Impact of salinization and pollution of groundwater on the adaptation of mosquito vectors in the Jaffna peninsula, Sri Lanka

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Abstract: Mosquito-borne diseases are a major health concern in many tropical and sub-tropical countries. In the absence of specific treatment for many mosquito-borne diseases, vector control in the form of eliminating preimaginal development sites and insecticide application has an important role in controlling these diseases. Anthropogenic environmental changes have become important driving forces causing the adaptation of many major mosquito vectors to such changes. Anthropogenic activities are major contributors to global warming that is causing rise in sea levels. Sea level rise along with over exploitation of groundwater results in sea water intrusion to fresh water aquifers causing fresh water salinization in coastal zones. Human activities, including the extensive use of fertilizers and agrochemicals, also cause groundwater pollution. Mosquito vectors that normally lay eggs and undergo preimaginal development in fresh water are now seen to be adapting to develop in brackish and polluted water habitats. This article reviews recent findings that show the adaptation of mosquito vectors of human diseases to lay eggs and undergo preimaginal development in groundwater that is undergoing rapid salinization and pollution in the Jaffna peninsula.

Keywords: anthropogenic environmental changes, groundwater pollution, Jaffna peninsula, mosquito adaptations, mosquito range expansion, mosquito vector-borne diseases, salinization of groundwater.

INTRODUCTION

Mosquito-borne diseases are the largest contributor to human vector-borne disease burden with more than 80% of the world population at risk (Franklinos et al., 2019). Mosquito vectors transmit many parasitic (e.g. malaria, filariasis) and arboviral (e.g. dengue, chikungunya, yellow fever and Zika fever) diseases that cause significant mortality and morbidity in many countries. It has been estimated that in 2018 there were approximately 228 million malaria cases and 405, 000 deaths worldwide, and of these, the Southeast Asia had an estimated 8 million cases and nearly 12,000 deaths (WHO, 2019). Malaria is caused by protozoan parasites of the genus Plasmodium, predominately by Plasmodium vivax and P. falciparum, and is transmitted by several species of Anopheles mosquitoes (Sinka et al., 2012). Lymphatic filariasis is caused by the nematode parasites Wuchereria bancrofti, Brugia malayi and Brugia timori and is a disease that is present in 49 countries with an estimated 893 million people at risk (WHO, 2020a). Different species of mosquitoes transmit lymphatic filariasis. Mosquito species belong to genus Culex are the widespread vectors while Anopheles and Aedes mosquitoes transmit the disease in rural areas and some Pacific islands respectively (WHO, 2020a). Dengue is an arboviral disease with an estimated 100-400 million infections worldwide. A fifteen-fold increase has occurred in the number of dengue cases worldwide during the last two decades (WHO, 2020b). There were 146, 914 confirmed cases of chikungunya worldwide in 2016 (WHO, 2020c). Zika fever outbreaks have been recorded from 87 counties with a peak in transmission during 2015-2016 in Africa, the Americas, Southeast Asia and Western Pacific and there has been over 2 million reported cases in the Americas alone since 2007 (WHO, 2020d). Yellow fever is prevalent across 47 countries in Africa and the Americas with approximately 170, 000 cases and 60, 000 deaths (WHO, 2020e). Mosquito species Aedes aegypti and Aedes albopictus are considered as the primary and secondary mosquito vectors respectively that transmit dengue, chikungunya, yellow fever and Zika in tropical and subtropical countries. The mosquito Aedes aegypti originated in the forests of tropical Africa but its subsequent adaptation to blood-feed on humans and oviposit and undergo preimaginal development in water collections near human habitations has facilitated its global spread (Crawford et al., 2017). Aedes aegypti has long been regarded to develop only in fresh water collections near human habitations (Barraud, 1934). Aedes albopictus is also considered to be an invasive sylvatic species that has also adapted more recently to develop in freshwater habitats in the urban and semi-urban environment in many countries, including Sri Lanka (WHO, 2012; Ramasamy et al., 2011). Aedes albopictus has recently expanded its range to temperate countries by developing a diapausing egg stage that has enabled it to survive winters (reviewed in Ramasamy and Surendran, 2012).

The mosquito-borne diseases malaria, dengue and filariasis have been historically prevalent in Sri Lanka. In 2016, WHO declared Sri Lanka free of malaria and...
Mosquito vectors adapting to anthropogenic environmental changes

Anthropophilic mosquito vectors have, therefore, evolved over a long-time period to take advantage of changes in the environment caused by human population increase and expansion, and societal features such as agriculture and urbanization, and these have markedly impacted the transmission and prevalence of mosquito-borne diseases (Vora, 2008; Ramasamy and Surendran, 2016). Many of the dominant mosquito vectors are anthropophilic (WHO, 2015 a,b) and have evolved behavioral traits and ecological features to lay eggs and undergo preimaginal development in freshwater habitats in close proximity to human dwellings. For example, *Ae. aegypti* can develop in water collections within houses (Juliano and Lounibos, 2005) and malaria vectors in freshwater pools and water storage tanks near houses and other buildings (Barraud, 1934; Thomas et al., 2016; Surendran et al., 2020).

