EFFECT OF VARIOUS SODIUM CHLORIDE CONCENTRATIONS ON THE DEVELOPMENT OF THE MOSQUITO *CULISETA INCIDENS* (THOMSON) (DIPTERA: CULICIDAE)[1]

FRANCIS C. LEE[2]

REVIEW OF LITERATURE. The effect of salinity on the development of mosquitoes and the range of tolerance of salinity have been investigated by a number of workers since 1914. Macfie (1914) reported that the destructive action of saline solution of 2 percent or higher on the larvae of *Stegomyia fasciata* (= *Aedes aegypti*) is due to the hypertonicity of the solution. In more dilute solutions, the destruction of the natural food supply of the larvae may have some influence. The same author (1922) repeated his experiments with seawater and found that undiluted seawater killed the larvae in 2 to 4 hours, and even if the seawater was diluted 50 percent or more by tap water it still caused death within 24 hours. It was found that seawater acts in the same manner as a solution of common salt of equivalent strength. It was also shown that 1.0 to 1.4 percent solution of common salt, or an equivalent strength of seawater, would prevent the larvae of *Stegomyia fasciata* from maturing. It was suggested that common salt or seawater might be used as a larvicide under certain conditions. This idea was put into practice by Banez (1963), who employed ordinary table salt to control breeding of mosquitoes. He found that in both laboratory and field tests concentrations of 10 percent would kill all larvae within an hour or two, but when test concentrations are lower than this a longer period of time is needed in order to achieve a 100 percent kill. He also discovered that the 10 percent salt concentrations had little or no effect on pupae. Kligler and Theodor (1925) made a study on the effect of salt concentration on the

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[2] Present address: Joaquin Miller School, 6151 Rainbow Drive, San Jose, Calif. 95129.

development of *Anopheles* larvae. They found that:

> *Anopheles elutus, A. superpictus* and *A. sergenti* show a decided selective capacity for egg laying, depositing more than 75 percent of their eggs in fresh water or water of low saline content.

It was also stated that "salt concentrations of M/4 and higher are toxic to these eggs and larvae." Wigglesworth (1933c) observed that larvae of *Aedes argenteus* Poir (= *Aedes aegypti*) reared in fresh water were killed by 1.1 percent NaCl or by "sea-water" (equivalent to 1.3 percent NaCl) but a gradual increase in the concentration causes the larvae to become resistant to 1.1 percent NaCl and to "sea-water" (1.75 percent NaCl). Bates (1939) demonstrated in a series of experiments that six species of *Anopheles* all have clear and readily definable differences in their tolerance for salt. He suggested that these differences may be useful in determining the different subspecies of the *Anopheles maculipennis* complex in Europe. Woodhill (1941) reported that the development of *Aedes concolor* Taylor is influenced by small quantities of salt in the water. He reported that *A. concolor* will not develop in distilled water plus food, but it will develop if NaCl is added to the distilled water at the rate of 0.1 or 0.05 gm. per liter. When only 0.025 gm. NaCl per liter was added, however, a high mortality occurred during the fourth instar. Marchal (1959) found variation in the anopheline population of a pond in which salinity varied greatly during the rainy season, even though the fluctuations in salinity showed no direct influence on larval morphology.

The purpose of this experiment was to determine the effects of various NaCl concentrations on *Culiseta incidens.*

MATERIALS AND METHODS. The following NaCl concentrations were prepared: 0 part per thousand (p.p.th.) (i.e., 0 gm. of NaCl per 1000 c.c. of water—Control), 1 p.p.th., 3 p.p.th., 5 p.p.th., 7 p.p.th., 9 p.p.th., 3 p.p.th., 11 p.p.th., and 13 p.p.th. Twenty-five first instar larvae, 24 hours old, of *C. incidens* were distributed into each of several enamel pans (6″ x 8″ dimensions) containing 500 c.c. of these respective concentrations. Each pan was covered with a window glass but no air was bubbled in (evaporation was thus kept at a minimum). However, the pellicle which formed on the water surface was removed daily by dragging strips of paper toweling over the surface. Observations were made daily, and all dead larvae recorded and removed with a clean forceps. The numbers of different instars were recorded during each observation.

