied when the permit for import is issued.

Before amending the Live Import List, the Minister must consult with appropriate agencies and other persons, and consider a report assessing the potential environmental impacts of the proposed amendment. When applying to the department to amend the Live Import List, all applicants are required to provide an accompanying report that addresses specific terms of reference.

The department undertakes a risk assessment using the information in the applicant's report and any other sources of relevant information. The department also considers comments and information received through the public consultation process (including states and territories). The application and accompanying report for the proposed import of Asian Tiger Mosquito *(A. albopictus)* was released for public comment in 11 September 2020.

Biology and Ecology of *Aedes albopictus*

Mosquito description

The genus *Aedes* (*Ae.*) belongs to the family Culicidae, order Diptera (GBIF Secretariat, 2020). The Asian Tiger Mosquito - *Aedes albopictus* Skuse, 1894 is originally indigenous to the forests of South-east Asia, islands of the Western Pacific and Indian Ocean (Gratz, 2004; Paupy et al., 2009). Similar species belonging to the same genus include *Aedes aegypti* (GISD, 2020) and *Aedes polynesiensis*. Synonyms for *Aedes albopictus* Skuse, 1894 include *Culex albopictus*, Skuse, 1895; *Stegomyia nigritia* Ludlow, 1910; *Stegomyia quasinigritia* Ludlow, 1911; *Stegomyia samarensis* Ludlow, 1903 (Catalogue of Life, 2020; GBIF Secretariat, 2020) and *Aedes (Stegomyia) albopictus* (Skuse, 1894) (Eritja et al., 2005). The Asian Tiger Mosquito is also commonly known as Forest Day Mosquito (Medlock et al., 2015).

Mosquitoes are blood sucking insects responsible for the transmission of many diseases throughout human and animal populations. Asian Tiger Mosquitoes are aggressive, outdoor day-time biters (Hartman, 2011). In their native environment in the forests of South-east Asia, they were likely zoophilic (i.e., feeding on wildlife). However, this species progressively adapted to anthropogenic changes to the environment, which provided alternative blood sources such as domestic animals and humans, in addition to water collections for larval habitats (Paupy et al., 2009). It has now adapted to many rural and suburban areas around the world (Gratz, 2004; Paupy et al., 2009).

Adult Asian Tiger Mosquitoes have conspicuous patterns of very black bodies with white stripes. They have a distinctive single white band (stripe) down the length of the back. They are small, fragile insects with slender bodies, one pair of narrow wings, and three pairs of long, slender legs. The wing length is used as a proxy for body size in mosquitoes and adult Asian Tiger Mosquitoes have an average wingspan of 2.7 mm. The body length averages about 4 mm, ranging from 2 to 10 mm (Hartman, 2011). The dry mass of Asian Tiger Mosquito adults from Florida, USA were weighed as 0.22-0.26 and 0.35-0.38 grams for males and females respectively (Lounibos et al. 2002). These mosquitoes have an elongated proboscis with which the female bites and feeds on blood (GISD, 2020).

Life cycle

The reported Asian Tiger Mosquito lifespan is variable, depending on the study. This could be due to different experimental or climatic conditions, as well as different strains being investigated. Löwenberg Neto and Navarro-Silva (2004) reported the average longevity of the species to be 35 days, with no differences between sexes. The experimental conditions for this study were 27/20°C, photoperiod of 12 hours and 75-95% relative humidity (RH). Other studies on longevity modelling estimated average life expectancy for females to be longer than for males, and to be affected by temperature. The estimated average adult life expectancies were the longest at 15°C for females and males (38.59 and 31.31 days , respectively) and the lowest at 35°C for females and males (19.86 and 14.9 d, respectively) (Delatte et al., 2009). Hartman

(2011) reported a much higher longevity , with females living up to 117 days in laboratory conditions. The age of sexual reproductive maturity is reported to be 8 days for female mosquitoes and 7 days for males (Hartman, 2011).

The Asian Tiger Mosquito has four distinct life stages, which consist of egg, larva, pupa, and adult. The first three stages occur in water. The adult is the free flying insect that feeds on humans, other animals, and the juice of plants (Lutz, 2002).

The female adult returns to water habitats briefly to lay each batch of eggs, which are shaped much like cigars. They are blunt at the anterior end and taper at the posterior end. Each egg is spotted with large, smoothly rounded outer tubercles, with small cell fields scattered around the rest of the egg. The eggs eventually hatch into larva, sometimes called wigglers, which are very small and must be studied under a microscope. They are active feeders, and thus are equipped with mouth parts. They also have long, protruding breathing siphons used for oxygen acquisition (Hartman, 2011).

Once females emerge from pupae, they take in a blood meal within the first two to three days, which is a vital source of protein for the development of eggs (Hartman, 2011). After a blood meal, females lay a batch of eggs (Lwande et al, 2020). Under favourable conditions, especially at high temperatures and flooding, eggs will hatch within a few days into larvae (Lwande et al, 2020). The larvae undergoes four moults, which may take between 9 and 13 days (Lwande et al, 2020). The male mosquitoes develop faster than the females and moult earlier into pupae. After a period of approximately 2 days, the pupae develop further into adult mosquitoes (Lwande et al, 2020).

Females can mate up to four times in a lifetime, depending on length of lifespan. Males typically have slightly shorter lifespans but have fewer restrictions on mating. They can both mate multiple times with multiple individuals. Males will form leks, or swarms, a few feet off the ground, which will attract females. Males will secrete stimulants which provide one stimulus for ovarian development (blood meal provides the other stimulus). The mating system is polyandrous (promiscuous) (Hartman, 2011). Mating will occur in flight and last for 5 to 15 seconds. At the end of the female's gonotrophic cycle she oviposits her eggs, placing them at a few various locations (Hartman, 2011).

The eggs from strains colonizing temperate regions resist lower temperatures than those from tropical areas (Hanson and Craig 1995). The mosquito strains adapted to temperate regions can lay diapausing eggs, which allows eggs to hibernate and survive colder temperatures. The phenomenon of diapause is induced by shorter photoperiods and lower temperatures (Hanson and Craig, 1995).

There is no one mating season for Asian Tiger Mosquito, but the species is likely to mate during the rainy season which varies geographically. This assures the quickest development time for the eggs, which begin hatching once submerged in a stagnant pool of water (Hartman, 2011). Females can lay from 42 to 110 eggs three or four days after a blood meal and 300-345 eggs over a lifetime (Davis, 2013; Lounibos et al., 2002).

Mating and competition with other *Aedes* species

An early study conducted by Leahy and Craig Jr. (1967) have shown that Asian Tiger Mosquitoes (*Aedes albopictus*) are not able to hybridise with *Aedes aegypti,* another species of the same genus. The researchers tested a total of 156,466 eggs which were the results of 30 combinations of species crosses, and no viable hybrid was produced.

More recent studies suggested that although 1.12 to 3.73% of interspecific mating between *Ae. albopictus* (Asian Tiger Mosquito) and *Ae. aegypti* happens in regions where both species co-exist, satyrization (failure to produce hybrids resulting in depressing reproductive output) also occurs, resulting in a reduction of the fitness of both species (reproductive interference) (Bargielowski et al., 2015, Giatropoulos et al., 2015; Tripet et al., 2011). In addition to *Ae. aegypti*, the Asian Tiger Mosquito is also capable of mating with other species of the *Aedes* genus (Tripet et al., 2011). In cage experiments, male Asian Tiger Mosquito mated with female *Aedes polynesiensis*, which resulted in female sterilization.

It has been suggested that the arrival of the Asian Tiger Mosquito in Guam after World War II may have led to competitive reductions of the native *Aedes guamensis* (Rozemboom and Bridges, 1972). It is important to note that many endemic *Aedes* species occur in all states and territories in Australia (ABRS, 2020d). Therefore, it is possible that the introduction of Asian Tiger Mosquitoes in the Australian environment could result in competition between this species, which has enormous physiological and ecological plasticity, and endemic *Aedes* species, which do not display such adaptability.

In conclusion, although it is unlikely that Asian Tiger Mosquitoes would be able to produce viable hybrids as the result of interspecific mating with Australian endemic *Aedes* species, satyrization could still occur, resulting in the reduction of the reproductive output of the native species. This could potentially interfere with the local native mosquito ecology dynamics. The possible satyrization and/or competition for habitats could result in Asian Tiger Mosquitoes displacing Australian endemic mosquitoes.

<u>Climate</u>

Different studies report different thresholds and optimum temperatures for Asian Tiger Mosquito development. This is likely due to different experimental designs, different natural conditions, different populations being studied, and their tolerance to climatic variations.

In general, Asian Tiger Mosquitoes thrive in a warm and humid climate (Lwande et al, 2020), with an optimal temperature of 29.7°C (Reinhold et al., 2018; Delatte et al., 2009). Adult mosquitoes have been reported to have a minimum development threshold temperature for survival and activity estimated at 10.4°C (Delatte et al., 2009). Another study has shown that male mosquitoes exposed to 2°C for 15 days had an 80% survival rate (Culbert et al., 2019). This species has also been recorded to occasionally reach the -5°C isotherm in the north of Asia and as far north as 52.15°N in the Netherlands (Scholte et al., 2007). Although mosquitoes can survive more extreme temperatures, they have been reported to fully develop between 15°C and 35°C. The longevity of individuals has been shown to be higher at lower temperatures (15 °C vs. 35 °C) in both females and males (Culbert et al., 2019).The maximum threshold for adult survival has been reported as 40°C (Delatte et al. 2009).

