ROLE OF SALTS IN OVIPOSITION SITE SELECTION BY THE BLACK SALT-MARSH MOSQUITO, *AEDES TAENIORHYNCHUS* (WIEDEMANN)[1]

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Brackish waters throughout the world are inhabited by species of mosquitoes evidently restricted to saline conditions, and the mosquitoes may be distinctly classified on this basis (Bates 1949). Many investigators have examined the role of inorganic salts in oviposition site selection, but the results have been inconsistent; tolerance of saline conditions rather than preference has been the general conclusion (Bates 1949). Most of this work has been reviewed by Bates (1949), Muirhead-Thomson (1951), and Clements (1963).

The salinity factor has been correlated with the abundance of various species (Cory and Crosthwait 1939, Vogt 1947, Darsie and Springer 1957, Dixon 1957, Chapman 1959, Micks and McNeil 1963, Knight 1965, Petersen and Rees 1966), but the role of individual salts remains unknown. Hudson (1956) correlated the responses of two fresh-water mosquito species, *Culex pipiens molestus* and *Aedes aegypti*, with the osmotic pressures of several salts. However, such studies with *Aedes taeniorhynchus* (Wiedemann), which breeds almost exclusively in salt-marsh or high saline situations (Knight 1965) were lacking.

Aedes sollicitans has been reported breeding in salt water from swimming pools, oil wells, and coal mines in Illinois (Fellton 1944) as well as in similar situations in Kentucky (Dixon 1957). The latter report was accompanied by analyses of larval breeding waters from two sites differing in the amounts of chloride present. Levels of sulfate reported were comparable, however, and were approximately the same as those reported by the same author for a Florida habitat. This suggests that a laboratory investigation of the role of sulfates in oviposition site selection by a salt-marsh mosquito might be fruitful.

Previous work has been conducted with various behavioral aspects of oviposition site selection (McGaughey and Knight 1967), and the chemical nature of natural breeding sites has been determined (Knight 1965) for *A. taeniorhynchus.* The laboratory study reported here was conducted to determine the response of *A. taeniorhynchus* to selected salt solutions in concentrations approximating those found in natural habitats (Knight 1965), to isolate a common factor determining this response, and to examine certain aspects of the laboratory technique that might alter the response.

METHODS AND MATERIALS

A laboratory colony of *A. taeniorhynchus,* obtained from the U. S. Department of Agriculture laboratories at Gainesville, Florida, in July 1963, was utilized in this work. The mosquitoes were reared and tested in a controlled-environment cabinet at 29.50° C. with a photoperiod of 16 hr light and 8 hr darkness by using the procedures described by McGaughey and Knight (1967).

Adult mosquitoes were maintained in cubical cages of the type described by Chao (1959). These cages were 51 cm on a side and were covered with transparent sheet acetate stapled and taped to wooden frames. They were made airtight by cov-

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ering the 17 x 20 cm sleeved opening with a removable section of sheet acetate. Three cheesecloth wicks were suspended in 250-ml beakers of distilled water inside each cage to maintain the relative humidity above 85 percent. A peeled apple was provided for an energy source for the mosquitoes.

Mosquitoes to be tested were transferred to separate cages immediately after they had been given a blood meal. Tests were performed during the first 5 hr in darkness on the third day after the blood meal, the period of maximum oviposition under the environmental conditions used (McGaughey and Knight 1967). Aqueous solutions of the materials tested were applied to cheesecloth pads in 10-cm petri dishes. Each pad weighed 2 gm, and 6 ml of test solution were applied to each, giving a substrate moisture content of 75 percent (Knight and Baker 1962). The liquid was measured into the dish with a pipette, and the pad was placed in the liquid. Immediately before placing the dishes in the cage, the pads were turned over to insure even wetting. The dishes were placed in the cages in a circular arrangement in random positions, and all other moisture sources were removed at the time of testing.

Experience indicated that ten blood-fed mosquitoes per experimental dish in each test cage would lay about 400 eggs per dish. This number provided fairly reproducible results. The exact number of mosquitoes was not critical; however, all results are expressed as numbers of eggs per blood-fed female, and an effort was made to keep the number of mosquitoes per dish constant so far as was practicable.

Seven salt solutions were tested: sodium chloride, magnesium sulfate, sodium sulfate, ferrous sulfate, sulfuric acid, sulfuric acid in sodium chloride, and an artificial marine salt mixture. The chemical composition of this marine salt mixture (available from Rila Products, Teaneck, New Jersey) approximates that of sea water. The highest concentration of the material used in this work was approximately 19 percent of the concentration equivalent to sea water. This concentration had a specific conductance of 10.5 to 11.0 millimhos/cm^3 (measured with an Industrial Instruments, Inc., Conductivity Bridge, Model RC-16B2), which corresponds closely with the average specific conductance of water extracts of salt-marsh breeding sites of *A. taeniorhynchus* (Knight 1965), and was chosen on this basis. Other concentrations tested were dilutions of this 19 percent solution.

