SALINITY EFFECTS ON THE DISTRIBUTION OF *Aedes aegypti* AND *Aedes albopictus* IN ST. JOHNS COUNTY, FLORIDA

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ABSTRACT

The distribution of *Aedes aegypti* in St. Johns County (SJC), Florida is suggested to be coastal along the inter-coastal waterway. Anastasia Mosquito Control District (AMCD) conducted a study to investigate the effects of salinity on the distribution of container-inhabiting *Aedes* in SJC. Mean weekly abundances of *Aedes aegypti* and *Aedes albopictus* at 7 different distances along the coastal-inland gradient, ranging from the SJC coast to the St. Johns River, were monitored for 10 weeks. Bi-weekly salinity measurements of potential *Aedes* breeding containers were obtained at each distance. A laboratory test was conducted by allowing preferential oviposition at different salinity levels and adult emergence was monitored. Both container salinity and *Ae. aegypti* abundance were significantly higher up to 5.0 km from the coast compared to the other distances. *Ae. aegypti* abundance was positively and negatively associated with the container salinity and distance from the coast respectively. *Ae. albopictus* abundance was significantly different (lower) only at the distance at which the highest salinity was recorded. The association of abundance (positive) was significant only with the distance from the coast. The adult emergence rate of *Ae. aegypti* in the laboratory was higher at higher salinity levels up to 5 ppt while there was no significant difference in *Ae. albopictus* emergence rate at different salinities. The study demonstrated a possible salinity effect on the distribution of *Ae. aegypti* but not *Ae. albopictus* in SJC, FL. The results warrant further studies on salinity effects with other confounding environmental factors that would contribute to the distribution of the two species in the county.

Key words: *Aedes aegypti; Aedes albopictus; container-inhabiting; abundance; distribution; salinity

INTRODUCTION

Container-inhabiting *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse) are of a public health concern as they vector important arboviruses such as yellow fever, dengue, Zika, and chikungunya (Gubler 2010). Due to the rampant occurrence of these diseases worldwide, much more attention is being paid to vector control. Successful vector control requires knowledge of the spatial distribution of the target species. A number of macro-level (e.g. environmental and climatic conditions) as well as micro-level factors (e.g. immature habitat preferences) influence the spatial distribution of the two species (Juliano et al. 2002, Loumibos et al. 2010). It is well documented that the two species follow a habitat segregation associated with characteristics of urban and rural areas, in which *Ae. aegypti* is dominant in urban areas and *Ae. albopictus* is dominant in rural areas (Braks et al. 2003, Rey et al. 2006, Tsuda et al. 2006). Barbosa et al. (2020) reported different observations from Brazil that populations are not concentrated in clearly distinct territories, as has been observed in other studies. The mean temperature of the coldest month, dry season duration, and annual rainfall were identified as the major factors affecting the distribution of the two species in Madagascar (Fontenille and Rodhain 1989). Loumibos et al. (2010) demonstrated hotter and drier habitat tolerance of *Ae. aegypti* relative to *Ae. albopictus*. In addition, micro-level habitat characteristics of breeding containers effect on the spatial distribution as well. *Aedes aegypti* is known to oviposit in many types of artificial water-holding containers (Chareonviriyaphap et al. 2003) such as boats, pontoons, cranes, and other associated paraphernalia (e.g., machinery and equipment), and in any place with items that can hold water (e.g., motor-parts, tires, discarded boilers, air conditioners, etc). (Cheong 1967). *Aedes albopictus* select similar containers (Gao et al. 2019), as well as natural water-holding containers such as tree holes and plant axils (Cheong 1967, Okogun et al. 2003, Paupy et al. 2009, Dom...
et al. 2013b, Bashar et al. 2016). Many factors including physical, biological, and chemical characteristics of breeding containers influence the oviposition habitat preferences and the immature stage survivorship in those habitats (Reji et al. 2013, Thangamathith et al. 2014). One of the key influential characteristics to be considered in breeding containers is the quality of the water (Oyewole et al. 2009, Dom et al. 2017, Sultana et al. 2017). The amount of salt, dissolved organic and inorganic matter, degree of eutrophication, turbidity, presence of suspended mud, presence or absence of plants, temperature, light and shade, and hydrogen ion concentration are some of those water quality parameters (Mogi 1978, Amerasinghe et al. 1995, Gimnig et al. 2001).

