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Original Article

Salinity tolerance of freshwater-derived *Aedes aegypti* and *Aedes albopictus* from the artificial containers of Dumaguete, Philippines

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Abstract

This study examined the response and tolerance of freshwater-derived *Aedes aegypti* and *Aedes albopictus* on salinity. Pre-imaginal stages of both species were exposed to salt concentration ranging from 3.5, 7, 10.5 ppt under laboratory conditions. The maximum salinity tolerance of *Ae. aegypti* and *Ae. albopictus* was at 7 ppt. Survival rate ranged from 53% to 93% and 63% to 90% for *Ae. aegypti* and *Ae. albopictus*, respectively. Percent emergence of *Ae. aegypti* ranged from 50% to 90%; 60% to 90% for *Ae. albopictus*. As salinity increases, larval duration increases while growth rate decreases. The LC50 for *Ae. aegypti* ranged from 6.1-7.1 ppt while LC90 ranged from 9.8-11.6 ppt. The LC50 of *Ae. albopictus* ranged from 6.5-7.1 ppt while LC90 ranged from 10.4-11.6 ppt. Findings of this study confirmed the need to implement control programs for mosquito vectors that also target brackishwater.

Keywords: development, *Aedes aegypti*, *Aedes albopictus*, salinity tolerance, brackishwater

1. Introduction

Aedes aegypti (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894) are major arboviral vectors that are regarded to undergo pre-imaginal development in natural and artificial freshwater habitats in urban and peri-urban environments (Ramasamy, Surendran, Jude, Dharshini, & Vinobaba, 2011). Since both artificial and natural containers strongly influence the density of resulting adult population, larval source reduction efforts worldwide against these two vectors have focused mainly on freshwater habitats (Surendran, Jude, Thabothiny, Raveendran, & Ramasamy, 2012). Eliminating or larviciding the freshwater habitats of these vectors are considered important strategies for controlling the transmission of arboviral transmission diseases such as dengue, chikungunya, and yellow fever, worldwide. However, recently these measures have failed to eradicate such diseases. Anthropogenic activities that eliminate or minimize the pre-imaginal habitats preferred by mosquito vectors were observed to induce adaptation to less optimal and underutilized habitats such as brackish water (Ramasamy & Surendran, 2016). Several studies have shown that both *Ae. aegypti* and *Ae. albopictus* have salinity tolerance and undergo pre-imaginal development in brackish water (Arduino, Mucci, Serpa, & Rodriguez, 2015; Clark, Flis, & Remold, 2004; Jude *et al*., 2012; Ramasamy *et al*., 2011; Surendran *et al*., 2012). Salinity tolerant *Ae. aegypti* and *Ae. albopictus* have been observed in Brunei Darussalam (Idris, Usman, Surendran, & Ramasamy, 2013), Southern Brazil (Arduino *et al*., 2015), and Sri Lanka (Surendran *et al*., 2018). It has been found out that *Ae. aegypti* and *Ae. albopictus* developing in brackish water can vertically transmit the dengue viruses to their progenies. Furthermore, density of salinity tolerant freshwater breeding mosquito vectors may increase especially in coastal localities due to rising sea level associated with global warming (Ramasamy & Surendran, 2011). Worldwide increase in the population density in coastal areas may likely exacerbate the situation by increasing human-vector contacts (Ramasamy *et al*., 2011)

Philippines has one of the longest coastlines in the world, estimated at 36,289 km with 25 major cities lying on the coast. Ninety-one percent (64/79) of its provinces are coastal. It is estimated that more than 60% of the nation's total population lives in the coastal zone. As of year 2000, the

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population density in coastal areas is 286/km^2 (worldbank.org/ INTPHILIPPINES/Resources/PEM05-ch1.pdf). It is expected that population density in coastal areas has increased from 2000 to 2019. With physiological and genetic adaptations of Aedes mosquitoes to breed in brackish water, dengue incidence in the Philippines may continue to increase. As of April 2019, the cumulative dengue cases were at 59,139 with 237 deaths, higher than those reported during the same period last year which were 32,611 cases and 175 deaths (WHO, 2019). Thus, this study examined the response and tolerance of *Ae. aegypti* and *Ae. albopictus* larvae to salinity during pre-imaginal development, with emphasis on growth rate, larval duration, emergence and survival. Findings of this study could be used as a basis for implementing dengue and other arboviral disease control programmes in the Philippines that would target the brackish water habitats of *Ae. aegypti* and *Ae. albopictus* in urban and peri-urban environments.

