# Chapter 3
# Adaptation of Freshwater Mosquito Vectors to Salinity Increases Arboviral Disease Transmission Risk in the Context of Anthropogenic Environmental Changes

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**Core Message** *Aedes aegypti* and *Aedes albopictus*, the dominant vectors of dengue, chikungunya and yellow fever, have been regarded to undergo pre-imaginal development only in freshwater. Vector control efforts therefore presently target only their freshwater habitats. The two *Aedes* vectors have recently been shown to develop in brackish water with associated biological changes in *Ae. aegypti*. Anthropogenic environmental changes have produced habitats that favour brackish water adaptation in the two *Aedes* and other vector species and increase the risk of arboviral disease transmission. Such changes can also increase disease transmission by other salinity-tolerant arboviral vector mosquitoes. Appropriate strategies are needed to address the associated health risks, particularly in the context of rising sea levels increasing coastal groundwater salinity.

## 1 Human Arboviral Diseases and Their Mosquito Vectors

Dengue is the most common arboviral disease of humans, with 50–100 million annual cases in more than 100 countries and an increasing incidence and spread worldwide that places 2.5 billion people at risk according to the World Health Organisation (WHO) [1]. Severe dengue or dengue hemorrhagic fever has a case fatality rate of 2.5 % [1]. There is presently no licensed vaccine or specific antiviral drug for dengue [2]. Yellow fever has a zoonotic reservoir, is endemic in Africa and South America with the potential to spread to Asia and is responsible for 200,000 cases and 30,000 deaths in the world every year [3]. An effective live attenuated

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vaccine is however available against yellow fever. Chikungunya, a debilitating arboviral disease, is endemic in Southeast Asia and has produced epidemics in tropical Africa and Asia, and more recently in temperate Europe and the Americas [4–6]. A vaccine against chikungunya is not yet available. Many other arboviruses have animal reservoirs and can be transmitted to humans with serious risk to health. These include viruses causing West Nile fever, Japanese encephalitis, St. Louis encephalitis and Eastern and Western equine encephalitis [7]. However the viremia that develops in humans is typically not high enough to permit human-to-human transmission but this situation can change if the viruses undergo appropriate genetic alterations.

*Aedes aegypti* (Linnaeus) (Diptera: *Culicidae*) is the principal mosquito vector of arboviruses causing yellow fever, dengue and chikungunya in populated areas of tropical and subtropical regions [7, 8]. *Aedes albopictus* Skuse is however becoming an increasingly important vector of dengue and chikungunya because of its recent global spread, the evolution of an ability to survive winters and a propensity to outcompete *Ae. aegypti. Aedes albopictus* has been responsible for the recent transmission of dengue and chikungunya in temperate regions [4, 5, 7, 9]. Both species are able to bite outdoors and during the day making the use of bed nets less effective than for malaria control. Therefore the control of dengue and chikungunya in tropical countries mainly relies on surveillance for *Ae. aegypti* and *Ae. albopictus* larvae, elimination of their pre-imaginal development habitats, use of larvicides and the space spraying of insecticides in areas of high transmission [1, 2, 5].

## 2 Salinity-Tolerant Mosquito Vectors of Human Arboviral Diseases

While most mosquito species lay eggs and undergo pre-imaginal development in freshwater, approximately 5 % of mosquito species undergo pre-imaginal development in brackish and saline waters. Water with less than 0.5 ppt or parts per thousand, 0.5–30 and greater than 30 ppt of sodium chloride are commonly termed fresh, brackish and saline, respectively [10]. Many mosquitoes that transmit human arboviral diseases, including *Ae. aegypti* and *Ae. albopictus,* have been long considered to undergo pre-imaginal development only in freshwater habitats [1, 2, 8, 11]. However, some salinity-tolerant mosquito species are known to transmit human arboviral diseases [8, 10, 12]. Common examples of salinity-tolerant mosquito vectors and the arboviral diseases that they transmit are presented in Table 3.1. Salinity-tolerant mosquito larvae and pupae have evolved various physiological and structural mechanisms to cope with high osmolarity in their aqueous environment [13–15].