St. Johns County (SJC), Florida (FL) currently records the presence of both Ae. aegypti and Ae. albopictus. The field observations suggested a restricted distribution of Ae. aegypti in areas along the inter-coastal waterway, the most populated and urbanized area of the county. The persistence of Ae. aegypti and Ae. albopictus in an urban-rural gradient in FL is well documented (Braks et al. 2003, Rey et al. 2006). The urban-rural gradient-based distribution was explained by the effects of temperature, relative humidity, and the relative availability of wet containers along a coastal-inland gradient in Palm Beach County, Florida (Reiskind and Lounibos 2012). The same study reported that Ae. aegypti was found in greater abundance closer to the intracoastal waterway whereas the opposite pattern was observed for Ae. albopictus. However, the authors did not observe the effects of salinity gradient on the distribution of the two species. Although both species have been widely considered as freshwater species (Bradley 1987, WHO 2009, Walter Reed Biosystematics Unit 2011, Roberts and Irving-Bell 1997), the salinity tolerance has been extensively explored. A number of studies reported on the salinity tolerance of Ae. aegypti; in Pakistan (Hai et al. 2021), in Sri Lanka (Ramasamy et al. 2011, Jude et al. 2012, Surendran et al. 2012, Ramasamy et al. 2014), in the USA (Yee et al. 2014), in Brazil (Arduino et al. 2010, 2015), and in Mexico (Galaviz-Parada et al. 2019). Some studies reported salinity tolerance of Ae. albopictus (Ramasamy et al. 2011, Jude et al. 2012, Ramasamy et al. 2014, Idris et al. 2023), while Yep et al. (1995) reported freshwater preference in a laboratory study. The final outcome of oviposition habitat preferences and immature survivorship in those habitats is the adult mosquito abundance and the spatial distribution (Reisen et al. 1981). Thus, the breeding container salinity could be another important contributory factor for the spatial distribution of container-inhabiting Aedes species. The present study was conducted to determine (i) the spatial distribution of Ae. aegypti and Ae. albopictus populations in SJC, FL, and (ii) the effects of container salinity on that distribution. Understanding the salinity effects on spatial distribution would provide better insights for planning control operations.

**MATERIALS AND METHODS**

The field test was conducted from June to August (2022), the usual peak season of container-inhabiting Aedes in the county. Three parallel transects running across the county from the Atlantic coastal line to the St. Johns River were selected for sampling. Transects were 4-4.5 km apart from each other and each was marked with 7 different distances from the coast (approximate distances 0.7 km, 3.0 km, 5.0 km, 7.0 km, 11.0 km, 21.0 km, 28.0 km) (Fig. 1). A location conducive for container-inhabiting Aedes at each distance of each transect was selected as the sampling point. The adult abundance of the two species was monitored once weekly for 10 weeks using one Biogents Sentinel trap (BG) baited with a BG lure (Biogents AG, Regensburg, Germany) and dry ice that was left out for 24 hr at each sampling point. Salinity (Elite CTS tester, ThermoFisher Scientific Inc., USA), total dissolved solids (TDS) (Elite CTS tester, ThermoFisher Scientific Inc., USA), and pH (Elite pH and pH spear tester, ThermoFisher Scientific Inc., USA) measurements were taken once in 2-weeks from 1-3 containers (e.g., tires, bird baths, toys, ornamental cement ponds, ornamental water fountains, leaf axils) from around each sampling point.