2. Materials and Methods

2.1 Identification and Rearing of *Aedes aegypti* and *Aedes albopictus* larvae

Pre-imaginal stages of *Ae. aegypti* and *Ae. albopictus* were collected from the artificial containers in Dumaguete, Negros Oriental, Philippines. The samples were then immediately brought to and identified in Negros Oriental State University-Biology Laboratory using a stereo microscope. Larval stages of *Ae. aegypti* and *Ae. albopictus* were identified by the shape of the comb spines on the eighth segment of the abdomen, shape of the pecten teeth on the siphon, and the number of setae at the ventral brush. Identification of larval instar for each species was done under a compound microscope using identification references (Andrew & Bar, 2013; Harrison & Rattanarithikul, 1973; Rueda, 2004; Teng 1996). Identified larval instars were then acclimated in the laboratory for 24 hrs. Since the larval growth rate is influenced by several factors, the exposure of *Ae. aegypti* and *Ae. albopictus* to changes in a particular environmental parameters in the laboratory was carefully controlled. In this regard, the instar larvae were reared at the same temperature, density, water volume, container, photoperiod, and feeding protocol. Ten larval instars in 500 ml capacity beaker with 100 ml of water of different salinities were maintained at room temperature (28±2°C) and 12h:12h L: D photoperiod until their emergence to adults. Larvae were fed twice daily with powdered fish pellet.

2.2 Evaluation of response and tolerance of *Aedes aegypti* and *Aedes albopictus* to salinity

Ten 1st to 4th larval instars of *Ae. aegypti* and *Ae. albopictus* were exposed to different salt concentrations choices from 3.5, 7, and 10.5 ppt. The salinity of the water was determined using a refractometer-salinometer (Atago Japan). The effects of various salinity levels on the development of *Ae. aegypti* and *Ae. albopictus* larvae were monitored in both experimental groups and control group from the day of exposure till adult emergence. Three replicate tests were run in parallel for each salinity. Distilled water was used as a control. Every 24 hrs, one randomly selected larva from each concentration was viewed under a stereomicroscope (ImageFocus4 under 40x and 100x magnifications) to determine the effects of salinity on development in terms of growth rate. In this study, the growth rate of each instar by salt concentration is represented by the increase in length and width of the head, thorax and last two abdominal segments of each instar per species. Morphometric measurements of head, thorax, segment VII and siphon were done under the stereomicroscope using ImageFocus 4 Software. Dead larvae were removed from each experimental unit. Dead larvae can be distinguished by their failure to respond in the presence of an investigator since mosquito larvae are usually mobile.

Salinity tolerance was determined by the durations of larval stages, emergence and survival. The durations of larval stages for each species in each salinity level were determined by hour. Percent emergence of pupa and adults for every instar exposed to each salt concentration were determined. Survival rate was calculated based on the number of adult emergence for each instar by salinity level. Mean larval mortality was determined after exposure.

One-way ANOVA was used to determine the significant differences in growth rates of *Ae. aegypti* and *Ae. albopictus* when exposed to different salinity levels. Chi-square test was used to determine the significant differences in the salinity tolerance of *Ae. aegypti* and *Ae. albopictus*. Probit analysis was used to determine the LC_{50} and LC_{90} of *Ae. aegypti* and *Ae. albopictus.*

3. Results

3.1 Growth rates of *Aedes aegypti* and *Aedes albopictus* in different salinities

In general, the growth rate of *Ae. aegypti* and *Ae. albopictus* larvae decreased with salinity. Figures 1-4 show the growth rates as represented by length and width of head, thorax, segment VIII, and siphon. For *Ae. aegypti*, the length of head, thorax, segment VIII and siphon of 1st instar to 4th instar were observed to decrease from 3.5 to 10.5ppt. However, the 2nd instar tends to increase its siphon length from 3.5 to 7 ppt, but it still decreased at 10.5ppt. The shortest length was observed at 10.5ppt for the four instars. There is no statistically significant difference in the length of the head (±SE; $p=0.243$), thorax (±SE; $p=0.109$), segment VIII (±SE; $p=0.067$) and siphon (±SE; $p=0.228$) of *Aedes aegypti* larvae by salinity level.

Likewise, the width of head, thorax, segment VIII and siphon of 1st instar to 4th instar decreased with salinity (Figure 2). However, it is interesting to note that although the width of segment VIII in 2nd and 3rd instar decreased at 3.5ppt, it slowly increased in width from 7.5 to 10ppt. Except for the width of the siphon (±SE; $p=0.017$), the width of the head (±SE; $p=0.167$), thorax (±SE; $p=0.074$) and segment VIII (±SE; $p=0.130$) of *Aedes aegypti* larvae exposed to different salinity levels had no significant difference by salinity.