Asian Tiger Mosquito eggs can survive for months at 4°C in laboratory conditions. In nature, sheltered eggs were able to survive Tennessee, USA, winter temperatures of -18°C, as evidenced by the abundance of adult mosquitoes found in the late spring (P. Reiter, pers. comm., cited in Eritja et al., 2005). Tropical Asian Tiger Mosquito eggs also survived and hatched after being reared in laboratory conditions (under tropical settings) and being exposed to field winter temperatures of central Europe for weeks. In the first year, the recorded temperatures over a two weeks period varied from as low as -10°C to as high as -3°C. During this period, the eggs endured a constant low of -8°C for 4 hours. Six per cent of the eggs hatched once they were taken back to the laboratory and were artificially flooded. In the second year the field temperatures varied between -6°C and -1°C and the experiment lasted for 16 weeks. The lowest temperature that eggs endured was -6°C for 2 consecutive hours. A good percentage of eggs were able to hatch following 16 weeks under these conditions (Tippelt et al., 2019). This research also demonstrated that hatching rates of the tropical eggs substantially increased when the temperature decreased slowly over a period of time, allowing the eggs to acclimate to the cold temperatures (Tippelt et al., 2019). In nature, the acclimation would be more likely as temperatures normally start decreasing slowly as the autumn progresses towards winter. This acclimation process would confer further cold tolerance to the mosquito eggs. At the other extreme, studies have shown that eggs fail to hatch above 40°C (Waldock et al., 2013).

Larvae and pupae have optimal survival at 25 – 30°C and high mortality at 15°C and 36°C. Larval survival is estimated to cease at 10 and 40°C, while for pupae, survival is estimated to cease at 10 and 37°C (Dellate et al., 2008).

Precipitation is another important environmental variable affecting Asian Tiger Mosquito populations. However, whilst the ancestral tree hole dwelling insects would have been dependent on rainfall to provide breeding water sources, modern and urbanised Asian Tiger Mosquitoes often breed in water sources that are independent of rainfall, for example through watering of plants in urban gardens, or the provision of water in vases in cemeteries (Waldock et al., 2013). It has been stated previously that a minimum annual precipitation of 500 mm would be necessary for Asian Tiger Mosquito survival (Medlock et al., 2006), however, by overlaying the current insect distribution in Europe with precipitation data it is suggested that a threshold of 200-250 mm per annum is more accurate (Waldock et al., 2013). The immature Asian Tiger Mosquito stages are susceptible to desiccation; however, the eggs can survive for up to 243 days following desiccation once embryonation is complete (Waldock et al., 2013). Although relative humidity (RH) affects adults and eggs of Asian Tiger Mosquitoes, with survival rates increasing with RH, European populations are found in regions with RH as low as 35% in the summer (Waldock et al., 2013).

It is critical to consider that irrespective of the differences in values and thresholds reported by different studies, the current global distribution of this species as shown in Figure 1 is a good indication of this mosquito's capacity to adapt to different climates and spread and establish around the world. It is also worth noting that climate change may allow the Asian Tiger Mosquito to spread even further into temperate regions (Waldock et al., 2013).

Environment

The optimal pH of water for larval development has been estimated to be 4.8, however, the pH of natural and artificial breeding sites can vary from 2.3 to 9.8. Other water physico-chemical parameters include a practical salinity unit (PSU) of < 0.01 to 6.33, dissolved oxygen (DO ppm) <0.01 to 24.2 (Medeiros-Sousa et al., 2020) and temperature 10 to 37ºC for larval development (Dellate et al., 2008).

Habitat

The Asian Tiger Mosquito chooses a habitat based on availability of food resources and locations for reproduction and development. In nature the breeding places for this species are small, restricted, shaded bodies of water surrounded by vegetation (Moore, 1999). However, these mosquitoes have great adaptative ability (Gratz, 2004) and have adapted well to peri-urban and rural environments (Braks et al., 2003), particularly densely vegetated rural areas (Moore, 1999). Due to their great ecological plasticity for habitats, these mosquitoes are capable of finding indoors or outdoors breeding sites including dense urban settings such as houses, gardens, drains, storage facilities, parklands and building sites (Pereira-dos-Santos et al. 2020). The breeding sites can be natural or artificial and include cemetery flower pots, coconut shells, bird baths, bromeliads, bamboo stumps, clogged gutters, soda cans, tree holes, palm leaves, litters, rock holes, leaf axils, snail shells, palm bracts, dead leaves, dead cow horns, puddles, tyres, ground cavities, hollow logs, and any abandoned containers and water recipients (Hartman, 2011; Moore, 1999; Pereira-dos-Santos et al., 2020). They can also establish and survive throughout non-urbanized areas lacking any artificial containers, raising additional public health concerns for rural areas (Moore, 1999).

Tyres are particularly useful for mosquito reproduction as they are often stored outdoors and effectively collect and retain rainwater for a long time. The addition of decaying leaves from the neighbouring trees produces chemical conditions replicating tree holes, which provides an excellent substrate for breeding (Moore, 1999).

Asian Tiger Mosquitoes are weak fliers; therefore, they remain within the same habitat their entire lives. They have high dietary plasticity, therefore being able to find food in a range of environments (Hartman, 2011).

Diet

Larvae of Asian Tiger Mosquito feed on aquatic detritus, algae, and microorganisms (Richards et al., 2006). Adults are day feeders, feeding on nectar and sugar-rich plant juices. Females need a blood meal to lay eggs (Hartman, 2011).

Female mosquitoes are attracted to a potential host through a combination of different stimuli that emanate from the host. The stimuli can include carbon dioxide, body odours, air movement or heat. Upon locating a suitable host, the female will probe the skin for a blood capillary then inject a small amount of saliva containing chemicals which prevent the host's blood from clotting. This is often the pathway for potential pathogens such as viruses to enter a host. After engorging on the host's blood, the female will find a resting place to digest her meal and develop eggs before flying off to deposit them in a suitable aquatic habitat (NSW Health, 2020a).

Female Asian Tiger Mosquitoes are very efficient in finding sources of blood meal, being able to feed on different classes of animals including mammals, birds, reptiles, and amphibians (Eritja et al., 2005). Humans are the Asian Tiger Mosquito’s favourite food source; they feed on humans 60% of the time (Pereira-dos-Santos et al., 2020). Other food sources include dogs, rodents, rabbits, cats, bovines, chickens, horses, pigs, squirrels, opossums, raccoons, turtles, and rats (Richards, 2006; Hartman, 2011; Pereira-dos-Santos et al., 2020). This wide host variability allows this species to thrive in a wide range of environments (Hartman, 2011).

Given its high dietary plasticity, the Asian Tiger Mosquito would likely be able to find plenty of its favourite and alternative food sources in a range of different habitats in Australia. This would be of great relevance for the species' survival and for the possible spread of zoonoses.

Predation

Many different species from different phyla prey upon Asian Tiger Mosquito. Most of these predators consume mosquitoes in their larval phase. The predator copepods *Mesocyclops leuckarti and M. scrassus* have been studied as biological control agents for *Aedes*, including *Ae. albopictus* (Asian Tiger Mosquito) larvae control (Udayanga et al. 2019). The subspecies *Mesocyclops leuckarti pilosa* has been found to have the ability to take out an entire group of larvae in a container (Hartman, 2011). Another copepod, *Macrocyclops albidus*, which has a wide geographic range, can knock out a dense population in tyre piles in 8 to 10 weeks. Some flatworms in the phylum Platyhelminthes also prey on larvae. Other mosquitoes including various species in the *Toxorynchites* genus, have shown an excellent ability to maintain Asian Tiger Mosquito populations at low levels and are being considered as a possible biological control species (Hartman, 2011). It is worth noting that the genus *Mesocyclops* is widely distributed in Australia, with *M. albidus* only not being present in the Northern Territory (ABRS, 2020a, 2020b, 2020c). Adult mosquito predators include bats and birds.

Disease transmission

Mosquitoes are blood sucking insects that are responsible for the transmission of many diseases throughout human and animal populations worldwide (NSW Health, 2020a). They are amongst the major vectors capable of transmitting arboviruses (arthropod-borne viruses) (Artsob et al. 2017). Arboviruses are maintained in nature mainly through biological transmission between susceptible vertebrate hosts by hematophagous (blood-sucking) arthropods (Artsob et al. 2017). Out of the more than 500 known arboviruses, 100 of them can cause disease in humans (Artsob et al. 2017). Globalization has significantly increased the vulnerability of human and animal populations to emerging arbovirus diseases worldwide (Lwande et al., 2020). The spread of these diseases has become a major global health concern, and it is predicted that climate change will affect mosquito distribution, which will allow these insects to spread new pathogens to previously unaffected areas (Reinhold et al. 2018).

The Asian Tiger Mosquito is one of the primary vectors for emerging and re-emerging arboviruses (Lwande et al. 2020). These mosquitoes are day feeders which feed on a wide range of hosts as described above, are a serious public health threat as a bridge vector for many zoonotic pathogens to humans and also a significant biting irritant, constituting an economic burden worldwide (Benedict et al. 2007; Lwande et al. 2020).

The most relevant human diseases transmitted by the Asian Tiger Mosquito include the 4 serotypes of Dengue virus (Dengue 1, 2, 3 and 4 virus [DENV-1, DENV-2, DENV-3, DENV-4]), yellow fever virus, chikungunya virus, and Zika virus (Hugo et al. 2019; Lwande et al. 2020). Notably, the Asian Tiger Mosquito ranks second to *Ae. aegypti* in importance to humans as a vector of Dengue and Dengue haemorrhagic fever (Knudsen et al. 1996). Evidence suggests that it serves as a maintenance vector of Dengue in rural areas of Dengue-endemic countries in South-east Asia and the Pacific islands (Gratz, 2004). Vertical transmission in vectors occurs through infected eggs and horizontal transmission occurs during blood meals on a host, during which female mosquitoes ingest viral particles. Dengue and chikungunya have both been reported to be transmitted both horizontally and vertically (Ferreira-de-Lima et al. 2020; Monteiro et al. 2019).

