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Short Research Communications

Adaptation of *Aedes aegypti* to salinity: Characterized by larger anal papillae in larvae

S.N. Surendran[1], K. Sivabalakrishnan[1], T.T.P. Jayadas[1], S. Santhirasegaram[1], A. Laheetharan[2], M. Senthilnanthanan[3] & R. Ramasamy[4]

[1]*Department of Zoology,* [2]*Department of Mathematic and Statistics,* [3]*Department of Chemistry, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka;* [4]*ID-FISH Technology Inc., Palo Alto, USA*

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Aedes aegypti is considered the principal vector of several human arboviral diseases, including dengue, chikungunya, West-Nile encephalitis and Zika. Many of the approximately 5% of mosquito species that are salinity tolerant[1], are vectors of important human and animal diseases; and such vectors can increase in number and invade to new territories in the context of global warming and other anthropogenic environmental changes[2–4]. *Aedes aegypti* has previously been regarded to oviposit and undergo preimaginal development only in fresh water (FW) collections, and hence, larval control efforts are solely directed towards FW habitats in the vicinity of human dwellings[5–6]. We recently showed that *Ae. aegypti* and the closely related arboviral vector *Ae. albopictus* can undergo preimaginal development in brackish water (within discarded food and beverage containers) of up to 15 parts per thousand (ppt) salt and 14 ppt salt, respectively in northern Sri Lankan beaches[7–8]. Saline, brackish water (BW) and FW are defined as containing >30, 0.5–30 and <0.5 ppt salt, respectively in this context[2]. *Aedes aegypti* larvae have also been observed in brackish domestic wells of up to 9 ppt salt in the coastal Jaffna peninsula of northern Sri Lanka[9]. Similarly, BW development of *Ae. aegypti* or *Ae. albopictus* has been observed in coastal locations of Brunei Darussalam[10], USA[11] and Brazil[12]. Physiological changes accompanying BW adaptation in *Ae. aegypti* include the greater ability to oviposit in 10 ppt salinity; and significantly greater, and partly inheritable, larval salinity tolerance[13].

The anal papillae of *Ae. aegypti* are reported to transport Na^+ and Cl^- from a FW environment into the hemolymph[14–15]. There is also a salinity-related differential expression of specific aquaporins in *Ae. aegypti* anal papillae[16]. These findings suggest a possible role of anal papillae in short-term osmoregulation in *Ae. aegypti* larvae. In an attempt to explore the mechanisms, underlying the adaptation to BW, this study investigated the changes in anal papillae size in *Ae. aegypti* mosquitoes adapting to BW.

Self-mating laboratory colonies of *Ae. aegypti* were established from larvae collected from FW ovitraps in Thirunelvely (9° 41' N: 80° 1' E) and BW domestic wells in coastal Kurunagar (9° 39' N: 80° 1' E) in the Jaffna peninsula of Sri Lanka. The FW colony was maintained in potable tap water supplied from a dug-well located in the University of Jaffna campus in Thirunelvely, and the BW colony in sea water collected from Jaffna lagoon, diluted with tap water to yield ~10 ppt salt (measured by a refractor Salinometer, Atago, Japan). The adult and larvae were maintained at 28–30°C with relative humidity ~75% and 12 h dark and light conditions. Eggs from the BW and FW colonies were collected in 10 ppt saline BW and 0 ppt saline FW egg-laying surfaces, respectively and allowed to hatch into larvae in the corresponding BW and FW. Larvae were reared in 24 × 16 cm plastic trays with 1.5 litre water with maximum number of 150 larvae per tray and fed with fish meal powder thrice a day. Adult females were blood-fed on mice according to a protocol approved by the Institutional Animal Ethics Review Committee of the University of Jaffna (June, 2014; AERC/2014/02).

The FW colony maintained up to 20 generations in the laboratory and BW colony maintained up to 24 generations were used for experimental purposes. For experiments to determine the effects of reversing larval rearing salinity (salinity reversal experiments), FW colony eggs were collected on FW egg-laying surfaces and then reared in 10 ppt salinity BW, and also separately in FW in parallel. For the corresponding reversal of salinity, BW