**Table 3.1** Salinity-tolerant mosquito vectors of human viral diseases

| Species | Distribution | Examples of viruses transmitted |
|---|---|---|
| *Aedes dorsalis* | Pacific coast of North America, Temperate Eurasia | West Nile and Western equine encephalitis viruses |
| *Ae. (Ochlerotatus) taeniorhynchus* | North and South America | Eastern equine encephalitis virus |
| *Ae. togoi* | North Pacific rim | Japanese encephalitis virus |
| *Ae. (Ochlerotatus) vigilax* | Australasia, Southeast Asia | Ross River and Barmah forest viruses |
| *Culex sitiens* | Indian Ocean rim countries | Japanese encephalitis and Ross River viruses |
| *Cx. tarsalis* | North America | St. Louis encephalitis, Western equine encephalitis and West Nile viruses |
| *Cx. tritaeniorhyncus* | Russia, Middle East, Africa, India | Japanese encephalitis virus |

Common examples of mosquito vector species that undergo pre-imaginal development in brackish or saline water, their geographical distribution and the viruses that they transmit are listed in this table (modified with permission from Ramasamy and Surendran, BMC Infectious Diseases 11:18 (2011). doi:10.1186/1471-2334-11-18 and Ramasamy and Surendran, Frontiers in Physiology 3:198 (2012). doi: 10.3389/fphys.2012.00198)

## 3 Recent Evidence Shows That *Ae. aegypti* and *Ae. albopictus* Can Undergo Pre-imaginal Development in Brackish Water

There is now evidence that *Ae. aegypti* and *Ae. albopictus* can also lay eggs and undergo pre-imaginal development into adults in brackish water collections in coastal areas of the tropics [16–21]. Pre-imaginal stages of *Ae. aegypti* and *Ae. albopictus* were observed in brackish water in coastal areas of up to 15 ppt and 14 ppt salinity, respectively, in northern and eastern Sri Lanka [16–18] and up to 8 ppt salinity in Brunei Darussalam [19, 20].

The habitats where the larvae of the two vectors were found in Sri Lanka were brackish water collections in discarded plastic and glass food and beverage containers along beaches, disused fishing boats and coastal domestic wells that were either abandoned or used for domestic purposes other than drinking, e.g., washing clothes and bathing (Fig. 3.1). Discarded food and beverage containers were also found to provide suitable brackish water habitats for the development of *Ae. albopictus* along the South China sea coast of Brunei Darussalam [19].

In the Jaffna peninsula in northern Sri Lanka, recent data show that adaptation to brackish water is accompanied by greater tolerance of *Ae. aegypti* larvae to salinity, which is only partly reversible after transfer to freshwater for five generations [21]. This suggests that genetic and reversible physiological changes are in combination


**Fig. 3.1** Typical brackish water habitats of *Aedes* larvae in coastal areas of northern and eastern Sri Lanka. The photographs show the brackish water collections containing larvae in (**a** and **b**) disused boats; (**c** and **e**) abandoned wells; (**d** and **f**) discarded food and beverage containers (reproduced with permission from Ramasamy et al. PLoS Neglected Tropical Diseases 5(11): e1369. doi:10.1371/journal.pntd.0001369)

responsible for the adaptation to brackish water. Brackish water *Ae. aegypti*, unlike freshwater isolates in the Jaffna peninsula, tended to prefer laying eggs in brackish water [21]. But the hatching of eggs was significantly less efficient and the time taken for larvae to develop into pupae was prolonged in 10 ppt brackish water compared to freshwater with both brackish and freshwater-derived *Ae. aegypti* [21]. Brackish and freshwater *Ae. aegypti* isolates from the Jaffna peninsula were however shown to interbreed and produced viable offspring in the laboratory [21]. These findings also showed that there was restricted gene flow between coastal brackish and inland freshwater *Ae. aegypti* isolates even when separated by a distance of only 5 km in the Jaffna peninsula. The results suggest that there is an ongoing adaptive process involving genetic and physiological changes, which could potentially lead to speciation in the future if the two populations were to become reproductively isolated.

## 4 Significance of the Likely Role of Brackish Water *Aedes* Vectors in Dengue Transmission

The analysis of the relationship between monsoonal rainfall and the incidence of dengue in coastal areas of northern and eastern Sri Lanka has been performed to evaluate the contribution of brackish water developing *Aedes* vectors in dengue transmission in the island [20]. The findings showed that monsoon rains, that allow freshwater to collect and form suitable habitats for pre-imaginal development of

*Aedes* vectors in the vicinity of populated areas, are the dominant driver for the increased dengue transmission that follows the monsoons in Sri Lanka. This was also found to hold true for the dry zone coastal districts of Jaffna and Batticaloa in northern and eastern Sri Lanka that receive rainfall during the Northeast monsoon [20] and where pre-imaginal development of *Ae. aegypti* and *Ae. albopictus* in brackish water habitats was first observed [16].