In case of *Ae. albopictus*, the body length and width of 1st and 2nd instars decreased with salinity (Figures 3 - 4). For the 3rd instar, its body length and width tended to increase when exposed to 3.5 and 7ppt but decreased when exposed to 10.5ppt. In case of 4rth instar, its body length and width decreased from 3.5 to 7ppt but increased when exposed to 10.5ppt. The same pattern was observed in the width of

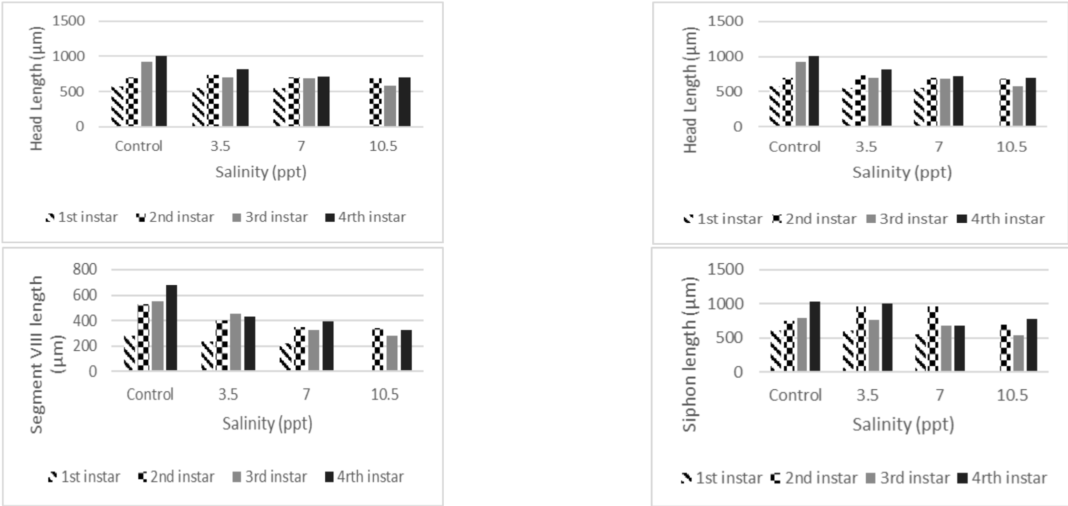

Figure 1. Length of the head (±SE; p=0.243), thorax (±SE; p=0.109), segment VIII (±SE; p=0.067) and siphon (±SE; p=0.228) of *Aedes aegypti* larvae exposed to different salinity levels

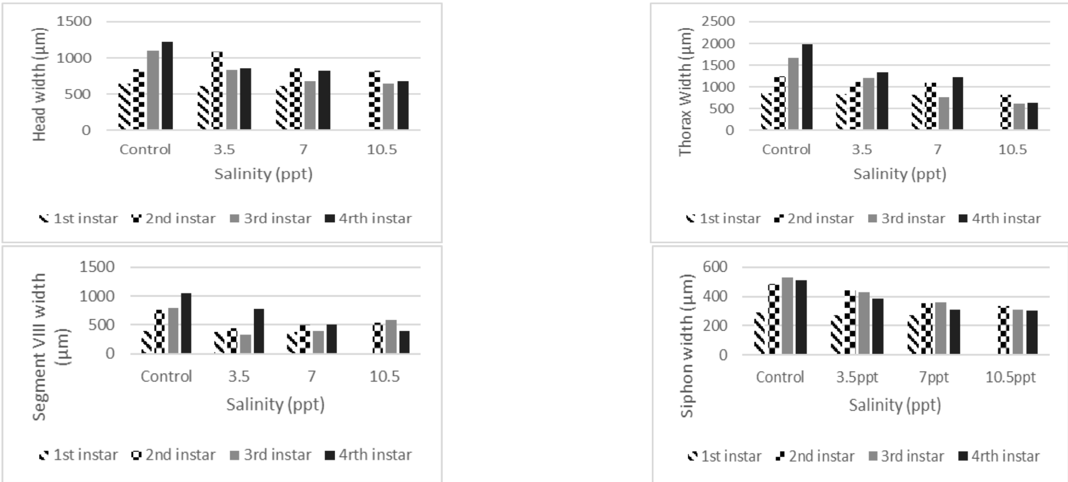

Figure 2. Width of the head (±SE; p=0.167), thorax (±SE; p=0.074), segment VIII (±SE; p=0.130) and siphon (±SE; p=0.017) of *Aedes aegypti* larvae exposed to different salinity levels