The food consisted of 80 mg. of yeast-breadcrumb mixture added to each pan before the larvae were introduced, and the same amount (80 mg.) added to each pan at 3-day intervals. The experiment was conducted at room temperatures of 72° to 80.5° F.

RESULTS. The findings of this experiment are summarized in Tables 1 through 4.

Analysis of data in Table 1 revealed the effect of various salt concentrations on the survival of the mosquito larvae in relation to time and instars. It was noted that during the first day of the experiment no first instars from any groups showed any ill effect, but from the second day onward the effect of higher salt concentration on the larval development became apparent. In the "control" group, 71.5 percent of the larvae reached the fourth instar in 6 days, but by that time the 1 p.p.th. NaCl group contained only 51 percent of 4th instar larvae, the 3 p.p.th. group contained 44.5 percent, the 5 p.p.th. group contained 12.5 percent, the 7 p.p.th. group contained 4 percent, and in the 9 p.p.th., 10 p.p.th., 11 p.p.th. and 13 p.p.th. groups there were no fourth instar larvae observed on the sixth day.

Table 2 shows that the length of time required by the first instar larvae to reach pupation increased as salt concentration was increased. Comparison between the "control" and 1 p.p.th. group and between

TABLE 1.—Effect of NaCl on the survival of larvae of *Culiseta incidens* in relation to time and instars.

		% Survival of indicated instars			
Treatment	Days	I	II	III	IV
0/1000 (control)	1	100.0	....	....	....
	2	100.0	....	....	....
	3	9.5	88.5	....	....
	4	....	73.5	22.5	....
	5	....	....	97.0	....
	6	....	....	25.5	71.5
	7	....	....	1.0	94.5
	8	....	....	....	95.5
1/1000	1	100.0	....	....	....
	2	99.0	....	....	....
	3	18.5	76.5	....	....
	4	0.5	85.5	6.5	....
	5	0.5	1.5	88.0	....
	6	....	....	36.0	51.0
	7	....	....	3.0	81.5
	8	....	....	....	81.5
3/1000	1	100.0	....	....	....
	2	99.0	....	....	....
	3	48.4	49.0	....	....
	4	8.4	66.5	....	....
	5	2.4	8.8	80.5	....
	6	1.4	4.0	62.4	24.5
	7	0.6	0.6	5.8	80.5
	8	....	0.4	1.6	84.2
5/1000	1	100.0	....	....	....
	2	97.5	....	....	....
	3	61.5	25.5	....	....
	4	5.5	74.0	2.0	....
	5	0.5	9.0	64.8	2.0
	6	....	1.5	56.5	12.5
	7	....	....	5.0	69.0
	8	....	....	1.5	69.5
	9	....	....	....	63.5
7/1000	1	100.0	....	....	....
	2	98.0	....	....	....
	3	63.0	25.5	....	....
	4	3.5	78.0	1.5	....
	5	....	15.5	65.5	....
	6	....	1.5	72.0	4.0
	7	....	....	12.5	63.0
	8	....	....	2.0	72.5
	9	....	....	1.0	71.0
9/1000	1	100.0	....	....	....
	2	99.5	....	....	....
	3	59.0	19.5	....	....
	4	22.5	44.5	....	....
	5	3.5	34.5	21.5	....
	6	1.5	10.5	41.5	....
	7	....	2.0	31.5	15.5
	8	....	0.5	11.0	32.0
	9	....	....	2.5	38.0
10/1000	1	100.0	....	....	....
	2	90.0	....	....	....
	3	38.0	18.0	....	....
	4	14.5	23.5	....	....
	5	3.5	24.5	5.0	....
	6	1.0	8.5	15.0	....
	7	0.5	1.5	19.0	2.0
	8	....	1.0	14.5	8.5
	9	....	0.5	4.5	11.5
	10	....	0.5	1.0	12.5
	11	....	0.5*	....	13.5
11/1000	1	100.0	....	....	....
	2	73.5	....	....	....
	3	26.5	4.5	....	....
	4	7.5	11.5	....	....
	5	2.0	13.0	....	....
	6	....	9.5	4.5	....
	7	....	3.5	7.5	....
	8	....	....	9.0	1.0
	9	....	....	5.5	3.5
	10	....	....	3.0	5.0
	11	....	....	1.5	6.0
13/1000	1	100.0	....	....	....
	2	4.0	....	....	....
	3	0.0	....	....	....

