Scientific Note

Detection of *Aedes albopictus* pre-imaginal stages in brackish water habitats in Brunei Darussalam

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The mosquitoes *Aedes (Stegomyia) albopictus* Skuse and *Aedes (Stegomyia) aegypti* (Linnaeus) are vectors of dengue, chikungunya, and other arboviruses and are widely regarded to undergo pre-imaginal development only in freshwater habitats. As there is no vaccine or specific drug for dengue, eliminating or larviciding the freshwater habitats of the vectors are important strategies for controlling dengue worldwide. However, such measures have failed to eradicate dengue from Brunei Darussalam, with an incidence of 73 cases per 100,000 persons in 2010, and other Southeast Asian countries with extensive coastal areas (Arima and Matsui 2011, Chun et al. 2007). They also did not prevent recent epidemics of chikungunya in Asia (Lobo et al. 2011, Surendran et al. 2007, Weaver and Reisen 2010). Two-fifths of the world's population is now at risk from dengue, which shows an increasing incidence and global spread. *Aedes aegypti* and *Ae. albopictus* were recently shown to oviposit and undergo pre-imaginal development in brackish water (fresh, brackish, and saline waters are defined as containing <0.5, 0.5 to 30, and >30 parts per thousand (ppt) salt, respectively) in discarded plastic and glass containers, abandoned boats, and wells in coastal areas of Sri Lanka in South Asia (Ramasamy et al. 2011, Surendran et al. 2012). Such brackish water habitats are potential sources of vectors that may contribute to the transmission of dengue and other arboviral diseases in coastal areas. We therefore investigated the possible presence of *Ae. aegypti* and *Ae. albopictus* larvae in brackish water collections along the South China Sea coast of Brunei Darussalam, a dengue-endemic Southeast Asian country where dengue is known to occur in coastal areas (Chun et al. 2007).

Approximately 300 potentially brackish stagnant water collections in natural pools or discarded food and beverage containers along beaches, estuaries, and lagoons were examined for the presence of *Ae. aegypti* and *Ae. albopictus* larvae and pupae at 12 locations along the South China Sea coast of Brunei Darussalam. This work was carried out between December and March in 2010/2011 and 2011/2012, which is a relatively dry season in Brunei Darussalam. The 12 locations in three of the four administrative districts of Brunei Darussalam were selected for the survey because of their position on the outskirts of major urban centers as well as ease of access. Inundations at high tides and sea spray were the likely cause of brackishness in the studied water collections.

When pre-imaginal stages were observed, they were collected with a Pasteur pipette, transferred with the habitat water to plastic containers, and brought to the laboratory for identification using standard keys (Barraud 1934, Rueda 2004). Salinity was measured with a salinity refractometer (Atago, Japan) and confirmed where necessary with a conductivity meter (Hach, CO, U.S.A.). The instruments were pre-calibrated with solutions of 0 to 30 ppt sodium chloride in the laboratory.

Salinity tolerance of *Ae. albopictus* in the laboratory was first investigated by allowing batches of 85 eggs each, collected from fresh water ovitraps, to hatch and develop into adults at salinities of 0 to 20 ppt, essentially as described by Ramasamy et al. (2011). Rain water was mixed in different proportions with 27 ppt salinity sea water in Petri dishes for the experiment. Based on the result, the development of 1st instar larvae from batches of 50 eggs each were subsequently tested in three replicates at both 8 and 10 ppt salinity essentially as described (Ramasamy et al. 2011). The approximate level of salinity producing 50% failure of 1st instar larvae to emerge as adults (LC_{50}) was determined by graphical analysis.

Pre-imaginal stages of *Ae. albopictus* were found in 13 brackish water collections of 0.5 to 8 ppt salinity at six sites: four beaches (Serasa, Tungku, Meragang, and Seri Kenangan) and two estuarine villages (Pelambaian and Melayu Asli) (Figure 1). The six sites were located on the suburban outskirts of the city of Bandar Seri Begawan, which is the national capital and the capital of the Brunei-Muara district, as well as the towns of Kuala Belait and Tutong that are respective district capitals of the Belait and Tutong districts (Figure 1). Immature stages of *Ae. albopictus* were also observed in fresh water collections in some of the surveyed sites. Table 1 summarizes relevant characteristics of the brackish water habitats containing *Ae. albopictus* with illustrative photographs in Figure 2. *Aedes aegypti*, although common in fresh water containers in houses in urban Brunei Darussalam (Chun et al. 2007 and our unpublished observations), was not found in brackish or fresh water at any of the 12 peri-urban locations studied. Culicine larvae that did not belong to the genus *Aedes* were also found in five brackish water collections of up to 10 ppt salinity at four sites as well as fresh water in different study sites.

We also observed that late stage larvae collected from the discarded plastic container in Serasa beach at 8 ppt salinity, pupated and became *Ae. albopictus* adults when maintained in their natural habitat water in the laboratory. An initial laboratory experiment showed that 33, 58, 68, 20, 14, and 4% of *Ae. albopictus* eggs collected from fresh water ovitraps hatched and 100, 85, 5, 0, 0, and 0% of 1st instar larvae completed development to adults at 0, 8, 10, 12, 15, and 20 ppt salinity, respectively. The survival of 1st instar *Ae. albopictus* larvae to adulthood was 83.4±1.4 and 6.7±1.3 (mean ± standard deviation of triplicate determinations) at 8 and 10 ppt salt, respectively, in a subsequent experiment, yielding an approximate LC_{50} of 9 ppt for this process.