A laboratory test (temperature 26±2°C, relative humidity 80%±10, light: dark cycle 14:10) was conducted with long-established insectary colonized Ae. aegypti (Orlando strain_1952) and Ae. albopictus (Gainesville strain_2003). Fifteen blood-fed females were released into separate BugDorms (60x60x60 cm) (MegaView Science Co., Ltd., Taiwan) to have three replicates of each species. Six ovicups (Solo®, 266 ml, Dart Container Cooperation, MI, USA) of different salinity levels (0.00, 0.05, 0.50, 1.00, 5.00, 10.00 ppt), each layered with a seed germination paper were placed in each BugDorm. Ovipapers were collected after three days, let dry for 24 hr and the eggs were counted under the microscope. Egg papers were re-immersed in the same respective ovi-cups for hatching and cups were covered with punctured lids to minimize evaporation. Hatching was not induced or synchronized purposefully so that it took the natural course of continuing to hatch over time. Larvae were fed with Tetramin fish food (Tetra, TetraMin® tropical flakes, Spectrum Brands, Inc. – one part powdered flakes dissolved in approximately 6 parts water), the frequency and the amount based on the visual...
Figure 1. Study area and sampling grid for the determination of salinity effects on *Ae. aegypti* and *Ae. albopictus* distribution in St. Johns County, Florida.

observation of larval density so that the scum formation by surplus food was prevented. Once the first pupation was observed, pupation was monitored daily, and the pupae were transferred to a different cup with the same salinity water for adult emergence. Adult emergence started in the second week after egg immersion in water and spanned out for several weeks. The adults that emerged from each cup were counted for 7 weeks. The procedure was repeated in three trials.

Descriptive statistics were used to summarize the mean weekly species abundance (mean weekly BG trap count per night) and mean weekly water quality parameters at different distances along the coastal-inland gradient. As the data failed to fulfill the requirements for parametric tests, the Kruskal-Wallis test with Dunn-Bonferroni *post hoc* pairwise comparisons and the Mann-Whitney U test were used to compare means at different distances. We assumed that the mean of three measurements of a particular water quality parameter at each sample point would represent the mean value of all possible immature *Aedes* habitats around that sampling point. Data of only corresponding weeks and of sampling points with three containers available for measurements were used to determine associations between species abundance and water quality parameters. The associations were determined by constructing Generalized Linear Models (GLM). As the initial construction of Poisson models to account for the count data of the response variable (i.e. species abundance) indicated over-dispersion, negative binomial models were constructed subsequently. Associations were expressed in terms of exponentiation of the regression coefficients (IRR: Incident Rate Ratio or risk level) and the corresponding probability of significance (P value). Associations between variables in the laboratory test were performed using either GLM (negative binomial) or Spearman’s rank correlation analysis. All analyses were
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performed using SPSS (IBM® SPSS® Statistics, V.20) with significance was set at P-value < 0.05.

**RESULTS**

Container salinity ranged from 0 to 3.3 ppt across the county with the highest mean salinity at 3.0 km followed by 5.0 km and 0.7 km respectively from the coast and the lowest was at 28.0 km (Table 1). Mean container salinity at different distances was significantly different ($\chi^2 = 52.628$, P<0.0005). *Post hoc* pairwise comparisons (Table 2) indicated three distinct salinity clusters with significantly different container salinity means along the coastal-inland gradient (see Table 2 for P-values). High salinity cluster contained distances from 0.7 – 5.0 km. From 7.0 – 21.0 km were included in the moderate salinity cluster and the distance 28 km was included in the low salinity cluster. The highest mean TDS was at 3 km and the lowest was at 28 km (Table 1) with significant differences in the distribution between different distances ($\chi^2 = 58.293$, P<0.0005). Corresponding to the salinity distribution, TDS distribution demonstrated the same three clusters. The high TDS cluster contained distances from 0.7-5.0 km. Due to high variance in the distribution TDS at 0.7 km.

**Table 1.** Means of salinity, total dissolved solids (TDS), and pH of containers at different distances along the coastal-inland gradient (n=total number of containers measured during the study).