The availability of water habitats where mosquitoes undergo preimaginal development determines numbers of adult mosquitoes produced and therefore their density and human-biting rate. Mosquito productivity is strongly influenced by various biotic (e.g. predation, competition) and abiotic (e.g. temperature, pH, salinity, dissolved solids, turbidity) factors and the interactions between these factors (Rejmánková et al., 2013).

Environmental changes orchestrated by human activities can create new habitats within the natural range of the mosquito vectors. Creation of new habitats can increase vector population and thereby disease transmission. Anthropogenic activities can also eliminate natural preimaginal habitats (e.g. *Aedes* source reduction in dengue vector control) of mosquito vectors. This can be a driving force for mosquitoes adapting to utilize less optimal unfavorable habitats within their natural range. In such a scenario mosquito vectors undergo physiological and genetic changes to develop in less suitable habitats within their natural range. Such adaptive changes allow mosquito vectors to expand their range to invade other areas and neighboring countries where similar habitats are available (Hufbauer et al., 2012; Ramasamy and Surendran, 2016). This ability has been termed as anthropogenically induced adaptation to invade in mosquito vectors (Ramasamy and Surendran, 2016). The process of developing such anthropogenically-induced adaptation to invade in mosquito vectors is illustrated in Figure 1.

Environmental changes due to deforestation and agricultural practices, and expansion create new preimaginal habitats for mosquito development. Deforestation resulting from agriculture expansion creates ecological heterogeneities that provide novel habitats for mosquitoes to adapt and undergo preimaginal development which in turn are capable of increasing disease transmission (Rejmánková et al., 2013). High incidence of chikungunya in Kerala, India has been associated with the adaptation of *Ae. albopictus* developing in latex-collecting containers attached to rubber
Ecological segregation of closely related taxa (Vittor et al., 2017) for preimaginal development within the natural range of the mosquitoes. The expansion of An. gambiae et al., 2017) and deforestation in Central Africa (Kamdem et al., 2012). Ecological segregation due to adaptation can lead to speciation. Anopheles coluzzi (formally known as the An. gambiae M form) and An. gambiae s. s. (formally designated as the An. gambiae S from) were shown to have adapted to more polluted urban habitats created after deforestation and less polluted rural habitats respectively in the Cameroons (Kamdem et al., 2012). Agriculture development associated with building infrastructure and irrigation facilities can lead to ecological changes that alter and/or create habitats for mosquito development that can influence disease transmission. A well-studied malaria outbreak along the Demerara river estuary in Guyana in South America was partly attributed to rice cultivation that provided preimaginal habitats for the locally prevalent salinity tolerant and highly exophilic and zoophilic An. aquasalis to become endophilic and anthropophagic (Giglioli et al., 1963). A high prevalence of malaria in the vicinity of newly constructed Mahaweli irrigation canals in north-central Sri Lanka was attributed to a high density of An. annularis developing in the canals that produced high entomological inoculation rates (Ramasamy et al., 1992).

Urbanization alters a natural ecosystem for human settlements. Urban developments that lack the infrastructure facilities to support large human populations are prone to public health challengers (Vora, 2008). Urbanization and human migration have also been attributed to the expansion of the range of vector mosquitoes into new territories. Habitat modification and fragmentation due to urbanization can lead to phenotypic trait selection in different taxa (Alberti, 2015; Alberti et al., 2017). The expansion of the range of Ae. aegypti from African forests and Ae. albopictus from Asia to many countries in the Americas and Europe is largely attributed to phenotypic trait selection from zoophagicity to anthropophagicity and adaptation to preimaginal development in domestic or peri-domestic environments (Powell et al., 2013; Scot et al., 2000) along with international trade (Benedict et al., 2007; Kraemer et al., 2019). Anopheles stephensi is a major urban vector of malaria in many Asian countries because it has become adapted to develop in cemented wells and cement overhead water storage tanks in the urban environment. The recent range expansion and invasion of An. stephensi to Djibouti in 2013 (Faulde et al., 2014), Ethiopia in 2016 (Carter et al., 2018) and Sri Lanka in 2017 (Dharmasiri et al., 2017) has been attributed to trade, urbanization and associated water storage practices (Surendran et al., 2019a).

It is in this contextual framework of mosquito-vectors of human diseases undergoing adaptation to anthropogenic changes in the environment, that the present article describes the impacts of increasing salinization and pollution of groundwater on mosquito disease vectors in the Jaffna peninsula.

**Global warming, sea level rise and salinization of groundwater in coastal areas**

Global warming, which has been largely attributed to human activity, can affect human health in multiple ways. Global climate change due to global warming has been predicted by modeling studies to increase the global spread of mosquito-borne diseases like malaria and dengue but also reduce their incidence in some presently endemic areas (Franklino et al., 2019; Ogden, 2017; McMichael et al., 2006; Confalonieri et al., 2007; Hunter, 2003). Various models predict the effects of changing temperature, rainfall and humidity on mosquito development and survival and shortening the extrinsic incubation period of pathogens in mosquitoes (Confalonieri et al., 2007; Githeko et al., 2000). Global warming is predicted to increase average sea levels by 18 to 59 cm by the end of the 21st century mainly...
due to the melting of glaciers and thermal expansion of seawater (UNIPCC, 2007). Rising sea levels is expected to increase the extent of saline or brackish water bodies (water with <0.5 ppt or parts per thousand which is approximately the grams per litre, 0.5–30 ppt and >30 ppt salt are termed fresh, brackish and saline respectively) in coastal areas such as coastal estuaries, lagoons, marshes and mangroves, and cause the intrusion of sea water into freshwater aquifers (Nicholls et al., 2007; FAO, 2007; Ramasamy and Surendran, 2011; 2012). As a consequence, freshwater bodies such as ponds, lakes and wells in coastal areas will become more brackish and cause potable water scarcity in coastal areas. The expansion of brackish and saline water bodies, mainly in coastal areas, can increase the transmission of mosquito-borne diseases (Ramasamy and Surendran, 2012).