The Asian Tiger Mosquito is also known to transmit other arboviruses including Japanese encephalitis virus and West Nile virus (Akiner et al., 2019; Pereira-dos-Santos et al., 2020; de Wispelaere et al., 2017). Additionally, the mosquito has been shown to experimentally transmit Cache Valley virus, Eastern equine encephalitis virus, Jamestown Canyon virus, Japanese encephalitis virus, La Crosse virus, Mayaro virus, Oropouche virus, Orungo virus, Potosi virus, Rift Valley fever virus, Ross River virus, Sindbis virus, San Angelo virus, Trivittatus virus, Western equine encephalitis virus and Venezuelan equine encephalitis virus (Brustolin et al., 2017; Medlock et al. 2015; Moore and Mitchell, 1997; Pereira-dos-Santos et al., 2020). Of the diseases vectored by the Asian Tiger Mosquito listed above, several are of relevance to Australia, including chikungunya virus, Dengue fever, Japanese encephalitis virus, Ross River virus disease, West Nile virus and Rift Valley fever virus (Department of Health, 2017; NSW Health, 2020a), Zika virus and Dog Heartworm (Jeremiah, et al. 2011).

Chikungunya virus is a mosquito-borne alphavirus associated with epidemics of acute and chronic arthritic disease in humans (Hugo et al. 2016). The infection causes fever and severe joint pain, muscle pain, joint swelling, headache, nausea, fatigue, and rash. The joint pain is debilitating and can vary in duration. There is currently no vaccine or medication against chikungunya virus, however, severe and fatal cases are very rare. The disease occurs mostly in Africa, Asia, India, and recently in the Americas (WHO, 2020a). The Asian Tiger Mosquito has emerged as an important new natural vector for chikungunya virus transmission (Hugo et al. 2016).

Dengue is the most important viral disease transmitted by mosquitoes afflicting humans in a world context. It is common in warm tropical climates. It is caused by any one of the four existing serotypes. According to the World Health Organization, this is the fastest spreading arbovirus; estimates show that 390 million people are currently infected per year, while 3.9 billion are considered at risk (WHO, 2020d). Clinical symptoms range from mild fevers, to a severe and potentially life-threatening haemorrhagic disease. Fatalities can occur. There are no treatments for the disease, however, symptoms can be managed. Epidemics usually happen after rainy seasons (WHO, 2020d). In Australia, Dengue fever is restricted to Queensland where the major vector *Aedes aegypti* occurs (NSW Health, 2020a). Vaccines to prevent Dengue infections are being developed, with one currently on the market (Dengvaxia® [CYD-TDV]), but only individuals who are seropositive are recommended to receive the vaccine. Vector control is still the main method to prevent the spread of this virus (WHO, 2020d).

Japanese encephalitis virus is a flavivirus related to Dengue, yellow fever and West Nile viruses (WHO, 2020e). It is a flavivirus vectored by mosquitoes from water birds or pigs to humans (van den Hurk et al., 2019). This virus is the leading cause of encephalitis in several countries of Southeast Asia, the Indian sub-continent and Indonesia, with estimated 68,000 clinical cases every year (WHO, 2020e). The disease is predominantly asymptomatic, with only 1% of the cases resulting in clinical disease. Symptoms range from mild febrile illness to meningomyeloencephalitis. Between 20-30% of the symptomatic cases are fatal, and among the survivors, 30-50% will have permanent neurological sequelae. The disease is vaccine preventable, however, there is no cure for infected patients. The treatment is focused on relieving the symptoms (WHO, 2020e). The disease is predominantly found in rural and peri-urban areas (WHO, 2020e). It is a major cause of neurological disease in Australia and emerged for the first time in the country in 1985, when an outbreak happened in the Torres Strait Islands (van den Hurk et al., 2019).

Ross River disease is the most commonly transmitted mosquito-borne viral disease in humans in Australia (over 6,500 cases in 1997) and occurs in all states. The virus appears to be endemic in most rural areas, and there has been an increasing incidence near major cities (NSW Health, 2020b). The disease shows a wide variety of symptoms including rashes with fevers, to arthritis that can last from months to years. There are occasional local epidemics with hundreds to thousands of infections, with many going unreported (NSW Health, 2020a). Serological studies and laboratory investigations have indicated that native mammals, most likely kangaroos, and wallabies, are natural hosts for Ross River virus (NSW Health, 2020b).

The West Nile fever is a viral infection caused by a flavivirus which causes neurological disease and death in people. This virus is maintained in nature in a cycle involving transmission between birds and mosquitoes, however, humans, horses and other mammals can be infected as well (WHO, 2020f). Patients present encephalitis, febrile disease, and meningitis (Chowers et al., 2001). Mortality rates increase with age, being as high as 29.3% in patients older than 70 years old (Chowers et al., 2001). West Nile virus has been isolated in Australia previously (Tsai et al. 1998).

Yellow fever is another arbovirus in the family of flaviviruses. It is an epidemic-prone and vaccine preventable disease. Mosquitoes can transmit the disease from person to person in heavily populated areas. The disease can present a wide range of symptoms and severity. Symptoms include fever, muscle pain, prominent backache, headache, loss of appetite, nausea, and vomiting. In most cases, symptoms disappear in 3-4 days, however, a small percentage of patients enter a second, more toxic phase within 24 hours of recovering from initial symptoms. High fever returns and several body systems are affected, usually the liver and kidneys. In this phase people are likely to develop jaundice (yellowing of the skin and eyes, hence the name yellow fever), dark urine and abdominal pain with vomiting. Bleeding can occur from the mouth, nose, eyes, or stomach. Half of these patients die within 7–10 days (WHO, 2020c). Although yellow fever does not occur in Australia, it could be transmitted by *Ae. aegypti* which is widely distributed in northern Queensland (Department of Health, 2019) and by the Asian Tiger Mosquito if it establishes in the country.

Zika virus is another flavivirus that resembles Dengue. It is transmitted from mother to foetus during pregnancy, through sexual contact, transfusion of blood and blood products, and organ transplantation. Symptoms are generally mild including fever, rash, conjunctivitis, muscle and joint pain, malaise, and headache. Zika virus infection during pregnancy is a cause of microcephaly and other congenital abnormalities in the developing foetus and newborn. Zika infection in pregnancy also results in pregnancy complications such as foetal loss, stillbirth, and preterm birth. It is also a trigger of Guillain-Barré syndrome, neuropathy, and myelitis, particularly in adults and older children. There is currently no vaccine to prevent the disease (WHO, 2020b). Areas of Queensland where *Ae. aegypti* and Asian Tiger Mosquitoes may be present could be vulnerable to Zika virus. The monitoring of international ports to prevent the entering of mosquitoes carrying the disease is a crucial part of the prevention of the spread of this disease (Department of Health, 2018). The Guillain-Barré syndrome, triggered by the Zika virus infection is a rare immunological disorder in which the body’s immune system attacks part of its peripheral nervous system, causing muscle weakness and sometime paralysis. Most people recover from even the most severe cases of Guillain-Barré syndrome; however, some people will sustain permanent damage (CDC, 2020).

In addition to vectoring several viruses, the Asian Tiger Mosquito can also vector nematodes causing veterinary filariasis, including *Dirofilaria immitis* (dog heartworm), *D. repens* (Gratz, 2004; Medlock et al. 2015) and *Setaria labiatopapillosa* (Paupy et al. 2009). These diseases can be fatal to dogs, and can also affect cats, wild animals, and humans (Gratz, 2004).

If Asian Tiger Mosquitoes were to escape the research facilities and establish in Australia, they could potentially act as vectors of the diseases described above, posing significant threat to public health as well as the health of domestic and native animals. Importation of Asian Tiger Mosquito would be subject to assessment and approval by the Commonwealth Department of Agriculture, Water and the Environment, under the *Biosecurity Act 2015*.

<u>Ecological and Physiological Plasticity</u>

The two main factors contributing to the success of the Asian Tiger Mosquito's spread in the last 30 years are thought to be its extremely high physiological and ecological plasticity (Paupy et al., 2013).

Physiological plasticity is observed in both ancestral type strains in Asia and recently expanded strains across the world. This plasticity is mostly conferred by the ability to survive in both tropical and temperate conditions by laying overwintering (diapausing) cold and desiccation resistant eggs (Paupy et al., 2013). Diapause is a dynamic state of low metabolic activity that is genetically determined and mediated by neurohormones that phenotypically affect individuals by decreasing morphogenesis, blocking reproduction and metamorphosis, and increasing tolerance to extreme environmental conditions (Diniz et al, 2017) such as low temperatures and reduced humidity (Lounibos et al, 2003).

The major stimuli inducing diapause in natural populations are changing photoperiod (short days and long nights) and gradual decreases in temperature (Hanson et al., 1994). Species exhibiting the phenotypic plasticity to undergo diapause have the required information encoded in their genomes (Diniz et al, 2017). Diapause has largely been reported to occur in temperate Asian Tiger Mosquitoes strains. However, it may also occur in strains which underwent selection pressure in temperate regions and later spread and established in tropical regions. One example of a tropically established Asian Tiger Mosquito population with ability to undergo diapause is found in the port city of São Luis, in the northern State of Maranhão in Brazil (Lounibos et al., 2003).