However it is likely that brackish water habitats provide a previously unappreciated source of vectors for maintaining dengue transmission in coastal areas that may be particularly important in a local context, e.g., coastal areas of Jaffna city in northern Sri Lanka [16–18]. Some brackish water habitats such as coastal wells constitute perennial habitats that are relatively independent of rainfall. Monsoonal and inter-monsoonal rains in combination with sea spray and tidal movements may also cause brackish water to accumulate in discarded plastic and glass containers and coastal rock pools that provide additional habitats for the dengue vectors. However monsoonal rains rapidly increase the extent of freshwater habitats for the *Aedes* vectors in coastal and inland areas leading to the greater transmission of dengue that follows soon after the rains. It seems possible therefore that brackish water vectors in coastal areas may play a role as a perennial reservoir of virus to support the post-monsoonal amplification of dengue transmission by freshwater vectors. Vertical or transovarial transmission of dengue virus in brackish water-adapted *Aedes* will enhance their capacity to serve a virus reservoir in the period between monsoons. Determining the relative vector competence of brackish and freshwater vectors, changes in adult vector densities and larval indices, temporal and spatial variation in dengue cases and rainfall and other pertinent epidemiological parameters during and between monsoons in selected coastal districts will be needed to confirm this hypothesis.

## 5 Anthropogenic Environmental Changes That Expand Brackish Water Habitats Increase the Potential for Transmission of Arboviral Diseases

### *5.1 Rising Sea Levels*

Global climate change is caused by long-term changes in common meteorological parameters that can be ascribed to anthropogenic influences, particularly the continuing accumulation of greenhouse gases like carbon dioxide in the atmosphere. Temperature, rainfall and humidity are primary climate changes and these have been studied for their impact on common vector-borne diseases, notably malaria and dengue (reviewed in [10, 12, 20]). These studies predict an expansion of the range of mosquito vectors due to climate change. Primary changes in global climate lead to secondary changes in the biosphere and geosphere, including an altered distribution of animals and plants and a rise in sea levels [10]. It has been proposed with supporting evidence that a rise in sea levels caused by global warming can potentially increase the transmission of vector-borne diseases [12].

The Intergovernmental Panel on Climate Change in its most recent report predicts a likely sea level rise of up to 82 cm by the year 2100 [22]. A rise in sea levels will produce greater saline intrusion into coastal freshwater aquifers [10, 12, 20, 23]. This will be particularly important for low-lying areas like the Jaffna peninsula in northern Sri Lanka which is largely composed of relatively porous limestone [10, 20]. The Jaffna peninsula also has a high population density and consequently a high rate of groundwater withdrawal rate from freshwater aquifers that are only replenished during the Northeast monsoon in October to December [10, 20]. A rise in sea level will therefore increase the availability of brackish water larval habitats for brackish water-adapted *Ae. aegypti* and *Ae. albopictus* as well as other brackish water mosquito vectors of human disease that are present in the peninsula and outlying islands [16–18, 21].

Tropical South and Southeast Asia have extensive coastlines bordering the Indian and Pacific Oceans and various seas. Southeast Asia also has the largest archipelago in the tropics encompassing populous countries like Indonesia and the Philippines with extensive coastal areas compared to their total land area. A similar situation prevails in the Caribbean countries and the Indian Ocean islands of Seychelles, Singapore and Reunion.

Countries with long coastlines and high coast to land area ratios are particularly vulnerable to the consequences of rising sea levels. One in ten persons worldwide live in coastal localities that are less than 10 m above sea level [24]. Such low-lying areas are prone to greater salinisation caused by rising sea levels. Many densely populated tropical countries have a large proportion of their populations living in such vulnerable areas, e.g., Vietnam, Bangladesh, Thailand and Indonesia [24].

## *5.2 Expanding Coastal Populations and Beach Litter*

Beach container litter provides excellent habitats for brackish water-adapted *Ae. aegypti* and *Ae. albopictus* [16, 19]. Increasing population densities along coastal regions will result in the construction of more wells that may become brackish and a greater tendency to litter the shoreline and beaches with discarded containers that can collect brackish water. Garbage collection and disposal mechanisms managed by local government authorities in coastal areas of resource-poor countries may find it hard to cope with such hazardous environmental changes posed by expanding populations.

Population increase in coastal areas can reduce vegetation cover, thereby driving vector mosquitoes, notably *Ae. albopictus,* to seek other habitats closer to human dwellings for laying eggs. Another likely consequence is a decrease in the relative numbers of animals and birds that can serve as alternative sources of a blood meal. The two factors combined with higher population density will increase the human biting rate and therefore the rate of propagation of arboviral diseases as outlined previously [10].

## 5.3 Agriculture in Coastal Zones

Intensive agriculture in dry coastal areas promotes salinisation of the land and causes inland water bodies to become more saline, thereby facilitating the expansion of salinity-tolerant mosquito vector habitats. For example, higher densities of *Aedes* (*Ochlerotatus*) *camptorhynchus*, a vector of Ross River virus, are associated with increasing salinisation of inland freshwater bodies caused by wheat farming in Western Australia [25]. Aquaculture, an increasing economic activity along tropical coasts, also creates new brackish water habitats for mosquito vectors. There is presently no data on its ensuing impact on the transmission of arboviral diseases though a consequential increase in malaria vector populations has been recorded [12].