Countries like Sri Lanka with extensive coastlines and high coast to land area ratios are particularly vulnerable to the impact of rising sea levels (Table 1). Coastal areas are highly populated and one in ten persons worldwide lives in coastal areas (McGranahan et al., 2007). Many countries in South and Southeast Asia have large proportion of their populations living in coastal areas that are vulnerable to the consequences of rising sea level. It has been postulated that rising sea levels due to global warming will increase the prevalence of many vector-borne diseases, including mosquito-borne diseases, initially along coasts before spreading inland (Ramasamy and Surendran, 2011; Ramasamy et al., 2015).

Jaffna peninsula has an extensive coastal line with many sea water inlets (Figure 2). It is undergoing rapid groundwater salinization due to over exploitation and sea water intrusion into fresh water aquifer systems. Rise in sea level due to anthropogenically induced global warming is expected to increase sea water intrusion to aquifer systems in the peninsula (Ramasamy and Surendran, 2011). This will extend salinization of groundwater to many parts of the peninsula and increase the availability of preimaginal habitats for mosquito vectors that are adapted to develop in brackish water.

**Increasing salinization and pollution in the Jaffna peninsula**

Sri Lanka is an island with a land area of 64,630 km². It has a 1,340 km coastline with coastline/land area ratio of 20.7 km² (Ramasamy et al., 2015). The Jaffna peninsula in northern Sri Lanka (Figure 2) lies in the island’s dry zone (Gunaalan et al., 2018). The temperature typically varies from 20.8 to 34.3 °C. Most of the rainfall in the peninsula is derived from the North-East monsoon (October to December) with a more minor and variable contribution to the rainfall from the South-West monsoon (April to July).

**Table 1:** Coastline in relation to land cover in selected countries in South Asia.
(adapted from Ramasamy et al., 2015)

| Country     | Land area Km² | Coastline Km | Coastline/Land area ratio m/Km² |
|-------------|---------------|--------------|---------------------------------|
| Bangladesh  | 130,168       | 580          | 4.5                             |
| India       | 2,973,193     | 7,000        | 2                               |
| Sri Lanka   | 64,630        | 1,340        | 20.7                            |
| Malaysia    | 328,657       | 4,675        | 14.2                            |
| Indonesia   | 1,811,569     | 54,716       | 30.2                            |
| Philippines | 298,170       | 36,289       | 121.7                           |

**Figure 2:** Map of Jaffna peninsula showing lagoons and freshwater aquifer systems. (Source: National Atlas of Sri Lanka)
yielding an annual average rainfall of 1,121mm (Annual Performance and Accounts Report: Jaffna District, 2018).

The Jaffna peninsula (which together with outlying islands constitutes the bulk of the administrative district of Jaffna) has a total land area of 1025 km² with average elevation of 5 m above sea level. The estimated total population in 2018 was 615,493 with the population density of 600.48 persons / km². The distance to the coast from any location in the peninsula is < 10 km and thus the entire peninsula can be considered to be a coastal zone. The population of the Jaffna district increased from 265,600 to 831,800 during the one hundred year period between 1881 and 1981. There was a sharp decline caused by the civil war from 1983 to 2009, to 541,380 in 2001. The population has now increased to 615,490 in 2018.

The Jaffna peninsula and adjoining islands have an underlying Miocene limestone formations inland and unconsolidated formations such as lagoonal and estuarine deposits, and coastal and dune sands near the sea (Sirimanne, 1952; Cooray, 1984). The limestone bedrock, which is primarily made up of calcite (Panabokke and Perera, 2005), extends below sea level and serves as an excellent aquifer (Arumugam, 1970; Joshua et al., 2013). There are four main limestone aquifers in the peninsula and the adjacent islands, viz. Chunnakam (Valikamam), Thenmaradchi, Vadamaradchi and Island (Figure 2) (Puvaneswaran, 1985). The fresh groundwater present in these limestone aquifers occurs in the form of a convex lens, which thins out towards the coast, above the seawater (Joshua et al., 2013) as per the Ghyben-Herzberg principle (Carlson, 1963). The thickness of the freshwater lens is about 25 m (Arumugam, 1970) and the lower part of the lens is transitional where the freshwater becomes increasingly brackish with depth (TDS 1,500 – 4,000 ppm) (Joshua et al., 2013; Kumara et al., 2013). Further, very limited elevation of the water table above sea level (<1 m - 12 m) (Kumara et al., 2013) reduces the depth of transitional zone (seawater – freshwater interface) in these aquifers and makes them shallow. The water table is lowered by groundwater extraction for irrigation and domestic use and the natural and continuous discharge of groundwater to the sea/lagoon along the coastline under the existing hydraulic gradient. The water table reaches its peak during the North-East monsoon rains. About 30 % of the rainfall is estimated to recharge groundwater in Jaffna peninsula and the rest of the rain water is lost mainly through direct runoff (about 10-15 %) and evaporation (about 40-48 %) (Navaratnarajah, 1994). The annual water table fluctuation in the unconfined limestone aquifers of Jaffna peninsula has been reported in the past to be 1-2 m (Arumugam, 1970; Kumara et al., 2013).