Ecological plasticity refers to the vast array of breeding habitats that Asian Tiger Mosquitoes can use, ranging from tree-holes and cut bamboo to a wide variety of man-made containers. In addition, despite a preference for humans and mammals, it can feed from a wide range of hosts if required, which makes this species a potentially important vector for zoonotic viruses.

Taken together, the capacity to spread to different climates and use different substrates for reproduction allow the Asian Tiger Mosquito to be highly invasive (Waldock et al., 2013).

Spread

The Asian Tiger Mosquito is in the top 100 invasive species list by the Global Invasive Species Database (GISD, 2020). This mosquito species is native to Southeast Asia (Lwande et al., 2020), and for many years it was geographically restricted to its original area in Southeast Asia and the African continent. These mosquitoes are non-migratory, and the adults' flight range is quite short. Most medium and long-range colonization is the result of passive transportation, where female mosquitoes deposit dormant eggs in goods which serve as larval habitats (Eritja et al., 2005). The spread of this species around the world has dramatically intensified since the 1970s thanks to globalization and the increase in the trade of used tyres (Eritja et al., 2005; Scholte et al., 2007) and other goods such as Lucky bamboo plants (*Dracaena* spp.) (Gratz, 2004; Scholte et al., 2007).

Presently, Asian Tiger Mosquito has spread to nearly all continents (Kraemer et al. 2015), including Africa, Europe, Australasia (Torres Strait), the Americas and Middle East (Gratz, 2004). In Asia, the mosquito is present in Taiwan, Malaysia, Indonesia, India, Japan, Thailand, Singapore, Lao People's Democratic Republic, Philippines, Viet Nam and numerous Pacific Ocean and Indic Ocean Islands (ECDC, 2020; Kraemer et al. 2015). The species was first detected in the Torres Strait in 2005 and is known to be present between Horn and Thursday Islands (Williams, 2012). The mosquito likely spread from Papua New Guinea into the Torres Strait Islands, and it is repeatedly detected at ports on mainland Australia, which makes invasion and colonisation highly likely (Williams, 2012).

Although the Asian Tiger Mosquito is originally a tropical species, it has become adapted to temperate regions, invading developed nations where it has been responsible for large outbreaks of diseases (Williams, 2012). It has invaded a large part of Southern Europe, and is currently spreading northwards (Medlock et al., 2015). It has been reported in the following European countries: Albania, Italy, France, Spain, Belgium, Switzerland, Greece, Serbia and Montenegro, Croatia, Bosnia, and Herzegovina, Slovenia and the Netherlands (Scholte et al. 2007).

The spread of the Asian Tiger Mosquito across new urban, peri-urban and natural areas could foster the interaction of this vector with wildlife which could act as local reservoirs of enzootic arboviruses, with implications for the potential zoonotic transfer of pathogens to new areas (Paupy et al., 2009; Pereira-dos-Santos et al., 2020). Consequently, the introduction of the Asian Tiger Mosquito into the Australian mainland could have serious consequences for the transmission of local vector-borne disease (Williams, 2012), particularly considering that this mosquito has a high dietary and ecological plasticity.

Establishment

Given the global distribution of Asian Tiger Mosquito in varying conditions (Figure 1), it may be able to adapt and establish in both temperate and tropical areas of Australia. This mosquito is highly matched to the Australian environment based on the Climatch output shown below, with a high proportion of the Australian mainland with a climate match score of 6 and above (Bomford, 2008) (Figure 2). This suitability to the Australian environment is likely due to the enormous ecological and physiological plasticity of this species, and its strong competitive aptitude (Medlock et al., 2015; Paupy et al., 2009; Waldock et al., 2013). Asian Tiger Mosquitoes can survive in a broad range of temperatures (Benedict et al., 2007; Delatte et al., 2009; Lwande et al, 2020; Paupy et al., 2009; Reinhold et al., 2018; Scholte et al., 2007), feed on the blood of a broad range of vertebrate hosts (Hartman, 2011; Pereira-dos-Santos et al., 2020) and can inhabit a wide range of environments ranging from natural, peri-urban, rural and urban areas (Braks et al., 2003; Gratz, 2004; Moore, 1999).

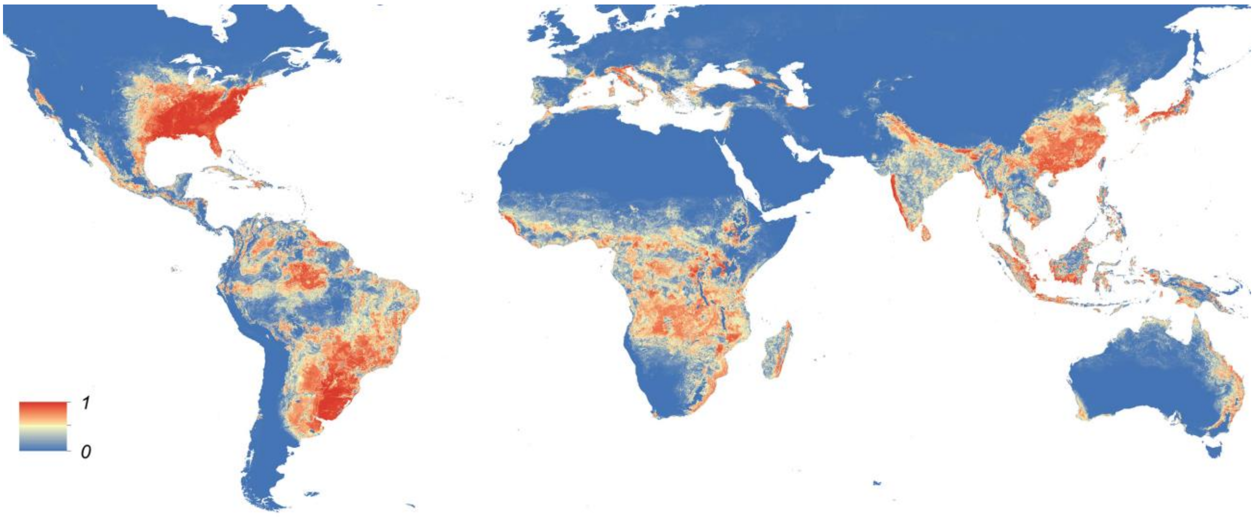

Figure 1. Global map of the predicted distribution of Ae. albopictus. The map depicts the probability of occurrence (from 0 blue to 1 red) at a spatial resolution of 5 km × 5 km. Source: Kraemer *et al*.(2015)

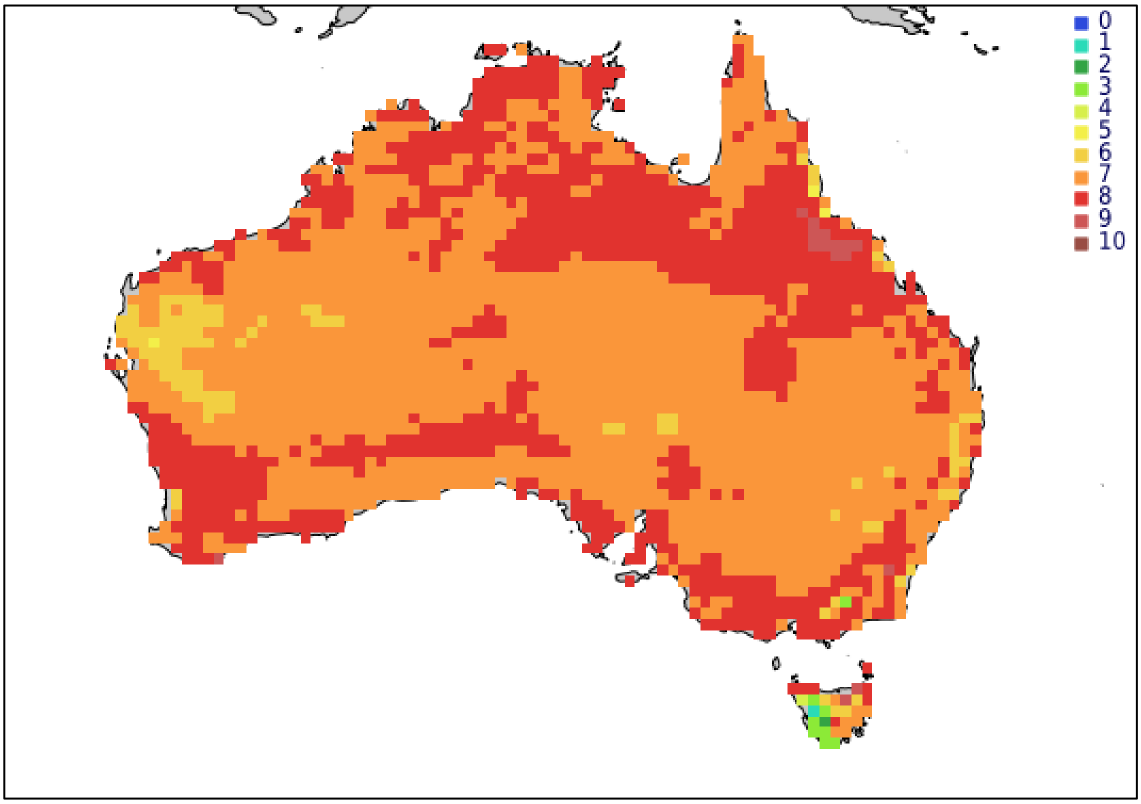

Figure 2. Climatch potential suitable habitat for the Asian Tiger Mosquito (*Ae. albopictus)* based on current range (as shown in Figure 1). The higher the number, the higher the climatic match.