## 5.4 Use of Insecticides and Larvicides in Inland Areas Can Drive the Adaptation of Freshwater Vector Mosquitoes to Coastal Brackish Water Habitats

Insecticides are used for agricultural pest control in inland areas. Malaria control programmes use indoor residual spraying of insecticides that target houses in endemic areas. Larvicides against malaria vectors are predominantly applied to freshwater collections in most countries and this has been exclusively the case for dengue control targeting *Ae. aegypti* and *Ae. albopictus.* Adaptation of vector mosquitoes to coastal brackish water habitats therefore has a selective advantage. Data from the Jaffna peninsula in northern Sri Lanka recently documented that coastal brackish water isolates of *Ae. aegypti* were significantly more sensitive to the organophosphate insecticide malathion than inland populations [21]. It was proposed that this was caused by the recent use of malathion for malaria control and organophosphate insecticides in general for controlling agricultural pests in inland areas of the Jaffna peninsula [21].

## 5.5 Increased Coastal Arboviral Disease Transmission Will Impact on Inland Areas

Any increase in the transmission of arboviral diseases in coastal areas due to conducive environmental changes will also act to increase disease incidence in inland areas through bridging freshwater vectors and inland movement of infected persons.

## 6 Implications for Other Arboviral Diseases Transmitted by Mosquitoes

*Ae. aegypti* and *Ae. albopictus* are potential vectors for arboviruses other than those causing dengue, chikungunya and yellow fever [8]. *Ae. albopictus*, for example, has been reported to be a vector of 26 different viruses from the Bunyaviridae, Flaviviridae, Nodaviridae, Reoviridae and Togaviridae families [26]. Brackish water-developing *Ae. aegypti* and *Ae. albopictus* may therefore make a hitherto unrecognised contribution to the transmission of many zoonotic arboviruses to humans in coastal areas. Besides *Ae. aegypti* and *Ae. albopictus*, other freshwater arboviral vectors also have the potential to adapt to brackish water and contribute to disease transmission.

The increased extent of brackish water habitats caused by anthropogenic factors outlined in Sect. 5 also heighten the risk of arboviral disease transmission by known salinity-tolerant mosquito vector species.

## 7 Strategies for Controlling the Transmission of Arboviral Diseases in Coastal Areas

Local government and national and international authorities responsible for concerned sectors, e.g., health, agriculture, coastal planning, environment, irrigation, local government and livestock development, need to be aware of the health risk associated with mosquito vectors developing in brackish water habitats in coastal areas. The potentially greater risk associated with rising sea levels needs to be appreciated and included appropriately in development plans. The WHO in particular needs to take cognizance of the likely role of brackish water mosquitoes in transmitting dengue and chikungunya and incorporate it into their international guidelines. Most countries depend on the WHO recommendations for formulating national dengue control programmes and the WHO guidelines are presently based on the assumption that the *Aedes* vectors of dengue only develop in freshwater habitats [2].

There is a need for more research at all levels into the bionomics of salinity-tolerant mosquito vector populations that has become more important in the context of the documented ability of the most important arboviral vectors to adapt to salinity. The underlying genetic and physiological mechanisms that facilitate salinity adaptation in *Ae. aegypti* and *Ae. albopictus* need to be elucidated. A readily usable molecular marker for salinity tolerance in the two species would be a useful epidemiological tool. Mathematical models of mosquito-borne disease transmission would need to incorporate the consequences of vectors undergoing development in brackish water habitats. Larvicidal formulations with *Bacillus thuringiensis* toxin that were primarily developed for freshwater use are less effective against *Ae. aegypti* in brackish water at high salinities [17]. Controlling pre-imaginal stages of the vectors in brackish water may therefore require the development of specific larvicides or larvicide formulations. Larvivorous fish and predatory mosquito larvae that are effective in controlling vector larvae in brackish water [18, 27] are other measures that can be utilised in brackish water habitats in coastal areas.

The monitoring of disease incidence in tropical coasts and mosquito vector development in coastal brackish and saline water habitats is a current deficiency that needs to be redressed. The immediate extension of vector source reduction and management programmes to the brackish water habitats of *Ae. aegypti* and *Ae. albopictus* may rapidly improve disease control in coastal areas, e.g., Kurunagar in Jaffna, Sri Lanka, that has a high dengue incidence [16]. Larval source reduction in defined habitats with the cooperation of affected communities has proved effective in controlling dengue and chikungunya in many countries. The likely role in disease transmission of salinity-tolerant mosquito vectors developing in brackish water habitats provided by beach litter and domestic wells need to communicated to coastal communities at risk and their cooperation sought for disease control.

**Conflict of Interest** The author declares no conflict of interest in this publication.

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