In the absence of any freshwater reservoirs of a perennial nature, the entire population of Jaffna peninsula relies on the groundwater stored in these aquifers to meet all its needs, including drinking, domestic, agriculture and other livelihood purposes (Navaratnarajah, 1994; Joshua et al., 2013; Mikunthan et al., 2013). Water from deep artesian wells in the Chunnakam aquifer is used to provide piped water supply to the city of Jaffna. Other areas of the Jaffna peninsula rely on domestic wells within premises for household water needs. However the groundwater is now brackish in many parts of the peninsula including the neighbouring islands due to complex hydrometeor, hydrogeologic and hydrogeochemical conditions prevailing in these areas, and an expansion of brackish groundwater has been observed in the past few decades (Nandakumar, 1983; Rajasooryiar et al., 2002). This is mainly caused by seawater intrusion due to excessive extraction of groundwater for domestic use and agriculture by the increasing population of the peninsula, as well as irrigation and leaching of evaporative salts has also been reported (Pathmaja et al., 2016).

Even though studies related to occurrence, quantity and quality of the groundwater of Jaffna peninsula had been carried out since 1938 with the available facilities and information at that time, systematic hydrological investigations were initiated only in 1965. After four years of continuous monitoring of over 400 representative observation wells, a preliminary report giving estimates of average freshwater storage (above MSL) during wet and dry seasons and the salinity status of groundwater in different regions of the Jaffna peninsula was first published in 1970 (Arumugam, 1970; Panabokke, 2007). Serious groundwater salinization in the shallow limestone and sandy aquifers in many parts of the Jaffna peninsula, especially in the coasts have since been reported (Gunasekaram, 1983; Nagarajah et al., 1988). Excessive extraction of groundwater for irrigation, anthropogenic activities such as high fertilizer inputs, improper sewage systems, landfills, etc. have also contributed to the groundwater salinity in Jaffna peninsula (Puvaneswaran, 1985; Navaratnarajah, 1994; Mikunthan et al., 2013). There has been no systematic monitoring of groundwater salinization the peninsula since the 1983-2009 civil war but limited observations suggest a severe deterioration of water quality (Rajasooryiar et al., 2002; Pathmaja et al., 2016; Chandrajith et al., 2016).

Groundwater in the coastal area is relatively vulnerable to contamination by seawater intrusion either by fall on the groundwater table near the coasts or increase in sea levels that can enhance the intrusion of seawater into the aquifer. Therefore, the groundwater in coastal areas can readily become brackish (Chandrajith et al., 2013). The chloride concentration in the coastal regions of the major aquifers of Jaffna peninsula reaches up to 2.8 g/L and makes those areas vulnerable to high salinity; but chloride concentration is less than 200 mg/L in the inland areas of these aquifers (Mageswaran et al., 2004). High electrical conductivity along with high chloride concentrations provide evidence for seawater intrusion (Rajasooryiar et al., 2002) and salinity in general in the Jaffna groundwater is inversely proportional to the distance from the sea (Puvaneswaran, 1987). Groundwater salinization via mixing of sea water due to low elevated groundwater table and highly porous geological formations in a neighbouring island (Pungudutivu) of Jaffna peninsula along with dissolution and leaching of evaporative salts has also been reported (Pathmaja et al., 2016).

Generally, groundwater in the limestone aquifers
possesses high electrical conductivity and high hardness due to dissolution of calcium carbonate from the limestone. As a consequence, high concentration of calcium ions too has been found in the drinking water of Jaffna peninsula (Kumara et al., 2013).

Although initiatives such as constructing earth bunds in coastal areas to prevent seawater intrusion during high tides and building a number of surface water tanks to harvest the excess rainwater that would infiltrate and reduce salinity in the limestone aquifers of Jaffna peninsula have been promoted for decades, a comprehensive long-term solution to the water shortage in the peninsula remains to be evolved.

Groundwater resources are extensively used for domestic as well as agricultural purposes in Jaffna peninsula are now severely polluted due to unsustainable levels of usage of fertilizers, agrochemicals and improper sewage management. There is no piped sewage disposal system in the Jaffna peninsula. Therefore sewage, even in the densely populated Jaffna city is treated in septic tanks within individual domestic and business premises. Household grey water wastes are directed to open drains in Jaffna city (Figure 3). The main drains discharge grey waste water into the Jaffna lagoon. However some of the grey waste water percolates into the groundwater within the peninsula. Additionally, many chemicals used in agriculture also percolate directly into the groundwater. As a result wells used for obtaining drinking water in most areas of the peninsula are heavily polluted. There are more than 100,000 dug wells which are used for agriculture and domestic purposes in Jaffna peninsula (Kraft, 2002). There has been increasing pollution of the groundwater in the peninsula and this has been reported for many decades (Mageswaran and Mahalingam, 1983; Mageswaran, 2003).