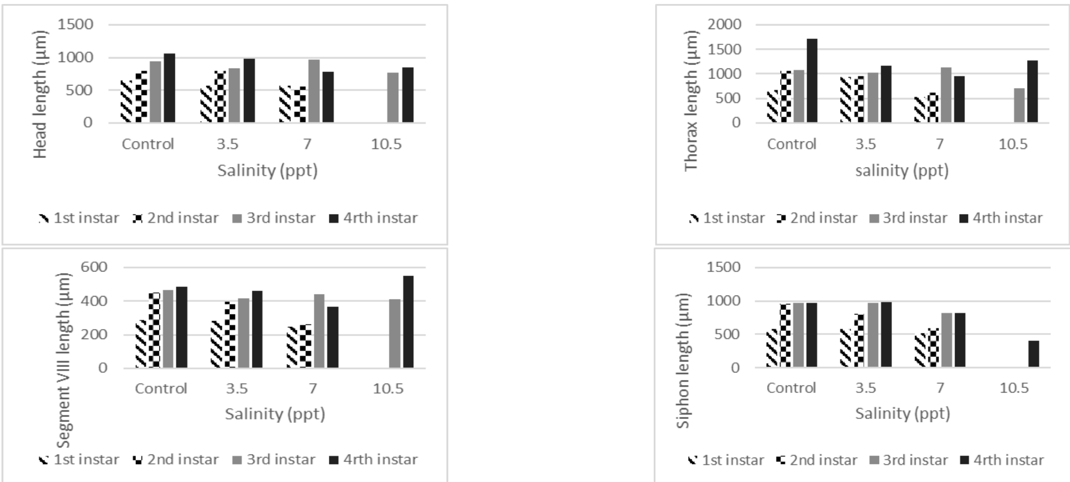

Figure 3. Length of the head (±SE; p=0.161), thorax (±SE; p=0.176), segment VIII (±SE; p=0.430) and siphon (±SE; p=0.000) of *Aedes albopictus* larvae exposed to different salinity levels

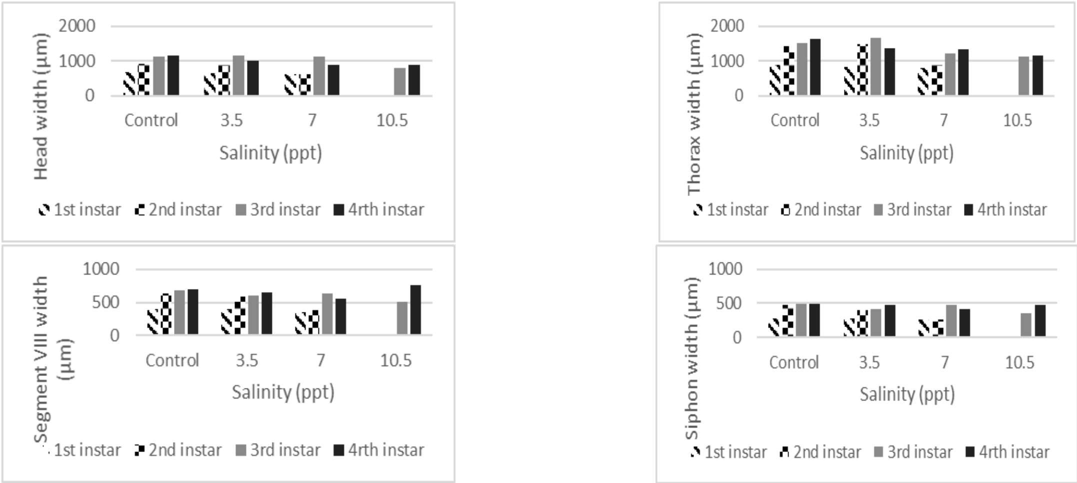

Figure 4. Width of the head (±SE; p=0.104), thorax (±SE; p=0.080), segment VIII (±SE; p=0.311) and siphon (±SE; p=0.036) of *Aedes albopictus* larvae exposed to different salinity levels

segment VIII and siphon of the 4th instar. The length and width of the head (±SE; p=0.161; p=0.104), thorax (±SE; p=0.176; p=0.080) and segment VIII (±SE; p=0.430; p=0.311) of *Aedes albopictus* larvae exposed to different salinity levels showed no significant differences, while the siphon length and the width (±SE; p=0.000; p=0.036) differed significantly by salinity level.

3.2 Larval duration, pupal and adult emergence

Figure 5 shows the larval duration of *Ae. aegypti* and *Ae. albopictus* exposed to different salinity levels. In *Ae. aegypti,* the larval duration of 1st and 2nd instars increased as salinity increased from 3.5ppt to 7ppt, but the 3rd and 4th instars had shorter larval durations than the control in the same salinity range. At 10ppt, the larval durations were all shorter than the control. In case of *Ae. albopictus,* the larval duration of 1st to 3rd instars increased as salinity increased from 3.5ppt to 7ppt. The same pattern with *Ae. aegypti* was observed for the 4th instar. Likewise, all the larval instars of *Ae. albopictus* had shorter durations at 10ppt than in the control group. The larval durations of *Ae. aegypti* and *Ae. albopictus* exposed to different salinity levels differed statistically significantly at p=0.039 and p=0.05, respectively.

Table 1 reveals the percentages of pupa and adults that emerged from each instar when exposed to different salt concentrations. At 3.5 ppt, 90% of 1st, 2nd and 3rd instars for both species emerged into pupae and adults. One hundred percent (100%) of the 4th instars for both species emerged into pupae and adults. When the salinity was increased, the percent of emerging pupa and adults decreased. At 7ppt, only 50% of *Ae. aegypti* 1st instars pupated and became adults. *Ae. albopictus* 1st instar had slightly higher percent of emergence than *Ae. aegypti* at 60%. It is also observed in this study that older instars of *Ae. aegypti* had higher percent emergence at 80% for 2nd and 3rd instars and 90% for 4th instar. The same trend was observed with 2nd to 4th *Ae. albopictus* instars. Highest percent of emergence was recorded for the 4th instar at 90%. At 10.5 ppt, only 10% of the 4th instars of *Ae. aegypti* and *Ae. albopictus* pupated and none became adults.