<table>
<thead>
<tr>
<th>Distance from the coast (km)</th>
<th>Salinity (mean±SE) (ppt)</th>
<th>Total dissolved solids (mean±SE) (ppm)</th>
<th>pH (mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7 (n=25)</td>
<td>0.42±0.10</td>
<td>728.08±208.03</td>
<td>7.77±0.25</td>
</tr>
<tr>
<td>3.0 (n=28)</td>
<td>0.73±0.13</td>
<td>1058.53±161.19</td>
<td>8.33±0.14</td>
</tr>
<tr>
<td>5.0 (n=19)</td>
<td>0.51±0.12</td>
<td>714.62±171.38</td>
<td>8.08±0.15</td>
</tr>
<tr>
<td>7.0 (n=37)</td>
<td>0.27±0.09</td>
<td>280.51±55.64</td>
<td>7.73±0.10</td>
</tr>
<tr>
<td>11.0 (n=18)</td>
<td>0.12±0.04</td>
<td>232.10±48.50</td>
<td>7.32±0.24</td>
</tr>
<tr>
<td>21.0 (n=31)</td>
<td>0.16±0.04</td>
<td>292.15±45.00</td>
<td>7.59±0.12</td>
</tr>
<tr>
<td>28.0 (n=31)</td>
<td>0.04±0.03</td>
<td>133.75±41.55</td>
<td>7.64±0.11</td>
</tr>
</tbody>
</table>

**Table 2.** The probability of significance of Kruskal-Wallis Dunn-Bonferroni *post hoc* pairwise comparisons between different distances along the coastal-inland gradient (* indicates significant differences between pairs).

<table>
<thead>
<tr>
<th>Salinity cluster</th>
<th>Distance pair (km)</th>
<th>Probability of significance (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salinity</td>
<td>Total dissolved solids</td>
</tr>
<tr>
<td>High</td>
<td>0.7/3.0</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>0.7/3.0</td>
<td>0.620</td>
</tr>
<tr>
<td></td>
<td>3.0/5.0</td>
<td>0.366</td>
</tr>
<tr>
<td>Moderate</td>
<td>5.0/7.0</td>
<td>0.025*</td>
</tr>
<tr>
<td></td>
<td>7.0/11.0</td>
<td>0.329</td>
</tr>
<tr>
<td></td>
<td>7.0/21.0</td>
<td>0.710</td>
</tr>
<tr>
<td></td>
<td>14.0/21.0</td>
<td>0.521</td>
</tr>
<tr>
<td></td>
<td>21.0/28.0</td>
<td>0.009*</td>
</tr>
<tr>
<td>Low</td>
<td>0.7/28.0</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>3.0/28.0</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>5.0/28.0</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>7.0/28.0</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td>11.0/28.0</td>
<td>0.101</td>
</tr>
</tbody>
</table>
km was significantly lower than that at 3.0 km, but it was still higher than those at 7 km and further. Moderate TDS cluster contained distances 7.0 - 21.0 km, and 28.0 km was included in the low TDS cluster (Table 2). The mean pH at each distance indicated alkalinity in containers with the highest pH at 3.0 km (Table 1) which was significantly different from that of all other distances except 0.5 km and there was no such clustering of pH levels (Table 2).

The highest mean weekly abundance (per trap number) of *Ae. aegypti* (33.31±6.49, n=29) was recorded along the coast at 0.7 km and the lowest was at 21.0 km (0.21±0.12, n=28) (Fig. 2). The mean weekly abundance of *Ae. aegypti* was significantly different between different distances ($\chi^2 (6) = 107.84$, $P<0.0005$, n=29, n=29, n=30, n=29, n=27, n=28, n=28) - the sample size at different distances was varied due to malfunction of traps). According to post hoc pairwise comparisons, the distribution of *Ae. aegypti* abundance indicated two marked clusters along the coastal-inland gradient. The high abundance cluster contained distances from 0.7 – 5.0 km, the low abundance cluster contained 7.0 – 21.0 km, and 28.0 km (Table 2). In contrast, the highest mean weekly abundance of *Ae. albopictus* was at 28.0 km (36.04±7.69, n=28) and the lowest was at 3.0 km (2.69±1.22, n=29) (Fig. 2) at which all the water quality parameters were at their highest. The only significant differences in the abundance of *Ae. albopictus* ($\chi^2 (6) = 40.25$, P<0.0005), were at 3.0 km and 28.0 km which were significantly lower and higher respectively than that at 0.7 km (see Table 2 for P values). The results indicate a broader distribution of *Ae. albopictus* across the coastal-inland gradient yet with a higher tendency to be away from the coast and towards the river.