A study based on samples collected from Jaffna peninsula revealed that out of 40 wells 38% were highly contaminated with coliform bacteria and the parameters recorded were not within the drinking water quality standards of WHO. Nearly 80% of wells were found not suitable for drinking based on the values specified in guidelines for drinking water quality on electrical conductivity (Mahagamage et al., 2019). Another study investigated nitrate-N contamination in wells associated with Chunnakam aquifer (Figure 1) revealed that 28% of the 44 wells examined exceeded the permissible level of Nitrate-N (Vithanagae et al., 2014). High concentration of Nitrate-N in the range of 7.1 to 15.3 mg/L was found in drinking water supply wells in Jaffna (Jeyaruba and Thushyanthi, 2009). The high nitrate levels recorded in the well waters is associated with agriculture practices in which inorganic fertilizers are heavily applied (Mikunthan et al., 2013). Total phosphate level in groundwater of the peninsula varied from 0 to 3 mg/L, exceeding the permissible level of 2 mg/L in some locations (Mageswaran, 2003). Rapid development of small industries, increasing agricultural activities together with a population increase that contributes nitrate contamination by domestic pit latrines, is expected to increasingly contaminate groundwater resources in the limestone strata of Jaffna peninsula (Vithanagae et al., 2014).

Figure 3: Open surface drain system in Jaffna city that increases ground water pollution and provide sites for preimaginal development of mosquito vectors.
Impact of increasing salinization of coastal groundwater on mosquito vectors of human diseases

There are numerous mosquito vectors of human diseases among the approximately 5% of mosquito species that naturally develop in brackish water and saline waters around the world. Examples of prominent salinity tolerant vectors are shown in Table 2. It is expected that the expansion of brackish water bodies will increase the densities of salinity-tolerant vector mosquitoes that undergo preimaginal development in brackish water and saline waterbodies (Ramasamy and Surendran, 2011; Ramasamy, 2015; Ramasamy et al., 2015; Surendran et al., 2012). Jude et al. (2010) observed the development of An. culicifacies, the major vector of malaria in the Indian subcontinent, in brackish water of up to 4 g/L or ppt salt in coastal areas of eastern Sri Lanka. It was subsequently observed that the many other mosquito vectors widely held to develop only in fresh water, are also capable of ovipositing and undergoing preimaginal development to adults in brackish water in coastal areas of the Jaffna peninsula and adjoining islands (Jude et al., 2012) (Table 3; Figure 2). The prominent freshwater vector species Ae. aegypti and Ae. albopictus have since our initial observation in Jaffna peninsula been reported to develop in coastal brackish water habitats in Brunei (Idris et al., 2013), the USA (Yee et al., 2014), Brazil (Arduino et al., 2015) and Mexico (Galaviz-Parada et al., 2019). Laboratory investigations showed that the development of Ae. aegypti in brackish water has both inheritable and reversible characteristics (Ramasamy et al., 2014). Aedes aegypti collected from brackish water habitats in the field and maintained in brackish water of 10 g/L salt tended to prefer brackish water to fresh water for oviposition and had greater LC50 for tolerating salinity in undergoing preimaginal development to adulthood than fresh water Ae. aegypti collected from and maintained in fresh water (Ramasamy et al., 2014). Further studies showed that brackish water Ae. aegypti developed larger anal papillae, organs that play a role in osmoregulation, than fresh water Ae. aegypti (Surendran et al., 2018b). These findings suggest that there are genetic differences exist between brackish water and fresh water Ae. aegypti in the Jaffna peninsula. Although interbreeding between brackish and fresh water Ae. aegypti from Sri Lanka was observed (Ramasamy et al., 2014), the findings demonstrate a potential for speciation in Ae. aegypti in the long term if fresh water and brackish water developing populations become reproductively isolated through efforts to eliminate their freshwater preimaginal habitats or geographically isolated in the Jaffna peninsula. Laboratory studies showed that the effect of salinity has a negative impact on the toxicity of Bacillus thuringiensis (Bt) to Ae. aegypti larvae (Jude et al., 2012). Since Ae. aegypti is adapted to salinity, it is expected that Bt will be less effective in controlling the brackish water population (Jude et al., 2012). Also it was found that both the brackish and fresh water developing female Ae. aegypti and Ae. albopictus were susceptible to DENV infection and the infected mosquitoes were able to vertically (transovarially) transmit DENV to their eggs and subsequent progeny (Surendran et al., 2018a). Vertical transmission maintains DENV among field Aedes vectors without a need to take a blood meal from dengue patients, a situation that is neither possible nor observed in anopheline vectors of malaria (Surendran et al., 2018a).