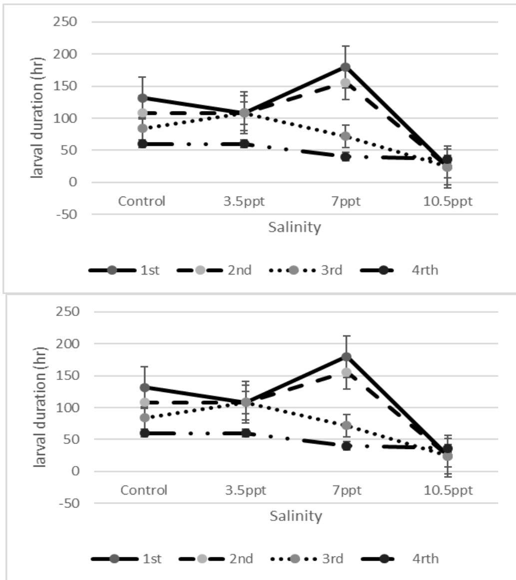

Figure 5. Larval durations of *Aedes aegypti* (p=0.039) and *Aedes albopictus* (p=0.05) exposed to different salinity levels. a) *Aedes aegypti* and (b) *Aedes albopictus*

3.3 Survival Rates

It was observed in this study that the survival rate of *Ae. aegypti* larvae decreased with salinity (Figure 6). However, older larval stages of development showed higher tolerance of salinity than the younger ones. At 3.5 ppt, the survival rate ranged from 90 to 100% for both species with 4th instar having the highest percentage. At 7ppt, the survival rate dropped, ranging from 53% for 1st instar to 93% for 4th instar of *Ae. aegypti*. In case of *Ae. albopictus*, the survival rate of 1st instar decreased to 63 %, 73 % in 2nd instar, 76 % in 3rd instar, and 90 % in 4th instar. None survived at 10.5 ppt.

Based on Probit Analysis, the LC_{50} of *Ae. aegypti* ranged from 6.1 to 7.1ppt while the LC_{90} ranged from 9.8 to

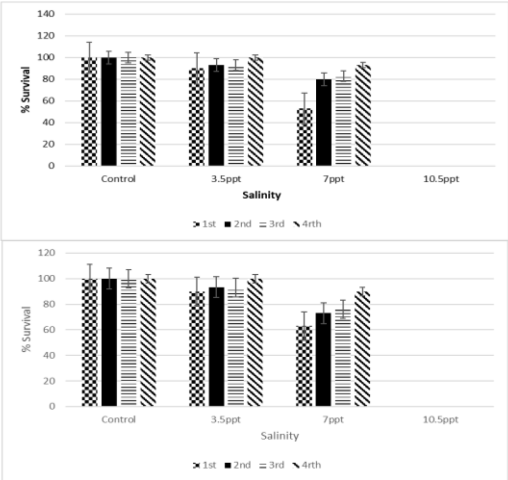

Figure 6. Survival rates of 1st, 2nd, 3rd and 4th instars of (a) *Aedes aegypti* (p=0.0114E17), and (b) *Aedes albopictus* (p=0.008 E16) exposed to different salt concentrations

11.6 ppt (Table 2). On the other hand, the LC_{50} of *Ae. albopictus* ranged in 6.5-7.1 ppt while the LC_{90} ranged within 10.4-11.6 ppt.

4. Discussion

Findings of this study showed the possibility of freshwater derived *Ae. aegypti* and *Ae. albopictus* in the Philippines to undergo pre-imaginal development in brackish water, thereby increasing the risk of transmission of arboviral diseases by these mosquito vectors even during the dry season. Results of this study support the occurrence of salinity tolerant *Ae. aegypti* and *Ae. albopictus* in Brunei Darussalam (Idris *et al*., 2013), Southern Brazil (Arduino *et al*., 2015) and Sri Lanka (Surendran *et al*., 2018). In Jaffina, Sri Lanka, *Ae. aegypti* and *Ae. albopictus* have been recently observed in artificial containers having brackish water with 15 and 14ppt salinities, respectively (Surendran *et al*., 2018). In Southeastern Brazil, *Ae. aegypti* was observed to develop in up to 14% salt concentration (Arduino *et al*., 2015). In this study, the maximum salinity tolerances of *Ae. aegypti* and *Ae. albopictus* were lower at 7 ppt. Differences could be attributed to higher salinity tolerance of brackish water derived Aedes larvae than in freshwater derived larvae.