Spearman’s correlation analysis demonstrated a strong correlation between salinity and TDS ($r=0.739$, $P<0.0005$, n=36), but none of the two parameters were significantly correlated to pH ($r=0.183$, P=0.286, n=36 and $r=0.283$, P=0.094, n=36 respectively). TDS was not included in the GLM models to prevent multicollinearity effects on salinity due to their strong linear correlation. The distance from the coast was included in models as a predictor with salinity and pH. The goodness of fit of the three-predictor model for *Ae. aegypti* was 1.568 and Likelihood ratio $\chi^2 (5) = 89.193$, P<0.0005). The model demonstrated significant positive (IRR=4.676, 95% CI: 1.673 – 13.068, P=0.003) and negative (IRR=0.721, 95% CI: 0.623 – 0.834, P<0.0005) associations of *Ae. aegypti* abundance with container salinity and distance respectively (Fig. 3) The mean weekly *Ae. aegypti* abundance would be increased by a factor of 3.676 for each additional unit of mean weekly container salinity and be decreased by a factor of 0.279 with each additional km from the coast while

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Distribution of weekly mean abundance of adult *Aedes aegypti*, *Aedes albopictus*, and weekly mean container salinity at different distances along the coastal-inland gradient (error bars = standard error of the mean).
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Figure 3. Association of distance from the coast (above) and container salinity (below) with the abundance of *Aedes aegypti* and *Aedes albopictus*.

controlling the other parameter interchangeably. There was no significant association between container pH and mean weekly *Ae. aegypti* abundance (IRR=0.53, 95% CI: 0.269 – 1.043, P=0.066). The goodness of fit of the three-predictor negative binomial model for *Ae. albopictus* was of 0.948 and the Likelihood ratio $\chi^2_{(3)} = 15.137$, P=0.003). The model was significant only for the distance (IRR=1.066, 95%CI: 1.024 – 1.11, P=0.002) indicating an increase in the mean weekly *Ae. albopictus* abundance by a factor of 0.066 with each additional km from the coast. There were no significant associations of mean weekly *Ae. albopictus* abundance with container salinity (IRR=0.843, 95% CI: 0.317 – 2.241, P=0.732) (Fig. 3) and pH (IRR=1.349, 95% CI: 0.657 – 2.769, P=0.415).

Adult emergence in the laboratory was observed from the second week after egg immersion and it was stretched over more than 7 weeks. Both species had no adults emerging at 10.00 ppt and it was not included in the analysis. Weekly emergence rates (cumulative percent eggs developed into adults—both males and females) of *Ae. aegypti* at any salinity level were not significantly different. However, the emergence rates at different salinity levels...
were significantly higher in the fifth week than in earlier weeks ($\chi^2_{(4)}=11.55$, $P=0.021$). Thus, the emergence rate in the fifth week after egg immersion (3 weeks of emergence) was selected for the comparisons between different salinity levels and the determination of correlation between variables. The emergence rate was significantly different at different salinity levels ($\chi^2_{(4)}=11.55$, $P=0.021$) with the highest rate at 5.00 ppt (16.16±6.19, $n=8$) (Fig. 4) Post hoc pairwise comparisons indicated that the emergence rate was not significantly different up to 0.50 ppt ($P=0.782$ for 0.00/0.05 ppt, $P=0.972$ for 0.050 ppt, $P=0.768$ for 0.05/0.50 ppt), but was significantly higher at 1.00 ppt than at 5.00 ppt ($P=0.045$) and continued to be higher at 5.00 ppt. Spearman’s correlation analysis confirmed a significant positive correlation between the emergence rate and the salinity level ($r=0.47$, $P=0.002$, $n=41$).