It has been shown that brackish water-derived Ae. aegypti remain relatively susceptible to malathion (an organophosphate insecticide) and permethrin (a pyrethroid). These and related insecticides of the two classes may

| Species | Distribution | Transmitted pathogens |
|---------|--------------|-----------------------|
| Aedes dorsalis | Temperate Eurasia, N America | West Nile virus and Western equine encephalitis virus |
| Ae. (Ochlerotatus) taeniorhynchus | N & S America | Eastern equine encephalitis virus |
| Ae. togoi | North Pacific rim | Japanese encephalitis virus and filarial parasites |
| Ae. (Ochlerotatus) vigilax | Australasia, SE Asia | Filarial parasites, Ross River virus, Barmah forest virus |
| Anopheles atroparvus | Coast of W Europe | Malaria parasites |
| An. farauti and An. annulipes | Australasia | Malaria parasites |
| An. melas and An. merus | Africa | Malaria parasites |
| An. multicolor | N Africa, Middle East | Malaria parasites |
| An. sacharovi | Russia, S Europe | Malaria parasites |
| An. subpictus | Asia | Malaria parasites |
| An. sundaicus | S Asia, SE Asia, China | Malaria parasites |
| Culex sitiens | Indian ocean rim countries | Japanese encephalitis virus and Ross River virus |
| Cx. tritaeniorhynchus | Russia, Middle East, Africa, India | Japanese encephalitis virus |
Table 3: Freshwater mosquito vectors of human diseases that have adapted to brackish water habitats in the Jaffna peninsula and islands in the Palk Strait.

| Species                | Transmitted disease/medical significance | Location       | Maximum salinity tolerance (g/L) | Reference                                      |
|------------------------|------------------------------------------|----------------|----------------------------------|-----------------------------------------------|
| *Aedes aegypti*        | Dengue, chikungunya                      | Jaffna city    | 15                               | Ramasamy et al., 2011, 2014                   |
| *Aedes albopictus*     | Dengue, chikungunya                      | Jaffna city    | 14                               | Ramasamy et al., 2011                        |
| *Anopheles culicifacies* | Malaria                                  | Delft, Jaffna city | 4                               | Jude et al., 2012; Surendran et al., 2020   |
| *Anopheles subpictus*  | Malaria                                  | Jaffna city    | 3.4                              | Surendran et al., 2020                       |
| *Anopheles stephensi*  | Malaria                                  | Jaffna city    | 3.5                              | Surendran et al., 2020                       |
| *Anopheles varuna*     | Malaria                                  | Jaffna city, Delft | 4                               | Jude et al., 2012; Surendran et al., 2020   |
| *Anopheles barbirostris* | Malaria                                 | Delft, Sarasali | 6                               | Jude et al., 2012                           |
| *Lutzia fuscanus*      | Not a vector but feeds on larvae of mosquito vectors | Nainativu | 10                               | Jude et al., 2012                           |

Therefore be useful for controlling brackish water-derived coastal populations of *Ae. aegypti*. The reported differential susceptibility to malathion also suggests that there are genetic differences between brackish water-derived *Ae. aegypti* and fresh water-derived *Ae. aegypti* in the Jaffna peninsula (Ramasamy et al., 2014).

*Anopheles culicifacies* is the major vector of malaria in Sri Lanka (Carter, 1930; Amerasinghe et al., 1991; Surendran et al., 2000). Studies reveal that *An. culicifacies* undergoes preimaginal development in brackish water in Jaffna peninsula, in Jaffna city and Delft (Table 3). *Anopheles stephensi*, the dominant urban malaria vector in India, is also found in other parts of Asia and Middle East. This invasive species was first detected in Mannar island in the Northern Province of Sri Lanka in 2017 and subsequently in domestic wells and water storage tanks in Jaffna city in 2018, with a genotype consistent with an origin in Tamil Nadu (Surendran et al., 2018c; Surendran et al., 2019a). The preimaginal stages of *An. stephensi* were found to tolerate brackish water of up to 3.5 g/L or ppt salt in Jaffna city (Surendran et al., 2020). *Anopheles subpictus* species A, a secondary vector of malaria in Sri Lanka, was observed developing in 3.4 g/L or ppt salt brackish water habitats in Jaffna city. The normally fresh water developing malaria vector *An. varuna* was also found capable of developing in brackish water of up to 1.4 g/L or ppt salt in Jaffna city (Table 3; Figure 4) (Surendran et al., 2020).

These observations in the Jaffna peninsula are
examples of the process where elimination of the natural habitats of vector mosquitoes by human activity has led the vectors to adapt to less desirable new habitats within their natural range. It has been hypothesized that the *Aedes* vectors developing in brackish water habitats is a globally widespread phenomenon favoured wholly or partly by the exclusive focus of dengue control measures on fresh water habitats (Ramasamy et al., 2011, 2015).

**Impact of increasing groundwater pollution on mosquito vectors of human diseases**

Anthropogenic activities can have great impact on ecosystems and this process is greatly exacerbated by a rapidly increasing population. Industrialization and rapid urbanization often necessitate rapid adaptation to changing complex environments. Heavy discharge of industrial waste, household wastes, unhygienic sewage system and widespread use of xenobiotics in agriculture and public health increases selective pressures on pathogens and their vectors (Hendry et al., 2017).