Elimination of preferred pre-imaginal habitats of mosquitoes could be a driving force for the anthropogenically induced adaptation of *Ae. aegypti* and *Ae. albopictus* to less optimal and under-utilized habitats within their normal range close to human habitation (Ramasamy & Surendran, 2016). It has been suggested that the use of insecticides to control agricultural pests and malaria has induced the adaptation of these vectors to less desirable habitats within their natural range. In southeastern Brazil, intensification of larval control activities seems to have changed the behaviour of selecting oviposition containers in *Ae. aegypti* (Arduino *et al*., 2015). In the Philippines, destroying mosquito breeding places and spraying and fogging in hotspot areas are part of the daily routine under the "4S Kontra Dengue Program". Thus, with the anthropogenically induced adaptation of *Ae .aegypti* and *Ae. albopictus* observed in Sri Lanka, Brazil, and Brunei Darrusalam, effectiveness of the vector control programs in the Philippines has to be monitored, especially in potential atypical habitats such as brackish water.

Table 1. Percentage of pupae and adults that emerged from each instar of *Aedes aegypti* and *Aedes albopictus* when exposed to different salinity levels.

Species	Instar	Percent emergence by salt concentration							
		Control		3.5 ppt		7 ppt		10.5 ppt	
		Pupa	Adult	Pupa	Adult	Pupa	Adult	Pupa	Adult
Aedes aegypti	1st	100	100	90	90	50	50	0	0
	2nd	100	100	90	90	80	80	0	0
	3rd	100	100	90	90	80	80	0	0
	4th	100	100	100	100	90	90	10	0
Aedes albopictus	1st	100	100	90	90	60	60	0	0
	2nd	100	100	90	90	70	70	0	0
	3rd	100	100	90	90	80	80	10	0
	4th	100	100	100	100	90	90	10	0

Table 2. LC_{50} and LC_{90} of (a) *Aedes aegypti*, and (b) *Aedes albopictus* larval instars

Species	Larval Instar	LC_{50} (ppt)	LC_{90} (ppt)	χ^2	Intercept	Slope
Aedes aegypti	1st	6.145	9.778	1.884	-0.010527	6.354164
	2nd	7.142	11.601	6.647	-0.193111	6.0824
	3rd	7.142	11.601	6.647	-0.193111	6.0824
	4th	-	-	-	-	-
Aedes albopictus	1st	6.461	10.402	3.015	-0.021498	6.197042
	2nd	6.793	11.011	4.55	-0.021498	6.197042
	3rd	7.142	11.601	6.647	-0.193111	6.0824
	4th	-	-	-	-	-

Culicidae have three osmoregulatory strategies, freshwater osmoregulator, euryhaline osmoregulator and euryhaline osmoconformist (Bradley, 1987). Observed salinity tolerance of *Ae. aegypti* and *Ae. albopictus* during this study could be due to the osmoconformist behaviour of the genus *Aedes* (Arduino *et al*., 2015). *Ae. aegypti* can ionoregulate by synthesizing extra osmolytes such as amino acid to increase internal osmotic pressure of their tissues and hemolymph. It is also equipped with osmoregulatory tissues which function to maintain the ionic level within the mosquito larvae (Akhter, unpublished). It is observed that hemolymph ion concentration and Na^+ and Cl^- transport kinetics of the anal papillae are altered with changes in salinity (Donini, Gaidhu, & Strasberg, 2017). Anal papillae were observed to be enlarged in an adaptation of *Ae. aegypti* to salinity (Surendran, *et al*., 2018). The syncytial epithelial tissues of the anal papillae are believed to have aquaporins which allow transport of water. The greater salinity tolerance of 3rd and 4th instars of *Ae. aegypti* and *Ae. albopictus* may be due to structural and physiological changes related to those that reduce ion permeability in pupae (Bradley, 1987) as observed in the study of Ramasamy *et al*. (2011). For instance, the increase in the length and width of segment VIII in the 4th instar of *Ae. albopictus* may confer salinity tolerance.

Maintaining osmoregulatory functions of the organism has been observed to reduce growth rate (Clarck *et al*., 2004; Hassell, Kefford & Nugegoda, 2006). It has been observed that a significant portion of the energy budget of *Ae. aegypti* larvae is used for ionoregulation at the higher salinities within the tolerable range (Clarck *et al*., 2004). In addition, at these higher salinities, Aedes mosquitoes have decreased feeding rate to avoid ingestion of ions at greater rate. These could be the reasons for the decreases in body width and length of *Ae. aegypti* and *Ae. albopictus* larvae exposed to 7ppt and 10.5ppt salinities. However, this decrease in body width and length could be a compensation mechanism of both species to balance with the salinity-induced changes in growth rate. To compensate for the negative effects of salinity on growth rate, development time is adjusted and pupation is delayed until the animal has acquired a critical mass for pupation (Clarck *et al*., 2004). In this study, longer larval duration for *Ae. aegypti* and *Ae. albopictus* was observed as salinity increased to 7ppt. This may show compromised rapid larval development in order to ensure pupation before they attain the mass they would have attained in less saline conditions. Delayed pupation can also be beneficial for mosquitoes since pupa have no ability of osmotic regulation (Hassell *et al*., 2006). Decreasing larval duration observed in 3rd and 4th instars of both species when exposed to 3.5ppt and 7ppt may suggest that older instars have distinct responses to salinity and are less affected by it. They tend to shorten their larval duration to minimize the time of exposure to unfavorable environmental conditions.