The weekly emergence rate of *Ae. albopictus* was significantly different between different salinity levels except 0.05 ppt ($\chi^2_{(5)}=13.206$, $P=0.022$ for 0.00 ppt, $\chi^2_{(5)}=8.204$, $P=0.145$ for 0.05 ppt, $\chi^2_{(5)}=22.386$, $P<0.0005$ for 0.50 ppt, $\chi^2_{(5)}=18.19$, $P=0.003$ for 1.00 ppt, $\chi^2_{(5)}=12.947$, $P=0.024$ for 5.00 ppt). At those salinities, the emergence rate was significantly higher in the third week than the second week ($P=0.025$ for 0.00 and 5.00 ppt, $P=0.009$ for 0.50 ppt and $P=0.042$ for 1.00 ppt) with no significant differences between other weeks. Thus, like in *Ae. aegypti* comparisons, the emergence rate at the fifth week was selected for the determination of correlation between variables. The emergence rate in the fifth week was highest at 1.00 ppt (20.81±6.41) and lowest at 5 ppt (8.21±1.81) (Fig. 4) with no significant difference between different salinity levels ($\chi^2_{(5)}=2.56$, $P=0.634$) and no significant correlation between the two variables ($r=-0.127$, $P=0.418$, $n=43$).

**DISCUSSION**

Salinity levels <0.5, 0.5 to 30, and >30 ppt are considered fresh, brackish, and saline waters respectively (Ramasamy et al. 2011). In the present study, the container salinity distribution of SJC, FL was almost within the freshwater salinity range with a slightly brackish tendency within the coastal stretch up to 5.0 km. Interestingly, the distribution of *Ae. aegypti* was almost restricted to the 5.0 km coastal stretch. However, *Ae. albopictus* did not demonstrate significant discrimination in distribution across the county although there was a tendency to have high abundances at distances away from the coast. Overall, the proportional distribution of the two species was markedly separated along the coastal-inland gradient with *Ae. aegypti* dominating the coastal stretch and *Ae. albopictus* dominating the inland area. The significantly lower abundance of *Ae. albopictus* at the distance with the highest container salinity could be an indication of its low preference for high salinities. The laboratory test results demonstrated similar trends in adult emergence. Both field and laboratory results matched with the findings of previous studies that *Ae. aegypti* achieved higher survival in higher salinity conditions (Yee et al. 2014, Clark et al. 2004). The present study demonstrates that, *Ae. aegypti* of SJC prefers to breed in high salinity freshwater or slightly

![Figure 4. Mean adult emergence rate (in the 5th week after egg immersion in water ± SE) of *Aedes aegypti* and *Aedes albopictus* at different salinity levels.](image-url)
brackish water whereas *Ae. albopictus* did not indicate any salinity choice for breeding. The study suggests that the container salinity could be a significant contributory factor for the abundance and distribution of *Ae. aegypti* in SJC, FL.

Notably, our study agreed with previous study results (Yee et al. 2014, Clarck et al. 2004) in the laboratory which demonstrated a prolonged larval development time of *Ae. aegypti* at higher salinities. The current laboratory study would have been improved with using with wild mosquitoes. However, the use of a long-established colony strain in the laboratory study does not appear to have affected any significant discrepancy in results. The possibility of larval competition was a limitation in the laboratory study and could have been eliminated if an equal number of larvae were used at each salinity level. Another limitation of this study was not selecting containers based on the presence of larvae and using emergence traps to collect adults so that direct correlations would be confirmed. As the availability of potential breeding containers was scarce around some sampling points, the introduction of ovi-cups at different distances would have been an alternative strategy to collect the data.

As per our knowledge, this is the first study that attempted to correlate breeding container salinity on the spatial distribution of container-inhabiting *Aedes* species. However, we considered this a preliminary study. Further studies are needed to determine combined effects of other factors such as the affinity of *Ae. aegypti* for urbanized areas with high human populations (Braks et al. 2003, Rey et al. 2006, Tsu da et al. 2006) and the effects of other abiotic factors (Reiskind and Lounibos 2012) such as average daily relative humidity, average daily temperature, and the availability of wet containers along the coastal-inland gradient.

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