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Detection of *Aedes albopictus* (Diptera: Culicidae) breeding in brackish water habitats in coastal Kerala, India and its implications for dengue scenario in the state

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Abstract
There is a long and widely held view that *Ae. albopictus*, the vector of Dengue, Chikungunya, Zika and Yellow fever breed only in fresh water habitats. Larval source reduction efforts worldwide, therefore focus on freshwater habitats of this vector. However, *Ae. albopictus* was recently shown to undergo larval development in brackish water with salinity up to 15 ppt in various parts of the world. In the present study breeding of *Ae. albopictus* was detected in three types of breeding habitats with brackish water *viz*., shallow ponds, plastic containers and discarded bottles in the coastal areas of Kannur and Kozhikode districts of Kerala, India. The salinity of water in these habitats ranged from 2.6 to 4.5 ppt. When tested in laboratory, the highest salinity tolerance was found to be 12 ppt. Since dengue is the most important mosquito-borne disease in the state, adaptation of *Ae. albopictus* to coastal brackish water habitats could have major consequences for the dengue scenario in the state. Besides dengue, Chikungunya and Zika are also prevalent in the state. This necessitates a need for constant monitoring of brackish water breeding habitats of the state and initiate appropriate control measures wherever necessary, in order to prevent the diseases vectored by *Ae. albopictus*.

Keywords: aedes albopictus, brackish water, salinity, vector, climate change

Introduction
Dengue is a mosquito borne viral disease transmitted mainly by *Aedes aegypti* and *Ae. albopictus*. These mosquitoes are also vectors of chikungunya, yellow fever and zika viruses. Dengue is widespread throughout the tropics, with local variations in risk influenced by climate parameters, as well as social and environmental factors [1]. India reported 123106 cases till October 2021of which the contribution of Kerala was 3794 cases (Source: National Vector Borne Disease Control Programme). Brackish water habitats are a neglected source of dengue vectors on tropical beaches in the world [2] *Aedes albopictus* breed and undergo larval and pupal development in natural and artificial fresh water collections in the urban and peri urban environment. Although Larval development of *Ae. albopictus* is known to exist in fresh water environment for many years, few recent findings have revealed the possibility of *Aedes* breeding and immature stage development in brackish water conditions [3]. Water with<0.5 ppt, 0.5-30 ppt and >30 ppt salt are termed as fresh, brackish and saline respectively [4]. Approximately 5% of mosquito species are adapted to undergo larval development in brackish and saline waters [5]. Many countries, particularly in Southeast Asia, have extensive coastlines, high coast to land area ratios and, a large proportion of their populations living in coastal areas. Such brackish water habitats are potential sources of vectors that may contribute to the transmission of dengue and other arboviral diseases in coastal areas [6]. The state of Kerala in South India has a total coastline of 589.5 kilometers, which forms 10% of India's total coastline. The present study was originally planned to investigate mosquito breeding in brackish water habitats, especially disease vectors and also to estimate the salinity tolerance of the local populations of the species.

Materials and Methods
Study area
Surveys for detecting mosquito breeding in brackish water was conducted randomly in all types of habitats in the coastal areas of Kannur and Kozhikode districts of North Kerala from January to December 2021. Surveys were restricted within 2 KM from the coastline. Ten localities were selected from each district for the study *viz*., Kurichiyil, Chalil, Thalayi, Edakkad, Dharmadam, Muzhappilangad, Mattool, Ezhome, Puthiyangadi, Thayyil (Kannur district), Azhiyoor, Chombala, Kuriyadi, Vadakara, Koyilandy, Thikkodi, Ezhukudikkal, Puthiyappa, Vellayil, Chaliyam and Beypore (Kozhikode district).

Larval collection and measurement of salinity
Before collecting larvae, salinity of the water was measured using a portable salinometer. Water with salinity ranging between 0.5 and 30 ppt were considered as brackish [5]. Larvae from large habitats were collected using 8 cm diameter and 240 ml capacity dipper. From smaller containers larvae were picked up by droppers. Fourth

instar larvae were identified using published identification keys in the field. Early instar larvae were reared in the laboratory to fourth intsar larvae. Identities were further conferred after emergence using adult keys [7].

Salinity tolerance study in the laboratory

For obtaining larvae for salinity tolerance study, female *Ae. albopictus* mosquitoes were fed on rat's blood and made to lay eggs in a rearing cage. Larvae were reared in plastic bowls of 300 ml capacity. Each bowl was fed on 2:1 mixture of finely ground fish pellets: baker's yeast. Solutions of commercial NaCl were prepared with distilled water following published procedures [8]. These preparations were placed in 500 mL plastic containers (each concentration was placed separately). Initially, larvae were exposed to six concentrations *viz*., 0 ppt, 10 ppt, 20 ppt, 30 ppt, 40 ppt and 50 ppt. There were 4 replicates for each concentration. NaCl solutions were left for 1hour before introducing mosquito larvae. Salinity tolerance was estimated following published procedures [5]. 20 fourth instar larvae were placed in each test solution. After 24 hours of exposure, larval survival was scored by eye. Larval death was confirmed by repeated stimulation with a plastic pipette to test for a motile response. It was repeated in 48 and 72 hours respectively. Once a tolerable salinity was obtained, they were further exposed to still narrower ranges of salinity in 1 ppt increments.