Results of this study further revealed that *Ae. albopictus* had slightly better tolerance of increasing salinity than *Ae. aegypti*, as shown in its higher percent emergence and survival. This result supports Kengne, Charmantier, Blondeau-Bidet, Constantini, & Ayala (2019) in that *Ae. albopictus* is the most tolerant among disease-vector mosquitoes. Among Aedes, Anopheles and Culex mosquitoes, *Ae. albopictus* was the only species with survivors after 24hr incubation in a test medium with 500mOsm/kg (50% SW). High resistance of *Ae. albopictus* was also observed to other environmental conditions, such as multiple commonly used insecticides (Su *et al*., 2019). This is of public health interest because *Ae. albopictus* demonstrated high vector competence for a number of highly pathogenic viruses, such as dengue fever under experimental conditions (Johnson *et al*., 2002). Moreover, it was observed that low rates (1.12-3.73%) of interspecific mating occur in nature among populations of *Ae. aegypti* and *Ae. albopictus* that have co-existed sympatrically over 3-150 years (Bargielowski *et al*., 2015; Braks, Honorio, Lourenc0-De-Oliveira, Juliano, & Louninos, 2003). Thus, further research focused on the vector ecology and evolution in the context of vectorial capacity of *Ae. aegypti* and *Ae. albopictus* is recommended.

5. Conclusions

This study assessed the pre-imaginal development of freshwater derived *Ae. aegypti* and *Ae. albopictus* in brackish water under laboratory conditions. Maximum salinity tolerance of *Ae. aegypti* and *Ae. albopictus* was at 7 ppt. At 7ppt, the survival rate ranged from 53% to 93% and from 63% to 90% for *Ae. aegypti* and *Ae. albopictus*, respectively. Older larval stages of development were observed to have higher salinity tolerance than the younger ones. The percent emergence of *Ae. aegypti* ranged from 50% to 90% while 60% to 90% for *Ae. albopictus*. For both species, the growth rate as manifested in body length and width was observed to decrease with salinity level. This could be a compensation mechanism in both species to the salinity-induced changes of growth rate. Larval durations of 1st and 2nd instars increased with salinity. In contrast, the larval durations of 3rd and 4rth instars decreased with salinity level. Decreased larval duration observed in 3rd and 4th instars of both species, when exposed to 3.5ppt and 7ppt, may suggest that the older instars have a distinct response to salinity and are less affected by it. The LC_{50} for *Ae. aegypti* ranged from 6.1 to 7.1 ppt and LC_{90} ranged from 9.8 to 11.6 ppt. On the other hand LC_{50} of *Ae. albopictus* ranged within 6.5-7.1 ppt and LC_{90} ranged within 10.4-11.6 ppt. Chi-square test showed that there was no significant difference between the mortality rates of *Ae. aegypti* and *Ae. albopictus* (p= 0.063089).

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References

Andrew, J., & Bar, A. (2013). Morphology and morphometry of *Aedes aegypti* adult mosquito. *Annual Review and Research in Biology, 3*(1), 52-69

Arduino, B. M., Mucci, F. L., Serpa, N. L. L. & Rodriguez, D. M. M. (2015). Effect of Salinity on the Behavior of *Aedes aegypti* populations from coast and plateau of Southern Brazil. *Journal of Vector Borne Diseases, 52*, 79-87

Bargielowski, I. E., Lounibos, L. P., Shin, D., Smartt, C. T., Carrasquilla, M. C., Henry, A., . . . Dennett, J. A. (2015). Widespread evidence for interspecific mating between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in nature. *Infection Genetics and Evolution, 36*, 456-461. doi:10.1016/j.meegid 2015.08.016

Braks, M. A., Honorio, N. A., Lourenco-De-Oliveira, R., Juliano, S. A, & Lounibos, P. (2003). Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. *Journal of Medical Entomology, 40*, 78

Bradley, T. J. (1987). Physiology of osmoregulation in mosquitoes. *Annual Review of Entomology, 32*, 43-462

Clark, M. T., Flis, B. J. Y. & Remold, S. K. (2004). Differences in the effects of salinity on larval growth and development programs of freshwater and euryhaline mosquito species (Insect: Diptera, Culicidae). *The Journal of Experimental Biology, 207*, 2289-2295

Donini, A., Gaidhu, M. P., Strasberg, D. R. &Donnell, J. O. (2007). Changing salinity induces alterations in hemolymph ion concentrations and Na+and Cl transport kinetics of the anal papillae in the larval mosquito, *Aedes aegypti*. *The Journal of Experimental Biology, 210*, 983-992