Results

Response to Salts. A series of concentrations of each salt was tested in several cages of mosquitoes. The responses obtained are given in Table 1, expressed as percentages of the total eggs that were laid on each concentration, along with the specific electrical conductance and osmolality of each concentration of each salt. The osmolality was calculated from values for freezing-point depression obtainable from the Handbook of Chemistry and Physics (Weast 1965). A one molal solution of a nonelectrolyte has a freezing-point depression of 1.86° C. and an osmolality of 1.

The responses to these salts are compared in Figures 1, 2, and 3 on the basis of total molar salt concentration, molar sulfate concentration, and osmolality, respectively. The marine salt mixture, sodium sulfate, and magnesium sulfate elicited maximum responses at approximately equal molar concentrations of salt (Figure 1) and at approximately equivalent osmolalities (Figure 3). However, maximum responses to ferrous sulfate and sulfuric acid occurred at concentrations equivalent to the molar concentration of sulfate in the marine salt (Figure 2). Sodium chloride was neutral or slightly repellent in the concentrations tested here, but was made attractive by the addition of small amounts of sulfuric acid. The response here was probably to the sulfuric acid. An interaction between the salts is indicated since a greater acid concentration was required to elicit a maximum response when sulfuric acid was tested alone.

These data seem to indicate that *A. taeniorhynchus* will respond to the sulfate content of the oviposition substrate. The differing optimal concentrations of sulfuric acid, ferrous sulfate, magnesium sulfate, and sodium sulfate may be due to properties of the compounds such as differences in ionic activity. However, the response to extremely low concentrations of sulfuric acid indicates that osmotic pressure, which is related to ionic activity, has little or no effect except at the upper extremity. The low pH of sulfuric acid may have prevented selection of higher concentrations of this material, however.

SELECTION FOR SALT PREFERENCE. The ability to distinguish between salt solutions exhibited in the preceding experiments falls

TABLE 1.—Percent eggs laid on each concentration of seven salts tested separately in ten experiments.

Salt	Molarity	Sulfate[a] Molarity	Sp. Cond.	Osmolarity	Percent[b] Eggs
Marine salt	0.001	0.0001	0.14	0.0019	20.5
	0.010	0.0010	1.25	0.0192	24.9
	0.052	0.0050	5.50	0.0962	27.2
	0.104	0.0100	10.50	0.1925	27.4
Marine salt	0.001	0.0001	0.14	0.0019	29.2
	0.052	0.0050	5.50	0.0962	36.9
	0.104	0.0100	10.50	0.1925	33.9
Sulfuric Acid	0	......	0.005	0	24.3
	0.00005	......	0.048	0.0001	26.4
	0.00050	......	0.440	0.0011	31.0
	0.00500	......	3.500	0.0108	18.3
Sulfuric Acid	0	......	0.005	0	26.3
	0.0025	......	1.850	0.0054	30.1
	0.0125	......	7.600	0.0269	17.1
	0.0250	......	14.000	0.0538	13.9
	0.0500	......	24.500	0.1075	12.6
H_2SO_4 in 0.125 M NaCl	0.12500	0	12.0	0.2305	25.7
	0.12505	0.00005	13.4	0.2306	28.3
	0.12550	0.00050	13.4	0.2316	26.2
	0.13000	0.00500	16.0	0.2413	19.8
H_2SO_4 in 0.2 M NaCl	0.20000	0	17.0	0.3688	21.6
	0.20001	0.00001	17.0	0.3688	16.4
	0.20010	0.00010	17.0	0.3690	24.6
	0.20100	0.00100	17.0	0.3710	19.1
	0.21000	0.01000	18.0	0.3903	18.3
$FeSO_4 \cdot 7H_2O$	0	....	0.005	0	18.9
	0.0001	....	0.028	0.00013	22.8
	0.0010	....	0.230	0.00128	24.6
	0.0100	....	1.525	0.01280	21.3
	0.1000	....	9.000	0.12850	12.4
Na_2SO_4	0.005	....	1.16	0.0131	25.4
	0.025	....	5.10	0.0653	23.9
	0.050	....	8.80	0.1305	30.9
	0.125	....	18.50	0.3264	19.8
$MgSO_4$	0.0025	....	0.265	0.0029	24.3
	0.0125	....	1.075	0.0144	21.9
	0.0250	....	1.900	0.0288	27.9
	0.0500	....	3.250	0.0576	25.9
NaCl	0	....	0.005	0	24.7
	0.05	....	5.25	0.0922	19.5
	0.10	....	10.00	0.1844	16.6
	0.15	....	13.2	0.2766	20.5
	0.20	....	17.0	0.3688	18.7

[a] Same as molarity for the pure sulfate solutions.