The preimaginal development of *Ae. aegypti* in highly polluted domestic septic tanks have been reported in Puerto Rico and Nigeria (Burke et al., 2010). A recent study showed that *Aedes* mosquitoes developing in human urine contaminated aquatic environments can acquire and transmit Zika virus (Du et al., 2019). Recently, *Ae. aegypti* in developing in polluted water in open surface drains were reported in Jaffna peninsula (Surendran et al., 2019b) and Colombo (Chandrasiri et al., 2020). *Anopheles culicifacies*, the primary malaria vector in Sri Lanka, has long been regarded to develop only in clear, sunlit and unpolluted, fresh water collections with high dissolved oxygen content, but its larvae were recently found developing in polluted water in blocked urban drains in eastern Sri Lanka (Gunathilaka et al., 2013). Range expansion and adaptation to atypical habitats have contributed for insecticide selection pressure to vector mosquitoes to become resistant to common insecticides. Resistance in the *Anopheles gambiae* complex to permethrin, have been associated with oil spills in Nigeria (Djouaka et al., 2007, 2008), and resistance to deltamethrin with the use of agricultural chemicals in Tanzania (Nkya et al., 2014).

Of considerable concern was the observation that *Ae. aegypti* adapted to undergo preimaginal development in polluted water in open surface drains in the Jaffna peninsula showed greater resistance in adult stages to the common pyrethroids deltamethrin and permethrin than fresh water *Ae. aegypti* (Surendran et al., 2019b). Furthermore the resistance to pyrethroids in polluted water *Ae. aegypti* adults was characterized by elevated levels of two groups of pyrethroid-detoxifying enzymes, viz. glutathione-S-transferases and monoxygenases. Therefore it seems possible that adaptation in preimaginal stages of *Ae. aegypti* to detoxify pollutants present in drain water can also result in an increased capacity of adults to detoxify pyrethroids (Surendran et al., 2019b). Since the application of pyrethroid insecticides is the mainstay for controlling epidemics of arboviral diseases worldwide, and open drains are common in congested urban areas of tropical arboviral disease-endemic countries, this observation is of great international concern (Surendran et al., 2019b).

A recent study revealed that the fresh water developing malaria vectors in Sri Lanka namely *An. culicifacies*, *An. subpictus*, *An. varuna* and *An. stephensi* had adapted to develop in polluted water in the Jaffna peninsula (Surendran et al., 2020). The polluted water developing *An. subpictus* in the Jaffna peninsula was resistant to deltamethrin with a characteristic L1014F (TTA to TTC) knock-down resistance mutation, found in Indian *An. subpictus* (Singh et al., 2015), in the IS6 transmembrane segment of the voltage gated sodium channel protein (Surendran et al., 2020).

Therefore, adaptation to polluted water in urban areas has two major detrimental effects on vector control. Firstly, the range and diversity of pre-imaginal habitats of polluted water-resistant vectors increases and this results in greater vector densities. Secondly, adapting to polluted water also causes greater resistance to commonly used insecticides thereby reducing the efficacy of insecticides used to control adult vector populations.

**Range expansion of vector mosquito species**

The westward range expansion of *An. stephensi* in the middle-east was attributed to the favorable conditions created by rapid social development and urbanization (Alahamed et al., 2012). The invasion into horn of Africa was postulated to have been caused by transportation of goods and movement of refugees across the Red Sea (Faulde et al., 2014). A southward range expansion of *An. stephensi* was observed in India along the west coast from Goa to Kanyakumari in South India in the 1980s. The range expansion was attributed to urbanization and associated water storage practices in India (Sharma and Hamzakoya, 2001). The urban malaria vector further expanded its range across Palk strait to northern Sri Lanka, initially to Mannar island (Dharmasiri et al., 2017) and Jaffna city (Figure 5) (Surendran et al., 2018c).

*Anopheles stephensi* exists as three biotypes namely *An. stephensi* mysorensis, *An. stephensi* intermediate and *An. stephensi* type. The rural *mysorensis* and *intermediate* forms have evolved to adapt to develop in freshwater ponds, stream beds, wells and seepage canals while the urban *An. stephensi* sensu stricto or type form populations develop mainly in artificial water containers, over ground water storage tanks, domestic wells, underground cement water storage tanks, evaporator coolers, cisterns, barrels, roof gutters, pits in construction sites and ornamental tanks (WHO, 2007; Sharma et al., 1993; Thomas et al., 2016).

The newly invasive *An. stephensi* in Jaffna peninsula has the intrinsic plasticity to develop in fresh water, brackish water and polluted water habitats. These characteristic were likely to have been developed in urban India and gives *An. stephensi* the advantage to expand its range to new territories, including North Sri Lankais an example of the proposed anthropogenically-induced adaptation to invade in mosquito vectors (Ramasamy and Surendran 2016). Studies have shown that *An. stephensi* has euryhaline characteristics and is able to develop in brackish water in Pakistan (Reisen et al., 1981; Roberts, 1996). Environmental
modifications that caused water logging and salinization favored salinity-tolerant *An. stephensi* (Reisen *et al.*, 1981) becoming the dominant malaria vector in South Punjab, Pakistan (Klinkenberg *et al.*, 2004). The northern Jaffna peninsula limestone geology and salinization of fresh water aquifers provide brackish water habitats for the preimaginal development mosquito vectors as stated above. It is therefore likely that *An. stephensi* that has evolved to undergo preimaginal development in brackish water, can utilize similar anthropogenic brackish water habitats to further extend its range within the Jaffna peninsula and other coastal areas of Sri Lanka.