Harrison, B. A. & Rattanarithikul, R. (1973). Comparative morphology of the early larval instars of *Aedes aegypti* and *A. seatoi* in Thailand. *Mosquito Systematics, 5*(4)

Hassell, K. L., Kefford, B. J. & Nugegoda, D. (2006). Sub-lethal and chornic salinitytolerance of three freshwater insects: *Cloeon* sp. and *Centroptilum* sp. (Ephemeroptera: Baetidae) and *Chironomus* sp. (Diptera Chironimidae). *The Journal of Experimental Biology*, 4024-4032

Idris, F. H., Usman, A., Surendran, S. N. & Ramasamy R. (2013). Detection of *Aedes albopictus* pre-imaginal stages in brackish water habitats in Brunei Darussalam. *Journal of Vector Ecology, 38*(1)

Johnson, B. W., Chambers, T. V., Crabtree, M. B., Filippis, A. M., Vilarinhos, P. T., Resende, M. C., . . . Miller, B. R. (2002). Vector competence of Brazilian *Aedes aegypti* and *Aedes albopictus* for a Brazilian yellow fever virus isolate. *Transactions of the Royal Society of Tropical Medicine, 96*, 611-613

Jude, P. J., Tharmasegaram, T., Sivasubramanoyam, G., Senthilnanthanan, S. V., Raveebdranm, S., Ramasamy, R., & Surendran, S. N. (2012). Salinity-tolerant larvae of mosquito vectors in tropical coast of Jaffna, Sri Lanka and the effect of salinity on the toxicity of *Bacillus thuringiensis* to *Aedes aegypti*. *Vectors and Parasite, 5*, 269

Kengne, P. G., Charmnatier, E., Blondeau-Bidet, C., Constantini, C., Ayala, D. (2019). Tolerance of disease-vector mosquitoes to brackishwater and their osmoregulatory ability. *Ecosphere, 10*(10), e02783.10.1002/ecs.2.2783

Philippine Coastal and Marine. (2019, May 5). An introduction. Retrieved from: http://siteresources.worldbank.org/INTPHILIPPINES/Resources/PEM05-ch1.pdf

Ramasamy, R. & Surendran, S. (2016). Mosquito vectors developing atypical anthropogenic habitats: Global overview of recent observations, mechanisms and impact on disease transmission. *Journal of Vector Borne Diseases*, 91-98

Ramasamy, R. & Surendran, S. (2011). Possible impact of rising sea levels on vector borne infectious diseases. *BMC Infectious Diseases*, 11-18

Ramasamy, R., Surendran, S. N., Jude, P. J., Dharshini, S. & Vinobaba, M. (2011). Larval development of *Aedes aegypti* and *Aedes albopictus* in Peri-Urban Brackishwater and its implications for transmission of Arboviral Diseases. *Plos Neglected Tropical Diseases, 5*(11), e1369. doi:10.1371/journal.pntd.0001369

Rueda, L. M. (2004). Pictorial keys for the Identification of mosquitoes (Diptera:*Culicidae*) associated with Dengue virus transmission. *Zootaxa*, 589

Su, X., Guo, Y., Deng, J, Xu, J., Zhou, G., Zhou, T., . . . Chen, X G. (2019). Fast emerging insecticide resistance in *Aedes albopictus* in Guangzhou, China: Alarm to the dengue epidemic. *Plos Neglected Tropical Diseases*. *13*(9), e007665. doi:10.1371/journal.pntd.0007665

Surendran, S. N., Jude, P. J., Thabothiny, V., Raveendran, S. & Ramasamy, R. (2012). Pre-imaginal development of *Aedes aegypti* in brackish and fresh water urban domestic wells in Sri Lanka. *Journal of Vector Ecology, 37*(2)

Surendran, S. N., Sivabalakrishnan, K., Jayadas, T. T. P., Santhirasegaram, S., Laheetharan, A., Santhilnan than, M. & Ramasamy, R. (2018). Adaptation of *Aedes aegypti* to salinity: Characterized by larger anal papillae in larvae. *Journal of Vector Borne Diseases*, 235-238

Surendran, S. N., Veluppillai, T., Eswaramohan, T., Sivabalakrishnan, K., Noordeen, F. & Ramasamy, R. (2018). Salinity tolerant *Aedes aegypti* and *Ae. albopictus*-Infection with dengue virus an contribution to dengue transmission in a coastal peninsula. *Journal of Vector Borne Diseases*, 26-33

Teng, H. (1996). Identification of larval instars of *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) (diptera: culicidae) based on head capsule size. *Journal of Vector Ecology, 21*(2), 186-191

World Health Organization. (2019). Update on dengue situation in Western Pacific Region. Retreived from: https://www.who.int/docs/default-source/wprodocuments/emergency/surveillance/dengue/dengue-20190425.pdf?sfvrsn=b42cfbd0_10