[b] Averages for all replications. Different salts were tested in separate cages and were not compared directly.

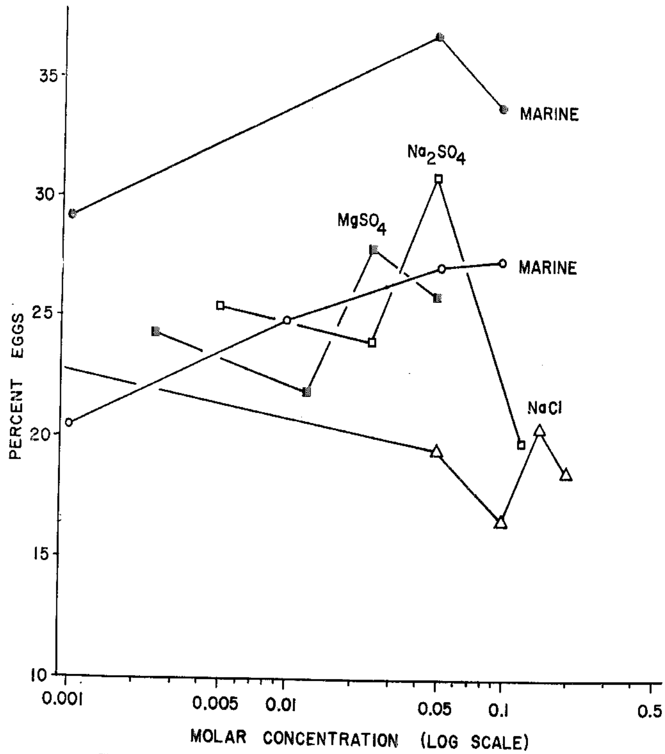

FIG. 1.—Oviposition response to total molar concentration of salt.

short of the virtually absolute distinction found in nature (Knight 1965). As an explanation for this, it is conceivable that some selective process may have occurred (either in the process of colonizing the mosquitoes or in subsequent laboratory rearing through many generations) which could have lowered the degree of preference of the laboratory colony for salt. Therefore, an effort was made to select from the laboratory colony a strain that preferred a saline oviposition substrate.

Beginning with a generation reared from the laboratory colony, four cheesecloth wicks were maintained in each parent cage. Two of these wicks were wet with distilled water and two with the marine salt solution (equivalent to 19 percent sea water). Eggs deposited on the salt-water wicks were retained and hatched, and the larvae were reared in tap water to produce the next generation, which was then selected in the same manner. This was continued through five generations. This selection procedure was utilized since the site of egg deposition must represent the ultimate oviposition preference of the mosquito.

The response to marine salt was tested for each generation from the selection process in which the larvae were reared throughout in tap water. In addition, a brood of each generation except the fifth was reared in the artificial marine salt solution and tested in the same manner. The results for each set of tests for each generation are presented in Figure 4. Examination of the figure reveals no consistent effect of rearing the larvae in salt water on the response to the salt solutions. However, microbial activity was reduced, and larval survival was better. Since no

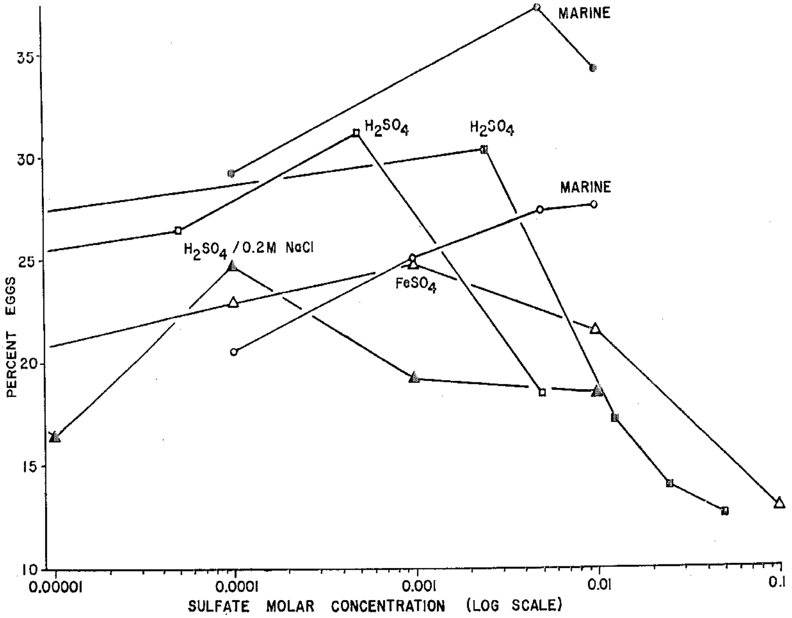

FIG. 2.—Oviposition response to molar concentration of sulfate.