The applicant states that in laboratory conditions Asian Tiger Mosquitoes are reared in controlled insectary colonies where they are normally conditioned to tropical conditions (>27°C) and on a 12:12h light cycle. According to the applicant 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. It is worth noting that research has shown that tropical strains of Asian Tiger Mosquito eggs reared in similar laboratory conditions are able to survive and hatch following exposure to the winter temperatures of central Europe, which are substantially colder than the winter temperatures of southern Australia (Tippelt et al., 2019).

Research conducted in Germany also shows that hatching rates greatly increased when the temperatures decreased slowly over a period of months, allowing the eggs to acclimate to the cold temperatures (Tippelt et al., 2019). In nature, acclimation would be more likely as temperatures normally start decreasing gradually as the autumn progresses towards winter. This would confer further cold tolerance to the mosquito eggs. If Asian Tiger Mosquito eggs reared in laboratory conditions were able survive the low winter temperatures of central Europe, it is plausible to assume that they could also survive the winter temperatures of southern Australia. Although adults and immature stages are not as cold-tolerant as the eggs, having a higher threshold of 10°C (Delatte et al., 2009), they would still be able to survive for a good proportion of the year in the southern regions of Australia.

The historic minimum average temperature for Geelong in Victoria, the location of the CSIRO ACDP, is approximately 6°C (Figure 3) (BOM, 2020a), and the lowest minimum temperature over the last 7 years was -2°C in July 2017 (Figure 4) (BOM, 2020b). Based on the study published by Tippelt et al. (2019) described above, a percentage of the tropical strain eggs reared in a laboratory could survive this field temperature, particularly if sheltered. Additionally, the lowest temperature threshold for larval and adult development and activity according to Delatte et al. (2009) is 10.4°C. Based on the monthly mean minimum temperature data from the Bureau of Meteorology (BOM, 2020a) (Figure 3) and the July lowest temperature for the last 7 years (BOM, 2020b) (Figure 4), Asian Tiger Mosquito immature stages and adults could possibly survive from October to May and eggs could survive all year in the Geelong area.

In conclusion, although egg, larval and adult development and survival rates are significantly affected by temperature, particularly low temperatures, evidence and climate data suggest that if Asian Tiger Mosquitoes were to escape the research facilities in Geelong, a percentage of individuals would potentially be able to survive. The mosquito's survival would depend on several factors such as minimum temperatures, duration of minimum temperatures, chance of acclimation, availability of shelter, availability of hosts and appropriate breeding sites. Therefore, this species of mosquito has the potential to adapt to colder weather conditions and establish in the region. The rising temperatures due to climate change could further increase the chances of insect survival (Waldock et al., 2013).

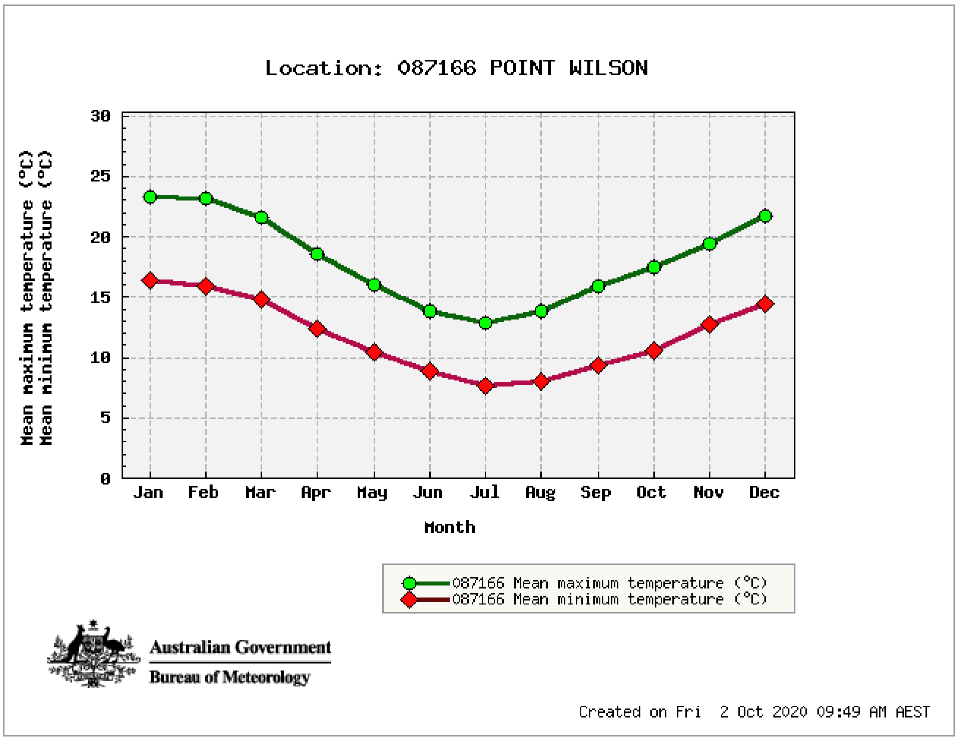

Figure 3: Source: BOM, 2020a.

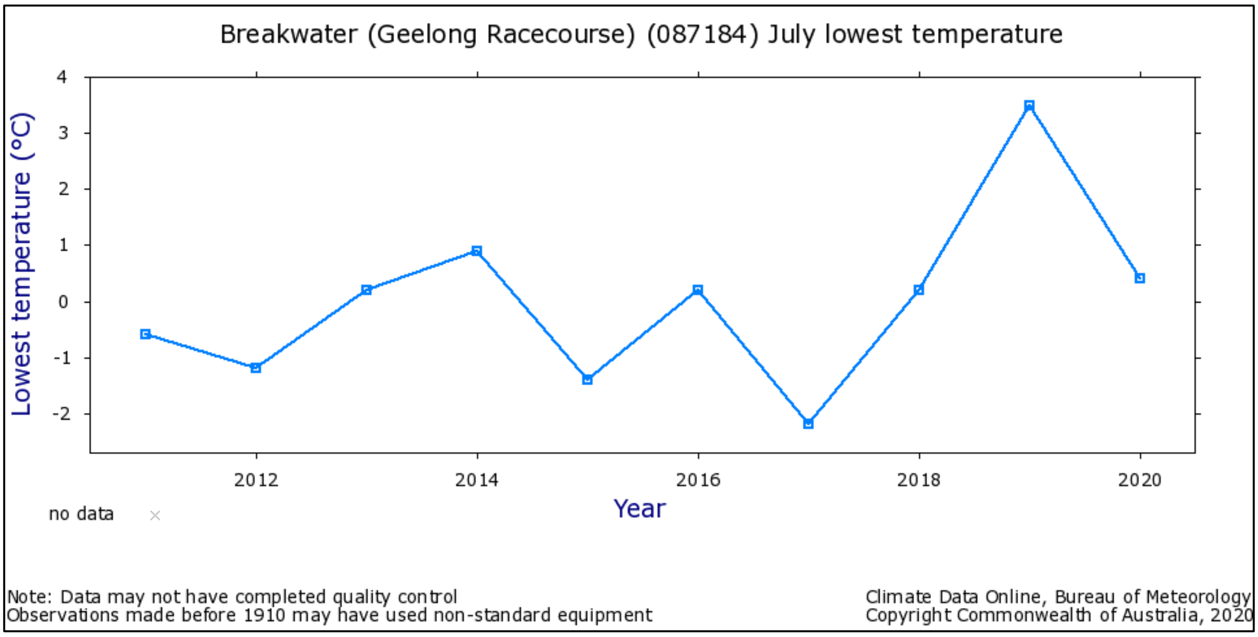

Figure 4: Source: BOM, 2020b.

Related Live Import List listings

Nine species of mosquito are listed on Part 2 of the Live Import List (*Aedes aegypti, Aedes polynesiensis, Anopheles farauti, Anopheles koliensis, Anopheles punctulatus, Anopheles stephensi, Culex quinquefasciatus, Halaedes australis* and *Ochlerotatus (Aedes) koreicus).* All with the conditions *'Eligible non-commercial purpose only, excluding household pets. High security facilities only*".

Conservation status

The Asian Tiger Mosquito *(Ae. albopictus)* is not listed on the International Union for Conservation of Nature's Red List of Threatened Species or the *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES) (CITES, 2020) list.

Risk assessment

Assessing the risk of the potential of introducing a new organism into the environment involves assessing the risk of it becoming established and spreading and the likely impacts if establishment occurred.

There are no accepted risk assessment models that can be used to calculate the establishment risk of invertebrates in Australia. Bomford (2008) found that for vertebrates, the level of risk can be assumed in accordance with the four key factors of establishment success. These factors are:

- Propagule pressure – the release of large numbers of animals at different times and places enhances the chance of successful establishment
- Climate match – introduction to an area with a climate that closely matches that of the species' original range
- History of establishment elsewhere – previous successful establishment
- Taxonomic group – belonging to a family or genus which has a high establishment success rate.

Although these factors apply to vertebrates, they have been used as a guide for this risk assessment of Asian Tiger Mosquitoes. In addition, using the information compiled from research into the above factors for Asian Tiger Mosquitoes the potential impacts of establishment of feral populations can also be assumed.

Risk of establishment

Propagule pressure – the release of large numbers of animals at different times and places

The application is for the listing of Asian Tiger Mosquitoes for research purposes in secure facilities only. This means all imports would be subject to an import permit which would limit the number of mosquitoes imported into Australia into quarantine approved facilities.

The likelihood of escape of specimens from an accredited biosecure facility is negligible. Quarantine facilities are required to have security measures in place to ensure that the movement of specimens in and out of the facility are tightly controlled and these measures should negate any chance of the specimens escaping.

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 their 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.