Data on dengue

Data on dengue cases and deaths were obtained from Directorate of Health Services, Thiruvananthapuram

Results and Discussion

***Ae. albopictus* breeding in brackish water**

Seven types of habitats were found having brackish water with salinity ranging between 0.5 and 30 ppt *viz*., cement tanks, plastic tanks, wells, shallow ponds, ditches, discarded bottles and different types of plastic containers. Breeding of *Anopheles subpictus* and *Culex sitiens* was observed in habitats having salinity ranging between 2.0- 28.0 ppt. *Ae. albopictus* breeding was observed in four shallow ponds and nine plastic containers in Ezhome (Kannur district) and three discarded bottles in Azhiyur (Kozhikode district). The salinity of water in these habitats ranged between 2.6 to 4.5 ppt (Table 1).

Table 1: Brackish water habitats with *Aedes albopictus* breeding

Locality / District	Habitat (Number)	Immature stages	Salinity range (ppt)
Ezhome/ Kannur	Shallow ponds (4)	All larval instars and Pupae	2.6 to 3.2
Ezhome/ Kannur	Plastic containers (9)	Third and Fourth Larvae	3.0 to 3.5
Azhiyur/ Kozhikode	Discarded bottles (3)	All larval instars	4.5

***Salinity tolerance of larvae* in laboratory**

In the first experiment, with broad ranges of salinity (0-50 ppt in 10 ppt increment), 100 % survival of larvae of *Ae. albopictus* was recorded in the samples with up to 10 ppt ((Table-2). In the second experiment, with narrow ranges of salinity (10 to 19 ppt in 1 ppt increment), 100% survival was recorded up to 12 ppt (Table-3).

Table 2: Salinity tolerance of fourth instar *Ae. albopictus* larvae (0 to 50 ppt)

Salinity (ppt)	Number of larvae exposed	Number of larvae survived		
		24 hours	48 hours	72 hours
0	20	20	20	20
10	20	20	20	20
20	20	0	0	0
30	20	0	0	0
40	20	0	0	0
50	20	0	0	0

Table 3: Salinity tolerance of fourth instar *Ae. albopictus* larvae (10 to 20 ppt)

Salinity (ppt)	Number of larvae exposed	Number of larvae survived		
		24 hours	48 hours	72 hours
10	20	20	20	20
11	20	20	20	20
12	20	20	20	20
13	20	16	10	8
14	20	4	3	1
15	20	0	0	0
16	20	0	0	0
17	20	0	0	0
18	20	0	0	0
19	20	0	0	0

Dengue situation

Both Kannur and Kozhikode districts reported dengue cases consistently (Table 4). From 2017 to 2021 the number of cases in Kannur ranged from 116 to 629. During the same period Kozhikode district recorded 69 to 1354. Deaths due to dengue in Kannur district ranged from 0 to 5 and the same was 2 to 7 in Kozhikode district.

Table 4: Dengue cases in Kannur and Kozhikode districts from 2017 to 2021

Year	Dengue cases (death)	
	Kannur	Kozhikode
2017	629 (0)	1354 (7)
2018	338 (3)	247 (3)
2019	212 (0)	405 (2)
2020	268 (5)	69 (2)
2021	116 (2)	209 (6)

The present study has brought the hitherto neglected brackish water habitats in the coastal areas of Kerala into lime light in the context of the epidemiology of diseases transmitted by *Ae. albopictus*. This is the first study demonstrating the adaptation of the predominantly freshwater breeding *Ae. albopictus* to brackish water in Kerala. In Sri Lanka, brackish water with salinity of 2 to 15 ppt in discarded plastic and glass containers, abandoned fishing boats and unused wells in coastal peri-urban environment were found to contain *Ae. aegypti* and *Ae. albopictus* larvae. In Jaffna city of Sri Lanka, higher incidences of dengue were reported from areas in the vicinity of brackish water habitats [2]. In the present study, though natural breeding of *Ae. albopictus* was found in brackish water with up to 4.5 ppt only, they could tolerate a salinity up to 12 ppt in the laboratory, which is comparable to the Sri Lankan strain of the species.

Since the number of brackish water habitats with *Ae. albopictus* in the present study was comparatively less, it could be argued that the phenomenon of adaptation to brackish water is in its initial stage. However, as time elapses, the chances of the saline tolerant population to proliferate cannot be ruled out.

Dengue is a regular phenomenon in all districts of the state including Kannur and Kozhikode with sizeable number of cases and even a few deaths every year. In the absence of an effective vaccine, dengue control strategies focus on the reduction and elimination of *Aedes* breeding in domestic and peri-domestic container habitats. Hence, the brackish water habitats are largely neglected. This could have serious consequences in the dengue scenario of the state in the near future. Besides dengue, two other *Aedes*-borne diseases *viz*., Chikungunya and Zika, are also prevalent in the state. Considering all these factors, serious efforts are needed to monitor and design control strategies to tackle this new phenomenon.

Conclusion

Breeding of *Aedes albopictus*, a predominantly freshwater species, was detected in three types of breeding habitats with brackish water *viz*., shallow ponds, plastic containers and discarded containers in two localities in the coastal areas of Kannur and Kozhikode districts of Kerala. The salinity of these habitats ranged from 2.6 to 4.5 ppt. In the laboratory the fourth instar larvae showed a maximum salinity tolerance of 12 ppt. The study is the first of its kind in the state. Adaptation of *Ae. albopictus* to brackish water could have serious consequences in the dengue scenario of the state. Hence, immediate strategies to monitor and contain the proliferation of saline tolerant populations of the species is recommended.

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