ENTOMOLOGY

To what extent does salt (NaCl) affect *Anopheles gambiae sensu lato* mosquito larvae survival?

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Abstract

The effect of salt (NaCl) on *Anopheles gambiae sensu lato* (s.l.) mosquito larval breeding was ascertained under laboratory conditions. No larval mortality occurred when the Cl^- concentrations were between 0.017 ppt (0.03 ppt salinity) and 7.371 ppt (13.25 ppt salinity). However, 9%, 24%, 73.5%, 91.5% and 99.5% larval mortality occurred at 10.828 ppt (19.49 ppt salinity), 16.069 ppt (28.95 ppt salinity), 18.739 ppt (33.77 ppt salinity), 32.587 ppt (58.82 ppt salinity) and 47.326 ppt (85.37 ppt salinity) NaCl concentrations respectively. The lower NaCl concentrations resulting in LC_{50}, (lethal concentration for 50% larval mortality), LC_{90} (lethal concentration for 90% larval mortality), LC_{95} (lethal concentration for 95% larval mortality), and LC_{99} (lethal concentration for 99% larval mortality) were 23.12 ppt (41.19 ppt salinity), 24.91 ppt (44.42 ppt salinity), 27.76 ppt (49.56 ppt salinity) and 33.87 ppt (60.568 ppt salinity) respectively. The upper NaCl concentration resulting in LC_{50}, LC_{90}, LC_{95} and LC_{99} were 32.89 ppt (58.83 ppt salinity), 37.21 ppt (66.63 ppt salinity), 44.79 ppt (80.32 ppt salinity) and 63.76 ppt (114.55 ppt salinity) respectively. In conclusion, the level of water salinity may indicate the presence or absence of *An. gambiae* s.l. mosquito larvae and this information can be used for disease control purposes.

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Introduction

Some mosquitoes are responsible for the transmission of several diseases of which malaria is one of them (McCord & Anttila-Hughes, 2017). Malaria is a major cause of morbidity and mortality worldwide with a lot of resources being channelled towards its control (WHO, 2016). Several species of Anopheles mosquitoes have been implicated as vectors of malaria in different continents of the world, including Africa (Wang *et al.*, 2016). In Zimbabwe, 50% of the country's population lived in malarious areas during the year 2012. A steady decline in malaria transmission was recorded between the years 2003 to 2013 in Zimbabwe (Sande *et al.*, 2016a)

Malaria transmission in Zimbabwe is associated with some members of the *Anopheles gambiae sensu lato* (s.l.) (Munhenga *et al.*, 2008) and *Anopheles funestus sensu lato* (s.l.) mosquitoes (Sande *et al.*, 2016b). Scaling up efforts in malaria control has been channelled towards the use of effective insecticides and anti-malarial drugs, with some efforts being placed on the distribution of Long Lasting Insecticide treated mosquito Nets (LLINs) (WHO, 2016). Larval Source Management (LSM) is a tool used to control the aquatic stages of vector mosquitoes (Walker & Lynch, 2007).

In the developmental stages of mosquitoes, the aquatic stage requires favourable conditions for fast development and this information can be used to improve control programmes (Meibalan & Marti, 2016). Scouting for mosquito larvae breeding habitats is crucial for the implementation of LSM, and understanding conditions for favourable mosquito breeding is an advantage that can be used to have tailor made programmes. Breeding habitats for Anopheles mosquito larvae vary, with most species being found in fresh water (Sanchez-Ribas *et al.*, 2015). However, some of the mosquitoes breed in brackish water with varying levels of salinity (Chirebvu & Chimbari, 2015). Some mosquitoes can tolerate certain levels of salinity and eventually survive to adulthood, fly away and transmit diseases (Patrick *et al.*, 2001). However, recent studies have shown that vector mosquitoes are adapting to unusual

environments for breeding, with elevated levels of salinity (Waniwa, 2011).

Several studies were conducted in order to understand the effect of salt on mosquito larval development in aquatic habitats. Larval development only occurred in salt concentrations between 3.5 ppt and 14 ppt (de Brito *et al.*, 2015). One hundred percent mosquito larval mortality was recorded at salinity of between 16-18 ppt in a study by Jude *et al*. (2012). Studies by Le Sueur & Sharp (1988) showed that *Anopheles quadriannulatus* mosquito larvae bred in saline water containing 0-2 g NaCl/L. *Anopheles melas* mosquito larvae were found in water containing 70.4 g NaCl/L (Gillies & De Meillon, 1968).