As the importation of the species would be restricted to research purposes only and the specimens will be kept in an accredited biosecure facility, all waste would be treated to a standard well beyond that which would kill all eggs or mosquitoes. A one-off deliberate or accidental release is unlikely and multiple releases would be highly improbable.

Climate match – introduction to an area with a climate that closely matches that of the species' original range

The native range of Asian Tiger Mosquito is the Southeast Asia region; however, the species has spread to many different areas of the world with different climates, displaying extremely high ecological and physiological plasticity. The Climatch map (Figure 2) shows that most of Australia is climatically suited to the mosquito. Under the Bomford model for exotic mammals and birds this high level of compatibility would result in a rating of extreme climate matching with a maximin score of 6 The Climatch score of 6 is based on there being more than 2700 grid squares in Australia within the 6 highest climate match classes out of a possible 2785 squares. The Asian Tiger Mosquito can acclimatise and reproduce at temperatures as low as 10°C and eggs are capable of surviving harsh and negative winter temperatures.

History of establishment elsewhere – previous successful establishment

The original location of the Asian Tiger Mosquito is Southeast Asia (Lwande et al., 2020). From its original location, this species expanded its range to Africa, the Americas, Europe, and the Middle East (Gratz, 2004). It is currently present in the Torres Strait in Australia (ABRS, 2020a; Williams, 2012). The species is known to be in the top 100 most invasive species in the world (GISD, 2019).

Taxonomic group – belonging to a family or genus which has a high establishment success rate

The *Aedes* genus has several members regarded as highly invasive. The Asian Tiger Mosquito is regarded as one of the top 100 invasive pests worldwide having established in 42 countries (GISD, 2019). *Aedes aegypti* is one of the most widespread mosquitoes worldwide (ECDC, 2019) and is present in Australia.

Potential impacts of established feral populations

The Asian Tiger Mosquito is likely to establish a feral population in Australia if it escapes the research facilities, given the availability of suitable habitats in the country and the extremely high ecological and physiological plasticity of the species. If a population did establish, the species could have an impact as a vector for Dengue fever, Japanese encephalitis virus, Ross River virus disease, West Nile virus, Rift Valley fever virus and Dog Heartworm. Additionally, it could also act as vector of chikungunya and Zika virus if these viruses entered Australia. Most of these viruses are zoonotic and hence may pose a risk to the Australian environment.

A full assessment of the disease risks posed by the Asian Tiger Mosquito would be undertaken by the Department of Agriculture, Water and the Environment under the *Biosecurity Act 2015* before the mosquitos could be imported.

The mosquito is an aggressive day biting species, being a major nuisance.

Risk summary and mitigation measures

The risk of animals escaping or being deliberately released into the wild is negligible given the strict security measures that would be implemented in research facilities. The imported mosquitoes would be required to remain in a quarantine accredited BIC3 for the duration of their lives. These premises are audited by the Commonwealth Department of Agriculture, Water and the Environment and must meet strict criteria to ensure that animals are kept in a highly secure environment that prevents escape of animals and any related pathogens. All waste products and contaminated materials are to be appropriately treated or disposed of as part of the certification of the facilities.

The applicants maintain the highest-level Biosecurity facility in the Southern hemisphere and are certified by the department under the Biosecurity Act, and by the Office of the Gene Technology Regulator, the World Health Organisation and the World Organisation for Animal Health (OIE).

Two Australian research institutions have safely held a strain of Asian Tiger Mosquitoes since 2016 with no incidents occurring. Discussions with the Animal Biosecurity Division within the department have confirmed that any permit to import or hold the species can be conditioned to reduce the level of risk to an acceptable level under the *Biosecurity Act 2015*.

Table 1: Summary of risks and mitigation measures

Risk	Likelihood	Impact	Mitigation measures	Overall risk
Release or escape of adult specimens	unlikely	minor	The species will only be held in a Quarantine Approved facility.	Low
Release or escape of immature specimens	unlikely	minor	The species will only be held in a Quarantine Approved facility.	Low
Disease transmission to native species populations	possible	unknown	The import of the mosquitoes will need to meet the conditions applied by the Department of Agriculture, Water and the Environment as part of the import permit. For the duration of the research, the mosquitoes will be housed in a quarantine approved facility and all specimens will be	low

			destroyed at the conclusion of the research. Therefore, there is a very low chance of interaction with native species.	
Theft and deliberate release	unlikely	unknown	Quarantine facilities are required to have security measures in place to ensure that access is controlled. The security procedures would mean that the risk of theft is extremely low. The species is not of any value. It is unlikely that they would be of interest to any groups apart from mosquito researchers.	Low

The listing of Asian Tiger Mosquito should only occur under Part 2 the Live Import List. The proposed import of mosquitoes for research purposes is unlikely to pose an environmental risk. Based on evidence of the mosquito's natural habitat and environmental requirements it is likely that the species would establish in Australia. The consequences of the mosquito establishing in Australia could be serious from the disease and social nuisance perspective and therefore importation of the mosquito should be limited to approved high security research facilities only.

Concerns raised about the proposal to import Asian Tiger Mosquito (*Ae. albopictus)* and responses

The department undertook consultation with relevant ministers (or their delegates), government agencies and the public in September-October 2020. The department received responses from two members of the public. None of the responses were supportive of the application. Below is a summary of the main concerns raised by each stakeholder:

> Stakeholder 1 is opposed to the amendment of the Live Import List due to concerns about the research facility not being able to properly contain the Asian Tiger Mosquito, and not being able to prevent their escape. The stakeholder also has concerns that the species could establish if they escape the research facilities.
>
>> Department response:
>>
>> Due to their high physiological and ecological plasticity, if Asian Tiger Mosquitoes were to escape the research facilities, a percentage of individuals would likely be able to survive and establish in any given season in the Geelong area in Victoria. However, the chances of mosquito escape are negligible.
>>
>> The research facilities described in the draft application are specially built to avoid insect escape. To be granted the status of Quarantine Accredited Biosecurity Insectary Containment Level 3 (BIC3), the research facility must meet minimum safety requirements and standards. These facilities are designed to contain goods subject to biosecurity control which pose significant risks to animals, plants or humans if a pest or disease associated with them spread outside the site and from which significant

economic impact would result to the community environment (Department of Agriculture and Water Resources, 2016). Additionally, these laboratories must meet Physical Containment Level 3 (PC3) design and construction requirements as specified in Australian/New Zealand Standard TM 2243.3:2002 and 2982.1:1997 (Department of Agriculture and Water Resources, 2016; OGTR, 2011).

Additionally, to maintain the BIC3 accreditation, the facility is audited annually, all staff must undergo rigorous training which needs to be refreshed periodically, and there are also strict guidelines and procedures to be followed.

Given the design and safety features, requirements and measures in BIC3 research facilities and the high-level of skill and training required of staff, the chances of Asian Tiger Mosquito individuals escaping are minimal.

Stakeholder 2 does not support the application to amend the Live Import List to add Asian Tiger Mosquitoes. The stakeholder raised concerns about the security of the facility and the potential for deliberate release of the mosquitoes should the facility be sabotaged.

Department response:

The mosquitoes will be kept in a high-security BIC3 facility (see response to stakeholder 1). The access to a BIC3 facility is restricted to accredited personnel only. Staff must undergo rigorous training and must maintain accreditation to be able to work in these research facilities. Given the extreme security procedures and design of the facility it is unlikely the facility could be sabotaged, and the mosquitoes removed from the BIC3 facilities.

A second consultation round was undertaken in March 2021 with state and territory government agencies**.** No responses were received.

Conclusion

The department has undertaken a risk analysis and reviewed the available information on *Ae. albopictus* and the proposed amendment to include this species on the Live Import List.

The biology and ecology of *Ae. albopictus* suggests that if released, the species is likely to establish populations in Australia due to its high ecological and physiological plasticity. Evidence from around the world indicates that the species is highly invasive and is a vector for many zoonotic diseases.

The listing of *Ae. Albopictus* can only be considered under rigorous containment and security arrangements. The applicants have experience with holding high risk mosquito species over an extended time with no escapes meaning quarantine facilities and procedures have been developed to reduce the risk to an acceptable level.

Based on the very low risk of the imported populations of *Ae. albopictus* escaping from the secure facilities and becoming established in the wild, it is recommended that *Aedes albopictus* be listed on Part 2 of the Live Import List, with conditions restricting imports to "**Research only. High security facilities only**".

References:

Akiner, M. M., Öztürk, M., Baser, A. B., Günay, F., Hacıoğlu, S., Brinkmann, A., Emanet, N., Alten, B., Özkul, A., Nitsche, A., Linton, Y-M., Ergünay, K. (2019). Arboviral screening of invasive Aedes species in north eastern Turkey: West Nile virus circulation and detection of insect-only viruses. *PLoS Negl Trop Dis*, **13** (5): e0007334. . https://doi.org/10.1371/journal.pntd.0007334

Artsob, H., Lindsay, R., Drebot, M. (2017). Arboviruses, Editor(s): Stella R. Quah, International Encyclopedia of Public Health (Second Edition), Academic Press, 2017, Pages 154-160.

Bargielowski, I. E., Lounibos, L. P., D. Shin, D., Smartt, C. T., Carrasquilla, M. C., Henry, A., Navarro, J.C., Paupy, C., Dennett, J. A. (2015). Widespread evidence for interspecific mating between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in nature. *Infect Genet Evol*., **36**: 456 - 461.

Benedict, M. Q., Levine, R. S., Hawley, W. A., Lounibos, L. P. (2007). Spread of the Tiger: Global risk of invasion by mosquito *Aedes albopictus*. *Vector-Borne Zoonotic Diseases*, **7** (1): 76-85.