* Became 4th instar on the 17th day.

5 p.p.th. group and 7 p.p.th. group did not indicate any significant differences.

The effect of various salt concentrations on sex ratio was especially interesting. It was found that only the 5 p.p.th. group showed any significant suppressive effect on the survival of female mosquitoes (see Table 3).

The LD_{50} of common table salt for the mosquito *C. incidens* is 4.7 p.p.th. (Reed and Muench method) (see Table 4).

DISCUSSION. Although studies of the effect of salt concentration on larvae of mosquitoes have been undertaken by a number of workers, investigations of this sort with *C. incidens* have not been seen in the literature. At the beginning of the present study, a preliminary test was conducted to determine which larval instar might be the most sensitive to salt, in order to select the instar most appropriate for these experiments.

Based on the statistical analyses of data

TABLE 2.—Effect of various concentrations on the length of larval developmental period of *C. incidens.*

NaCl concentration	No. of replicate	Length of time (in days)	Signif.
0/1000 (control)	12	7.58	a
1/1000	12	7.85	a
3/1000	10	8.50	b
5/1000	12	9.25	c
7/1000	12	9.42	c
9/1000	10	9.90	d
10/1000	7	11.29	e
11/1000	2	12.50	f

Analysis of Variance

Source	Sum of squares	D.F.	Est. variance
Within	39.7	69	0.618
Between	115.0	7	16.4
Total	154.7	..	....

Calculated F = 26.45.
Table F (5%) = 2.17.
L.S.D. = 0.35.

shown in Tables 1, 2, and 3, it can now be stated that there is a general delay in the time of larval development correlated with higher salt concentrations and that the greater survival rate is inversely proportional to the increase in salt concentration. The LD_{50} was found to be 4.7 p.p.th. (see Table 4). This table also shows that the LD_{99} concentration is 11 grams NaCl per 1000 c.c. of water (= 11 parts per thousand).

Salinity measurements taken directly in field waters (from which eggs of *C. incidens* were collected) showed that the range of salt concentrations in those waters varied from 7.0 to 7.5 parts per thousand. The results obtained from the present experiments showed that this range of salinity was apparently not tolerable to the larvae under laboratory conditions. This may be explained by the fact that pure sodium chloride will kill larvae at a lower concentration than higher concentrations of other salts found in the field water (Wigglesworth, 1933c).

In this study the author was not primarily concerned with the mode of action of various salts. It is interesting, however, to note the conclusion reached by Graham (1910): "Salt appears to inhibit the growth of very young larvae, probably by diminishing the supply of food, but the development of fully grown larvae appears to be hastened in a hypertonic medium, and they pass into and through the pupal stage with unusual rapidity." Later, it was further pointed out by Wigglesworth(1933c) that the toxic effects of hypertonic salt solution on mosquito larvae "are not due to osmotic pressure alone as originally suggested by Paul Bert (1871), but that the salt itself has a destructive action on the living tissue."

In view of field observations, it appears that direct osmotic action was not responsible for the destructive effect of salt on *C. incidens* larvae in the laboratory. It is possible that types of ions and concentrations of ions play a significant role here.

TABLE 3.—Effect of various NaCl concentrations on sex ratio of *C. incidens.*

Concentration	No. survival		Calculated χ^2	Table χ^2	Signi. diff.
	Male	Female			
0/1000 (control)	Ave. 10.8	9.8	0.50	3.8	no
1/1000	8.5	8.7	0.02	3.8	no
3/1000	10.0	7.7	2.99	3.8	no
5/1000	7.6	2.8	26.70	3.8	yes
7/1000	5.7	5.4	0.07	3.8	no
9/1000	2.7	1.8	1.80	3.8	no
10/1000*	.6	0	3.0	3.8	no

* The number of adult survival in this group was so few that the χ^2 value obtained (3.0) shows that there was no significant difference in sex ratio.