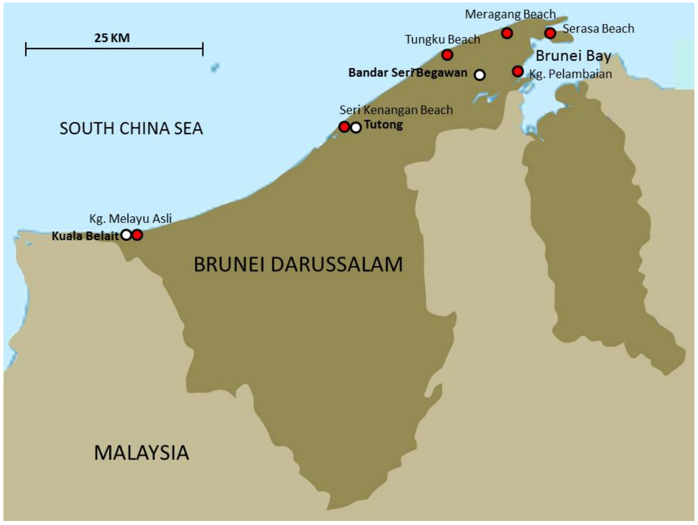

Figure 1. Map of Brunei Darussalam showing the six coastal sites with brackish water collections that contained *Aedes albopictus* pre-imaginal stages (red circles).

Figure 2. Brackish water collections where pre-imaginal stages of *Aedes albopictus* were found on the coast of Brunei Darussalam. (A) cut plastic bottle and (B) plastic cup in a rock pool at Serasa beach; (C, D) plastic cups in rock pools at Tungku beach; (E) coconut shell at Seri Kenangan beach.

The presence of the pre-imaginal stages of *Ae. albopictus* in brackish water of up to 8 ppt salinity in peri-urban settings in Brunei Darussalam builds on data from Sri Lanka that show *Ae. albopictus* in urban and peri-urban habitats of up to 14 ppt salinity (Ramasamy et al. 2011, Surendran et al. 2012). The LC_{50} for salinity in the transformation of 1st instar larvae of *Ae. albopictus* to adults in the Jaffna peninsula of Sri Lanka was 13 ppt, with 100% survival to adulthood seen at 10 ppt salinity (Ramasamy et al. 2011). The possible higher salinity tolerance of *Ae. albopictus* in the Jaffna peninsula compared with Brunei Darussalam and a coastal location in mainland Sri Lanka may be attributed to adaptation to the more extensive groundwater salinization in the peninsula (Ramasamy et al. 2011, Surendran et al. 2012, Ramasamy and Surendran 2012).

The salinity of the observed brackish water habitats along the Brunei Darussalam coast could have conceivably changed over time due to the combined effects of rainfall, sea spray, and tides. Hence, it is possible that the developmental sequence from oviposition to adult emergence may occur at different salinities. More detailed studies are needed to evaluate such possible salinity changes and their impact on mosquito development. Significant hatching of eggs and pre-imaginal development of *Ae. albopictus* to adulthood at up to 8 ppt salinity in the laboratory, however, supports the view that the same process can also happen in brackish water habitats in the environment in Brunei Darussalam.

The greater preference of *Ae. aegypti* to rest and oviposit indoors (endophilicity) or closer to houses than *Ae. albopictus* (Barraud 1934) may be one reason for not detecting *Ae. aegypti* in the present study. The Sri Lankan sites where *Ae. aegypti* were found in brackish water (Ramasamy et al. 2011, Surendran et al. 2012) had a greater population density and attendant shorter distances between the coastal habitats and houses than the sites investigated in Brunei Darussalam. Brackish-water development shown in the present study to occur in Brunei Darussalam for *Ae. albopictus*, and previously in Sri Lanka for *Ae. aegypti* and *Ae. albopictus* (Ramasamy et al. 2011, Surendran et al. 2012), could be a widespread phenomenon that can contribute to the transmission of dengue and other arboviral diseases in coastal areas. *Ae. albopictus* has been associated with recent outbreaks of dengue and chikungunya in continental Europe (Rezza et al. 2007, La Ruche et al. 2010), and dengue in southern China (Peng et al. 2012) and many islands worldwide (Rezza 2012). *Ae. albopictus* adapted to tolerate cold temperatures and/or salinity can therefore potentially become an increasingly important vector in the transmission of dengue and other arboviral diseases worldwide.

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Table 1. Characteristics of coastal brackish water collections containing *Aedes albopictus* in Brunei Darussalam.

Sites	No. of containers with *Ae. albopictus* in brackish water	Nature of containers (salinity/ distance from sea or estuary)	Numbers of larvae and pupae per 100 ml water	Pre-imaginal stages observed
Serasa beach	2	Plastic container (8 ppt/ 0.5m)	4	Late stage (3rd-4th instar) larvae
		Plastic container (1 ppt/3m)	<10	Late stage larvae, pupae
Tungku beach	7	Plastic containers (0.5-3 ppt/3-10m)	<20	Early (1st-2nd instar) and late stage larvae, pupae
Meragang beach	1	Plastic container (2 ppt/5m)	6	Late stage larvae, pupae
Pelambaian	1	Plastic container (0.7 ppt/15m)	5	Late stage larvae
Seri Kenangan beach	1	Coconut shell (2 ppt/10m)	4	Late stage larvae
Melayu Asli	1	Pit in stone fence (1 ppt/10m)	<20	Early and late stage larvae and pupae

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