Bomford M (2008). *Risk assessment models for establishment of exotic vertebrates in Australia and New Zealand*. Invasive Animals Cooperative Research Centre, Canberra.

Braks, M. A. H., Nildimar, Honório, N. A., Lourenço-De-Oliveira, R., Juliano, S. A., L., Lounibos, L. P. (2003). Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in South-eastern Brazil and Florida. *Journal of Medical Entomology*, **40** (6): 785-794.

Brustolin, M., Talavera, S., Nuñes, A., Santamaría, C., Rivas, R., Pujol, N., Valle, M., Verdún, Brun, A., Pagès, N., Busquets, N. (2017). Rift Valley fever virus and European mosquitoes: vector competence of *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*). *Medical and Veterinary Entomology*, **31**: 365-372.

Chowers, M. Y., Lang, R., Nassar, F., Ben-David, D., Giladi, M., Rubinshtein, E., Itzhaki, A., Mishal, J., Siegman-Igra, Y., Kitzes, R., Pick, N., Landau, Z., Wolf, D., Bin, H., Mendelson, E., Pitlik, S. D., Weinberger, M. (2001). Clinical characteristics of the West Nile Fever outbreak, Israel, 2000. *Emerging Infectious Diseases*, **7** (4): 675-678.

Culbert, N.J., Gilles, J.R.L., Bouyer, J. (2019). Investigating the impact of chilling temperature on male *Aedes aegypti* and *Aedes albopictus* survival. *PLoS ONE*, **14** (8): e0221822. https://doi.org/10.1371/journal.pone.0221822

Davis, T. (2013). Oviposition Strategies of Aedes Albopictus (Skuse) (Diptera Culicidae): Analysing Behavioural Patterns for Surveillance Techniques and Control Tactics PhD thesis, University of Florida, 159 p.

Delatte, H., Dehecq, J. S., Thiria, J., Domerg, C., Paupy, C., Fontenielle, D. (2008). Geographic Distribution and Developmental Sites of *Aedes albopictus* (Diptera: Culicidae) During a Chikungunya Epidemic Event. *Vector-Borne and Zoonotic Diseases*, 8 (1): 25 – 34.

Delatte, H., Gimonneau, G., Triboire, A. & Fontenille, D. (2009). 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.

Diniz, D. F. A., de Albuquerque, C. M. R., Oliva, L. O., de Melo-Santos, M. A. V., Ayres, C. F. J. (2017). Diapause and quiescence: dormancy mechanisms that contribute to the geographical expansion of mosquitoes and their evolutionary success. *Parasites & Vectors*, **10**: 310. DOI 10.1186/s13071-017-2235-0

Eritja, R., Escosa, R., Lucientes, J., Marquès, E. Molina, R., Roiz, D., Ruiz, S. (2005). Worldwide invasion of vector mosquitoes: present European distribution and challenges for Spain. *Biological Invasions*, **7**: 87-97.

Ferreira-de-Lima, V. H., Andrade, P. dos S., Thomazelli, L. M., Marelli, M. T., Urbinatti, P. R., Almeida, R. M. M. de S., Lima-Camara, T. N. (2020). Silent circulation of dengue virus in *Aedes albopictus* (Diptera: Culicidae) resulting from natural transmission. *Nature Scientific Reports*, **10** (1): 3855.

Giatropoulos, A., Papachristos, D. P., Koliopoulos, G., Michaelakis, A., Emmanouel, N. (2015). Asymmetric Mating Interference between Two Related Mosquito Species: *Aedes (Stegomyia) albopictus* and *Aedes (Stegomyia) cretinus*. *PLoS ONE*, **10**(5): e0127762. doi:10.1371/journal.pone.0127762

Guber, D.J. (1970). Induced sterility in *Aedes (Stegomyia) polynesiensis* marks by cross-inseminating with *Aedes (Stegomyia) albopictus* Skuse. *Journal of Medical Entomology*, **7** (1): 65-70.

Gratz, N. G. (2004). Critical review of the vector status of Aedes albopictus. *Medical and Veterinary Entomology*, **18**: 215-227.

Hanson, S. M., Craig JR, G. B. (1995). Relationship between cold hardiness and supercooling point in *Aedes albopictus* eggs. *Journal of the American Mosquito Control Association*, **11** (1): 35-38.

Hugo, L. E., Prow, A. P., Tang, B., Devine, G., Suhrbier, A. (2016). Chikungunya virus transmission between Aedes albopictus and laboratory mice. *Parasites & Vectors*, **9**: 555.

Hugo, L. E., Stassen, L., La, J., Gosden, E., Ekwudu, O., Winterford, C., Viennet, E., Faddy, H. M., Devine, G. J., Frentiu, F. D. (2019). Vector competence of Australian Aedes aegypti and Aedes albopictus for an epidemic strain of Zika virus. *PLoS Neglected Tropical Diseases*, **13** (4): e0007281. https://doi.org/10.1371/journal.pntd.0007281

Jeremiah, CJ, Aboltins, CA and PA Stanley (2011). Lymphatic filariasis in Australia: an update on presentation, diagnosis and treatment. *MJA* **194** (12): 655-657.

Knudsen, A. B., Romi, R., Majori, G. (1996). Occurrence and spread in Italy of Aedes albopictus, with implications for its introduction into other parts of Europe. *Journal of the American Mosquito Control Association*, **12** (2): 177-183.

Kraemer, M. U. G., Sinka, M. E., Duda, A. K., Mylne, A. Q. N., Shearer, F. M., Barker, C. M., Moore, C. G., Roberta G Carvalho, R. G. C., Coelho, G. E., Bortel, W. V., Hendrickx, G., Schaffner, F., Elyazar, I. R. F., Teng, H. J., Brady, O. J., Messina, J. P., Pigott, D. M., Thomas W Scott, T. W., Smith, D. L., Wint, G. R. W., Golding, N., Hay, S. I. (2015). The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus. Ecology, Epidemiology and Global Health*, 4:e08347. DOI: 10.7554/eLife.08347.

Leahy, M. G., Craig Jr., G. B. (1967). Barriers to hybridization between Aedes aegypti and Aedes albopictus (Diptera: Culicidae). *Evolution,* **21:** 41-58.

Lounibos, L. P., Suárez, S., Menéndez, Z., Nishimura, N., Escher, R. L., O'Connell, S. M., Rey, J. R. (2002). Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? *Journal of Vector Ecology*, **27** (1): 86-95.

Lounibos, L. P., Escher, R. L., Lourenço-de-Oliveira, R. (2003). Asymmetric Evolution of Photoperiodic Diapause in Temperate and Tropical Invasive Populations of *Aedes albopictus* (Diptera: Culicidae). *Ecology and Population Ecology*, **96** (4):512-518.

Löwenberg Neto, P., Navarro-Silva, M. A. (2004). Development, longevity, gonotrophic cycle and oviposition of *Aedes albopictus* Skuse (Diptera: Culicidae) under cyclic temperatures. *Neotropical Entomology*, **33** (1): 29 – 33.

Lwande, O. W.; Obanda, V., Lindström, A., Ahlm, C., Evander, M., Näslund, J., Bucht, G. (2020). Globe-trotting Aedes aegypti and Aedes albopictus: Risk factors for Arbovirus Pandemics. *Vector-Borne and Zoonotic Diseases*, **20** (2), 71-81.

Medeiros-Sousa, A. R., de Oliveira-Christe, R., Camargo, A. A., Scinachi, C. A., Milani, G. M., Urbinatti, P. R., Natal, D., Ceretti-Junior, W., Marrelli, T., M. (2020). Influence of water's physical and chemical parameters on mosquito (Diptera: Culicidae) assemblages in larval habitats in urban parks of São Paulo, Brazil. *Acta Tropica*, **205**, 105394.

Medlock, J. M., Avenell, D., Barrass, I., Leach, S. (2006). Analysis of the potential for survival and seasonal activity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom. *Journal of Vector Ecology*, **31** (2): 292 - 304.

Medlock, J. M., Hansford, K. M., Versteirt, V., Cull, B., Kampen, H., Fontenielle, D., Hendrickx, G., Zeller, H., Van Bortel, W., Schaffner, F. (2015). An entomological review of invasive mosquitoes in Europe. *Bulletin of Entomological Research*, **105** (6): 637-663.

Monteiro, V. V. S., Navegantes-Lima, K. C., de Lemos, A. B., da Silva, G. L., Gomes, R. de S., Reis, J. F., Rodrigues Junior, L. C., da Silva, O. S., Romão, P. R. T., Monteiro, M. C. (2019). Aedes-Chickungunya virus interaction: Key role of vector midguts microbiota and its saliva in host infection. *Frontiers in Microbiology*, **10**: 492.

Moore, G. C. (1999). *Aedes albopictus* in the United States: Current status and prospects for further spread. *Journal of the American Mosquito Control Association*, **15** (2): 221-227.

Moore, C. G., Mitchell, C. J. (1997). *Aedes albopictus* in the United States: Ten-year presence and public health implications. *Emerging Infectious Diseases*, **3** (3): 329-334.

Paupy, C., Delatte, H., Bagny, L., Corbel, V., Fontenille, D. (2009). *Aedes albopictus*, an arbovirus vector: From the darkness to the light. *Microbes and Infection*, **11**: 1177-1185.

Pereira-dos-Santos, T., Roiz, D., Lourenço-de-Oliveira, R., Paupy, C. (2020). A Systematic Review: Is Aedes albopictus an Efficient Bridge Vector for Zoonotic Arboviruses? *Pathogens*, **9** (266): doi:10.3390/pathogens9040266

Reinhold, J. M., Lazzari, C. R. & Lahondère, C. (2018). Effects of the Environmental Temperature on Aedes aegypti and Aedes albopictus Mosquitoes: A Review. *Insects* **9** (4): 158.