TABLE 4.—Effect of various NaCl concentrations (the estimation of LD_{50}) by Reed and Muench method.

Salt conc. tested	Alive	Dead	Total Alive	Total Dead	Total Alive & dead	% kill P'	P'-c	Adjust. & kill
14/1000	0	25.0	0	....	....	....	....	....
13/1000	0	25.0	0	142.5	142.5	100.0	95.1	100
11/1000	.2	24.8	.2	117.5	117.7	99.0	94.1	98.9
10/1000	1.2	23.8	1.4	92.7	94.1	98.5	93.6	98.4
9/1000	3.7	21.3	5.1	68.9	74.0	93.1	88.2	92.7
7/1000	11.1	13.9	16.2	47.6	63.8	74.6	69.7	73.3
5/1000	10.3	14.7	16.5	33.7	60.2	56.0	51.1	53.7*
3/1000	18.2	6.8	44.7	19.0	63.7	29.8	24.9	26.2**
1/1000	17.1	7.9	61.8	12.2	74.0	16.5	11.6	12.1
Control (c)	20.7	4.3	82.5	4.3	86.8	4.9	0	0

$$\frac{53.7 - 26.2}{5 - 3} = \frac{50 - 26.2}{x - 3}$$

$$\frac{27.5}{2} = \frac{23.8}{x - 3}$$

$$x = 4.7 \text{ parts}/1000\text{—}LD_{50}$$

A possible explanation for the fact that 5 p.p.th. NaCl concentration killed more females than males (see Table 3) might be that ions such as K^+ and Ca^{++} are not present, and lack of ion antagonism could be deleterious to some metabolic process. Since females require more energy in their development than males, they might have been more susceptible to the situation than the males.

SUMMARY. As the salt concentration in water increases there is a general delay in the speed of larval development, as well as an increase in the general mortality rate. At a concentration of five parts per thousand, NaCl was found to exert a greater inhibitory effect upon the development of female mosquitoes than males, but the other concentrations tested affected both sexes similarly. It was suggested that when ions such as K^+ and Ca^{++} are not present, the lack of ion antagonism could be deleterious to some metabolic process.

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References Cited

Banez, L. F. L. 1963. Use of ordinary table salt against breeding of mosquitoes in artificial containers. Philip. J. Sci. 92:447–48.

Bates, Marston. 1939. The use of salt solution for the demonstration of physiological differences between the larvae of certain European anopheline mosquitoes. Am. Jour. Trop. Med. 19:357–384.

Graham, W. M. 1910. The study of mosquito larvae. Bull. Ent. Res. 1:51–53.

Kliger, I. J. and O. Theodor. 1925. Effect of salt concentrations and reaction on the development of *Anopheles* larvae. Bull. Ent. Res. 16:45–49.

MacFie, J. W. S. 1914. A note on the action of common salt and sea water on *Stegomyia fasciata*. Bull. Ent. Res. 4:339–344. (Note: *Stegomyia fasciata* is now considered a synonym of *Aedes aegypti*-Banez).

———. 1922. The effects of saline solutions and sea water on *Stegomyia fasciata*. Ann. Trop. Med. Parasit. 15:277–280.

Marchal, E. 1959. Variation in the anopheline population of a pond of variable salinity in the region of Conakry (French Guinea) (In French). Bull. Inst. Afr. Morire (A)21(1): 180–203.

Wigglesworth, V. A. 1933c. The adaptation of mosquito larvae to salt water. J. Exp. Biol. 10:27–37.

Woodhill, A. R. 1941. The development of *Aedes (Pseudokusea) concolor* Taylor in relation to small quantities of salts in solution and to temperature of the water. Proc. Linn. Soc. N. S. W. 66:396–400.