In Zimbabwe, Masendu (1996) collected water samples from mosquito breeding sites in Bare Salt Pan and Kurima stream and found out that the water contained 1 g/L and 4 g/L NaCl respectively. Typical breeding water of *An. merus* was found to contain 64.71 g NaCl/L (Gilles & De Meillon, 1968), 14.7 to 38 g (Le Sueur & Sharp, 1988), 12.68 g (Jepson *et al.,* 1947) and 9.51 to 41.53 g. WHO defines seawater as containing 31.7g NaCl/L and this is equivalent to 100% seawater (Njogu & Kinoti, 1971). Studies done on the breeding requirements of *An. arabiensis* mosquito larvae showed that the mosquito could tolerate chloride levels of 4.44g/L (Jepson *et al.,* 1947). Studies by de Brito *et al.*, (2015) showed that *Aedes aegypti* mosquito larvae adapted to salinity levels between 3.5-14 ppt. The general objective of the study was to understand the effect of NaCl levels on the development of *An. gambiae* s.l. mosquito larvae and relate this to field conditions in order to improve malaria control programmes.

We studied the effect of analytical salt (sodium chloride) on *An. gambiae* s.l. mosquito larvae under laboratory conditions and related it to field conditions.

Materials and methods

Kamhororo village (17°51'S × 28°38'E) is a dry area whose rivers hardly run for 4 months in a year and this is located in Midlands province, Gokwe South district. The main sources of water are artesian wells (Figure 1A) that were dug during the construction of Gokwe-Binga road. Mosquito larvae were collected through larval scooping (Figure 1B) and placed in rearing dishes. Malaria transmission has declined over the years despite the fact that Indoor Residual house Spraying (IRS) is still being conducted.

Anopheles mosquito larvae were separated from other larvae on the basis of morphological identification as described by Gilles & De Meillon, (1968). The larvae were reared to adults where *An. gambiae* s.l. mosquitoes were separated from the other mosquitoes. The female *An. gambiae* s.l. were fed on guinea pig blood and made to lay eggs. The eggs where then reared to third instar mosquito larvae that were eventually used for bioassays.

Preparation of NaCl

A total of 35.7 grams NaCl was dried at 140°C in an oven and dissolved in 200 ml distilled H_2O. NaCl samples were prepared by pipetting 0.1 mL, 0.5 mL, 1 mL, 2 mL, 4 mL, 8 mL, 16 mL, 32 mL and 64 mL of NaCl solutions. Each aliquot was topped up to 200 mL distilled H_2O. The control contained 200ml distilled H_2O only. These preparations were placed in 500 mL plastic containers (each concentration was placed separately). There were four replicates for each concentration, including the control. NaCl concentrations were left for 1 hour before introducing mosquito larvae.

Determination of the NaCl concentration using the Mohr's method

Determination of NaCl was done using Mohr's method (Rand *et al*, 1976). The indicator solution was prepared by dissolving 50 g of $K_2Cr_2O_7^{-2}$ into 1 L distilled H_2O. The 0.0141N AgNO3 solution was made up by dissolving 4.79 g into 2 L of distilled H_2O. A 0.0141N $AgNO_3$ solution was added until a definite red precipitate was formed. The preparation was kept for 24 hours before filtering using a Whatman number 1 filter paper. Standardization of $AgNO_3$ was done by titration with 0.041N NaCl (prepared by dissolving 824.1 mg in 1 L distilled H_2O). The indicator blank was 0.3 mL of 0.0141N $AgNO_3$. The CrO_4^{2-} ions gave a red brown precipitate of $AgCro_4$. A total of 100 mL of the H_2O sample was used for all chloride determinations according to the method of Rand *et al*. (1976) as follows:

$$\frac{(\text{mL } AgNO_3 - \text{blank}) * 0.5*1000/L}{100 \text{ mL (sample used)}}$$

0.0141N * 35.45 (molecular weight of chlorine) is 0.5. The amount of calculated Cl^- was compared with mortality rates. The mean and range of chloride that caused mortality was then calculated.

Figure 1. A) Source of water for the breeding site. B) Larval scooping.

Calculation of water salinity

WHO defines seawater as containing 31.7 g NaCl/L and this is equivalent to 100% seawater (Njogu & Kinoti, 1971). We used this formula to calculate salinity:

$$\text{Salinity} = 0.03 + (1.805 * \text{chlorinity})$$

Effect of NaCl on *An. gambiae* s.l. mosquito larvae

A total of 50 *An. gambiae* s.l. third instar mosquito larvae were placed in each of the containers and exposed for 24 hours. A dead mosquito larva was classified as dropping on the floor of the exposure containers or moribund after being pricked by a needle.

Computing lethal concentrations

The LC_{50} (lethal concentration that killed 50% of the mosquito larvae), LC_{90} (lethal concentration that killed 90% of the mosquito larvae) and LC_{99} (lethal concentration that killed 99% of the mosquito larvae) were determined using the Environmental Protection Agency (EPA) Probit Analysis programme used for calculating LC/EC (lethal concentration/effective concentration) values, Version 1.5.