Richards, S. L., Ponnusamy, L., Unnasch, T. R., Hassan, H. K., Apperson, C. S. (2006). 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** (3): 543-551.

Rozeboom, L. E., Bridges, J. R. (1972). Relative population densities of *Aedes albopictus* and *A. guamensis* on Guam. *Bulletin of the World Health Organization*, **46**(4): 477-483.

Scholte, E-J., Jacobs, F., Linton, Y-M., Dijkstra, E., Fransen, J., Takken, W. (2007). First Record of *Aedes (Stegomyia) albopictus* in the Netherlands. *European Mosquito Bulletin*, **22:** 5-9.

Tippelt, L., Werner, D., Kampen, H. (2019). Tolerance of three *Aedes albopictus* strains (Diptera: Culicidae) from different geographical origins towards winter temperatures under field conditions in northern Germany. *PLoS ONE*, **14** (7): e0219553. https://doi.org/10.1371/journal.pone.0219553

Tripet, F., Lounibos, L. P., Robbins, D., Moran, J., Nishimura, N., Blosser, E. M. (2011). Competitive Reduction by Satyrization? Evidence for Interspecific Mating in Nature and Asymmetric Reproductive Competition between Invasive Mosquito Vectors. *Am. J. Trop. Med. Hyg*., **85** (2): 265–270.

Tsai, T. F., Popovivi, F., Cernescu, C., Campbell, G. L., Nedelcu, N.I. (1998). West Nile encephalitis epidemic in south eastern Romania. *Lancet*, **352** (9130): 767-771.

Udayanga, L., Ranathunge, T., Iqbal, M.C.M., Abeyewickreme, W., Hapugoda, M. (2019). Predatory efficacy of five locally available copepods on *Aedes* larvae under laboratory settings: An approach towards biocontrol of dengue in Sri Lanka. *PLoS ONE*, **14** (5): e0216140. https://doi.org/10.1371/journal.pone.0216140

van den Hurk, A. F., Pyke, A. T., Mackenzie, J. S., Hall-Mendelin, S., Ritchie, S. A. (2019). Japanese Encephalitis Virus in Australia: From Known Known to Known Unknown. *Trop. Med. Infect.Dis.*, **4** (38): doi:10.3390/tropicalmed4010038

Waldock, J., Chandra, N. L., Lelieveld, J., Proestos, Y., Michael, E., Christophides, G., Parham, P. E. (2013). The role of environmental variables on *Aedes albopictus* biology and chikungunya epidemiology. *Pathogens and Global Health*, **107** (5), 224-241.

de Wispelaere, M. , Desprès, P., Choumet, V. (2017). European *Aedes albopictus* and *Culex pipiens* are competent vectors for Japanese Encephalitis Virus. *PLoS Neglected tropical diseases*, **11** (1): e0005294. doi:10.1371/journal.pntd.0005294

Williams, C. R. (2012). The Asian Tiger Mosquito (*Aedes alpopictus*) invasion into Australia: A review of likely geographic range and changes to vector-borne disease risk. *Transactions of the Royal Society of South Australia*, **136** (2): 128-136.

Websites:

ABRS, 2020a – Australian Biological Resources Study – Australian Faunal Directory page for "*Aedes (Stegomyia) albopictus* (Skuse, 1894)". Found at: https://biodiversity.org.au/afd/taxa/Aedes_%28Stegomyia%29_albopictus. Downloaded 28/09/2020.

ABRS, 2020b – Australian Biological Resources Study – Australian Faunal Directory page for "*Mesocyclops* Sars, 1914". Found at: https://biodiversity.org.au/afd/taxa/Mesocyclops. Downloaded 29/09/2020.

ABRS, 2020c – Australian Biological Resources Study – Australian Faunal Directory page for "*Macrocyclops albidus* (Jurine, 1820)". Found at: https://biodiversity.org.au/afd/taxa/Macrocyclops_albidus. Downloaded 29/09/2020.

ABRS, 2020d – Australian Biological Resources Study – Australian Faunal Directory page for "*Aedes Meigen, 1818*". Found at: https://biodiversity.org.au/afd/taxa/Aedes. Downloaded 29/10/2020.

American Heartworm Society 2019 - https://www.heartwormsociety.org/pet-owner-resources/heartworm-basics. Downloaded 4/3/19

BOM, 2020a - Bureau of Meteorology – Mean minimum and maximum monthly temperatures for Point Wilson (Geelong): http://www.bom.gov.au/jsp/ncc/cdio/cvg/av. Downloaded on 08/10/2020.

BOM, 2020b – Bureau of Meteorology – Minimum July temperatures in Breakwater (Geelong) for 7 years: http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_display_type=dataGraph&p_stn_num=087184&p_nccObsCode=43&p_month=07. Downloaded on 08/10/2020.

Catalogue of Life page for "*Aedes albopictus*". Found at: http://www.catalogueoflife.org/annual-checklist/2019/details/species/id/5b34c2a09eaceb93d340a20f2019d4a6. Accessed 01/09/2020.

CDC 2019 - https://www.cdc.gov/parasites/dirofilariasis/biology_d_immitis.html. Downloaded 4/3/19

CDC 2020 – Centers for Disease Control and Prevention: https://www.cdc.gov/vaccinesafety/concerns/guillain-barre-syndrome.html. Downloaded 30/09/2020.

CITES, 2020 - Endangered Species of Wild Fauna and Flora - https://www.cites.org/eng/disc/text.php. Accessed 16/10/2020.

Department of Agriculture and Water Resources, 2016 - Approved Arrangements For 7.3 Biosecurity insectary containment level 3 Requirements - Version 3 -

https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/biosecurity/import/arrival/approved-arrangements/7.3-requirements.pdf. Downloaded 27/10/2020.

Department of Health, 2017 - Table of arboviruses of importance to Australia https://www1.health.gov.au/internet/main/publishing.nsf/Content/health-arbovirus-important.htm

Department of Health, 2018 – Zika Virus Factsheet – The Basics https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-zika-factsheet-basics.htm. Downloaded on 15/10/2020.

Department of Health, 2019 - Yellow fever - general fact sheet https://www1.health.gov.au/internet/main/publishing.nsf/Content/health-pubhlth-strateg-communic-factsheets-yellow.htm. Downloaded on 15/10/2020.

ECDC 2019 - https://ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-aegypti. Downloaded 12/3/2019.

ECDC 2020 - https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-albopictus. Downloaded 31/8/2020.

GBIF Secretariat 2019 - https://www.gbif.org/species/1651430. Downloaded 31/8/2020.

GISD, 2020- Global Invasive Species Database (2020) Species profile: *Aedes albopictus*. http://www.iucngisd.org/gisd/species.php?sc=109 Downloaded 31/8/2020.

Hartman, K. (2011). "Aedes albopictus" (On-line), Animal Diversity Web. Accessed September 15, 2020 at https://animaldiversity.org/accounts/Aedes_albopictus/

Health 2019a - http://www.health.gov.au/internet/main/publishing.nsf/Content/ohp-chikungunya-fact-sheet.htm. Downloaded 4/3/19

Health 2019b http://www.health.gov.au/internet/main/publishing.nsf/Content/ohp-zika-factsheet-basics.htm. Downloaded 4/3/19

Lutz, N. (2000). A North Carolina Summer Pest - The Asian Tiger Mosquito *Aedes albopictus*. EcoAssess. North Carolina Central University. http://www.ibiblio.org/ecoaccess/info/wildlife/pubs/asiantigermosquitoes.html. Downloaded 01/09/2020.

NSW Health 2020a - http://medent.usyd.edu.au/arbovirus/mosquit/mosqfact.htm#:~:text=Diseases%20transmitted%20by%20mosquitoes%20in,humans%20in%20a%20world%20context. Downloaded 15/10/2020.

NSW Health 2020b - http://medent.usyd.edu.au/arbovirus/viruses/rossriverbarmahforest.htm. Downloaded on 15/10/2020.

NSW Health 2020c - http://medent.usyd.edu.au/arbovirus/viruses/murrayvalleyencephalitisandkunjin.htm. Downloaded on 15/10/2020.

OGTR, 2011 – Australian Government, Department of Health and Ageing, Office of the Gene Technology Regulator - http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/PC3inv2.1-toc/$FILE/PC3INV2-1.pdf. Downloaded 27/10/2020.

SCI Journal 2020 – Science Journal Impact Factor for Infection, Genetics And Evolution - https://www.scijournal.org/impact-factor-of-infect-genet-evol.shtml. Accessed on 08/10/2020

Weather and Climate 2019. https://weather-and-climate.com/average-monthly-Rainfall-Temperature-Sunshine-in-Cook-Islands. Downloaded 11/01/19

WHO 2020a - https://www.who.int/news-room/fact-sheets/detail/chikungunya. Downloaded 14/10/2020.

WHO 2020b - https://www.who.int/health-topics/zika-virus-disease#tab=tab_1. Downloaded 14/10/2020.

WHO 2020c - https://www.who.int/health-topics/yellow-fever#tab=tab_1. Downloaded 14/10/2020.

WHO 2020d - https://www.who.int/health-topics/dengue-and-severe-dengue#tab=tab_1. Downloaded 14/10/2020.

WHO 2020e - https://www.who.int/news-room/fact-sheets/detail/japanese-encephalitis. Downloaded 14/10/2020.WHO 2020f - https://www.who.int/news-room/fact-sheets/detail/west-nile-virus. Downloaded 14/10/2020.