The movement of people has been an important factor in the geographic spread of insects to new territories (Palkovacs *et al.*, 2012). There has been relatively free movement across the 64 to 137 km-wide Palk Strait that separates northern Sri Lanka from Tamil Nadu (Figure 5) in the past. It is, therefore, reasonable to assume that *An. stephensi* arrived in Sri Lanka from Tamil Nadu. It appears likely that eggs and larvae in brackish water collections within small fishing boats was the probable method of arrival of *An. stephensi* in northern Sri Lanka. However wind-borne dispersion of *An. stephensi* from Tamil Nadu during the South-West monsoon is also a possibility in view of recent data from the Sahel region of Africa showing that anopheleline vectors can be passively dispersed over hundreds of kilometers by winds (reviewed in Surendran *et al.*, 2020).

The establishment of *An. stephensi* population in Jaffna peninsula has been facilitated by environmental and biological factors that include availability of urban domestic wells and cement water storage tanks as habitats to which it was already adapted in India, the adaptation to undergo preimaginal development in brackish water man-made habitats in coastal areas which may also have been previously acquired in coastal areas of Tamil Nadu, and resistance to pyrethroid, organophosphate and organochlorine insecticides which may also have been previously developed in Tamil Nadu (Surendran *et al.*, 2019b). The above factors are examples of mosquitoes adapting to changing environment and the anthropogenically-induced adaptation to invade in mosquito vectors of human disease (Ramasamy and Surendran, 2016).

**Implications for increased disease transmission**

More than half of the world’s population lives within 60 km of a shoreline. Population density in coastal areas is expected to increase from 87 persons per km² in the year 2000 to 134 persons per km² in 2050 (UNEP, 2007). This will place more people at risk of exposure to many mosquito-borne diseases by the increasing mosquito vector population in coastal areas.

Rising sea levels resulting from human induced global
warming will increase sea water intrusion and expand the extent of brackish water bodies in coastal areas that in turn can facilitate salinity adaptation in fresh water vectors and increase the numbers of existing salinity-tolerant vectors (Ramasamy and Surendran, 2011).

The intrinsic plasticity allows mosquitoes to develop in atypical habitats. Genetic changes associated with resistance to insecticides contribute to adaptation to salinity in Ae. aegypti (Ramasamy et al., 2014) and oil pollution in An. gambiae (Djouaka et al., 2008). This has implications for the worldwide prevalence of vector mosquitoes adapting to develop in atypical habitats and therefore the control of mosquito-borne diseases in many countries.

Mosquito vectors adapted to the new habitats therefore have the potential to extend their range to similar habitats in other areas. Brackish water-adapted Ae. aegypti and Ae. albopictus in Sri Lanka can expand their range to similar coastal brackish water habitats in Sri Lanka and neighbouring countries in the Indian Ocean. Similarly, pollutant-adapted An. gambiae from Lagos have been predicted to be able to spread to develop in polluted water habitats in adjacent territories (Costantini et al., 2009). Adaptation-associated physiological changes in mosquito vectors can influence the infectivity of pathogens to mosquitoes and thereby influences vectorial capacity (Ramasamy and Surendran, 2016). Further research on mosquito vector ecology and pathogen-vector interaction in the context of vectorial capacity is therefore needed to more effectively control mosquito-borne human diseases in the future.

Greater attention is needed on vector prevalence and monitoring of disease incidence in coastal areas. Counter measures should be introduced to prevent further range expansion of mosquito species developing in brackish water and polluted water habitats. Current vector control measures targeting fresh water habitats should be extended to brackish and polluted water habitats with community participation and awareness on mosquito vectors developing habitats.

There is a need for more research at local, national and international levels into impact of sea level rise, salinization and pollution on mosquito vectors population in coastal regions. The inherent physiological mechanisms associated with adaptation to develop in brackish water and polluted water needs to be further investigated.

CONCLUSIONS

Recent findings suggest that the rapid salinization and pollution of groundwater in the Jaffna peninsula and very likely elsewhere in Sri Lanka is leading to the adaptation of fresh water mosquito vectors of malaria and arboviral vectors to brackish water and polluted water with physiological changes that reduce the effectiveness of present vector control methods. There is presently a dearth of studies on the distribution, insecticide resistance, abundance and adaptations of major Culex species that are potential vectors of human diseases in other parts of the country. Furthermore, salinization and pollution of water have direct health impacts on people. Appropriate monitoring and mitigating measures to address these problems are urgently needed in the Jaffna peninsula and other coastal areas of Sri Lanka. A solution to stop and perhaps reverse groundwater salinization in the Jaffna peninsula proposed nearly a century ago was to construct tidal barriers to convert Thondamanaru lagoon (Figure 2) into a freshwater, rain fed reservoir. A sewer system and treatment plant for the city of Jaffna and adjacent suburbs can greatly help to reduce the present levels of groundwater contamination. It was proposed in 2012 that the Jaffna peninsula may constitute a model coastal zone for examining the impacts of global warming, sea level rise and salinization on mosquito-borne diseases (Ramasamy and Surendran, 2012). The recent findings, summarized here, further supports the view that the Jaffna peninsula serves as a global paradigm for the effects of multiple anthropogenic changes on mosquito vectors of human disease in resource-limited tropical countries.

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STATEMENT OF CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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