Results

No *An. gambiae* s.l. larval mortality was recorded with NaCl concentrations between 0.047-7.371 ppt (0.03-13.25 ppt salinity) (Table 1). *An. gambiae* s.l. mosquito larval mortality was recorded in NaCl levels between 10.828-47.326 ppt (19.49 ppt salinity – 85.37 ppt salinity). Mean percent *An. gambiae* s.l. larval mortality increased as the NaCl concentration increased. The standard deviations ranged from 0.3-3.2, with larger variations observed in higher concentrations (10.828-47.326 ppt). No mosquito larval mortality was recorded in the controls.

The range in LC and salinity increased as the LC and salinity values increased in both the lower and upper values (Table 2). The difference between the lower and upper values (range) increased as the LC increased. All the lower LC values were above 40 ppt salinity and all upper LC values were above 50 ppt salinity. The range in salinity (difference between the lower and upper values for each LC figures) was always above 17 ppt.

Discussion and Conclusions

The study on the effect of different NaCl concentrations on *An. gambiae* s.l. mosquito larvae showed concentration related responses that were pronounced from 19.49-85.37 ppt salinity, in agreement with studies by Patrick *et al*. (2001). Lower NaCl (hence lower salinity) concentrations did not kill *An. gambiae* s.l. mosquito larvae in this study although a study by Jude *et al*. (2012) showed 100% larval mortality. These results suggested that *An. gambiae* s.l. mosquito larvae could survive water salinities below 13.25 ppt, although this is specifies specific. However, studies by de Brito *et al*. (2015) gave a bigger margin of larval survival (up to 14 ppt salinity), in agreement with our studies. The study by Brito *et al*, (2015) showed 100% larval mortality at 17.5 ppt salinity, in contrast with this study when 4.8 times this salinity was required to cause very high mortality.

Our results have taken cognisance of variations in salinity levels that affect mosquito larval presence, as observed by Sanchez-Ribas *et al*. (2015). The study by de Brito *et al*, (2015) showed that salinity levels in breeding sites had no effect on *Ae. aegypti* mosquito egg laying tendencies, although we did not look at this aspect. However, such results indicate that vector mosquitoes will continue laying eggs in breeding sites irrespective of salinity levels, ruling out the incorporation of salinity in disease control programmes in order to deter egg laying, although the mosquito larvae are killed.

Our results are also important in understanding larval ecology for disease control as noted by de Brito *et al*. (2015), who documented a decrease in *Ae. aegypti* mosquito larvae as salt concentration increased in water found in breeding sites. The saline water preparations in our study partly depicted the saline water levels recorded by Masendu (1996) in selected localities in Zimbabwe where *An. gambiae* s.l. were found breeding sites with lower salinity concentrations those where *An. merus* bred. *An. merus* mosquito larvae were demonstrated to survive in breeding sites with high saline levels (Gilles & De Meillon, 1968; Le Sueur & Sharp, 1988). These results can be used in Zimbabwean settings that have *An. gambiae* s.l. mosquito breeding.

The LC_{90} of in our study was very high (44.42-66.63 ppt salin-

Table 1. Effect of NaCl on *An. gambiae* s.l. (N=200 per concentration).

Mean Cl^- (ppt)	Salinity (ppt)	Mean mortality, %
0.017±0.3	0.03	0.0
0.589±0.3	1.01	0.0
1.526±0.3	2.70	0.0
3.149±0.9	5.63	0.0
7.371±0.9	13.25	0.0
10.828±2.1	19.49	9.0
16.069±3.1	28.95	24.0
18.739±2.7	33.77	73.5
32.587±1.5	58.82	91.5
47.326±3.2	85.37	99.5

Table 2. Calculation of effective LC_{50}, LC_{90}, LC_{95} and LC_{99} on *An. gambiae* s.l. mosquito larvae.

Lethal Concentration	Lower value (ppt Cl^-)	Salinity (ppt)	Upper value (ppt Cl^-)	Salinity (ppt)	Range (ppt Cl^-)	Range salinity (ppt)
LC_{50}	23.12	41.19	32.89	58.83	35.71	17.64
LC_{90}	24.91	44.42	37.21	66.63	41.72	22.21
LC_{95}	27.76	49.56	44.79	80.32	17.03	30.76
LC_{99}	33.87	60.59	63.76	114.55	29.89	53.96

ity) as compared to that recoded in a study by Jude *et al.* (2012) (13.39 ppt), implying that mosquito larvae in a study by Jude *et al*. (2012) did not tolerate water with high salinity levels. Close to 100% mosquito larval mortality was achieved by Cl^- concentrations of 85.37 ppt salinity in our study as compared to 100% mortality found in water containing 16-18 ppt salt concentration (Jude *et al.*, 2012). The study by Namuchimba (2007) showed that salinity had an effect on mosquito larval density, although the trend was not very clear, and we did not study this aspect.

In conclusion, the level of saltiness in mosquito breeding sites may indicate the presence or absence of An. *gambiae* s.l. mosquito breeding levels in Zimbabwe, and elevated salt concentrations may favour breeding of salt water species.

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