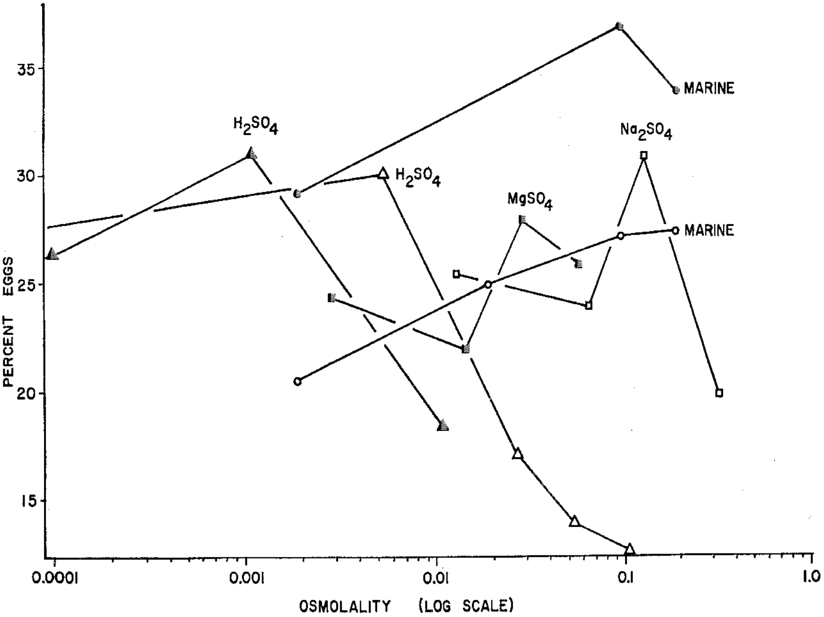

FIG. 3.—Oviposition response to osmolality of salt solutions.

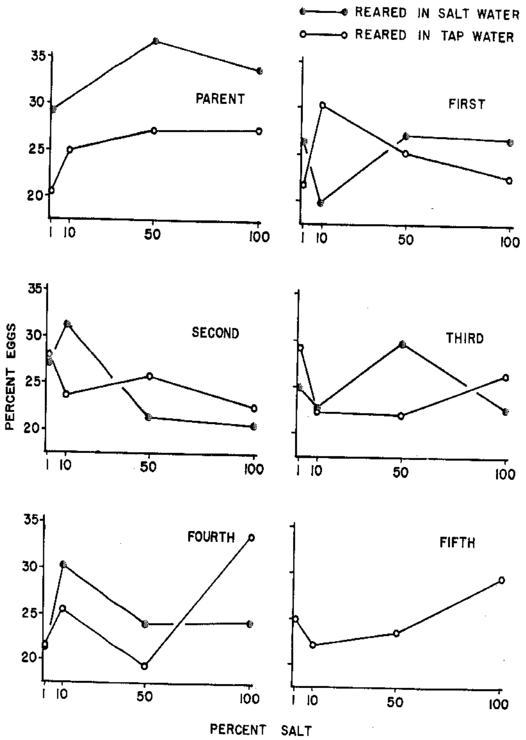

FIG. 4.—Oviposition response to marine salt by females reared in salt water and tap water from a strain selected for salt preference through five generations maintained in tap water (salt concentrations are expressed as dilutions of a concentration equivalent to 19% sea water).

consistent difference was noted between broods of each generation, the data for each generation were combined and plotted in Figure 5. (Data for the parent generation were not combined because different numbers of salt solutions were tested in each series.) These combined data indicate a decreasing response to the higher salt concentrations through the first and second generations and a gradual recovery to a level slightly higher than that of the parent colony in the fourth and fifth generations.

To examine further the possibility of selective larval survival in salt water, a second selection process was performed through three generations exactly as before, but larvae were reared in salt water throughout the selection process. Thus, any selective effects of the salt-water larval medium were combined with egg selection. The responses (Figure 6) were similar to those obtained in the previous selection process, however.

From these data it can only be concluded that the sensitivity of the laboratory colony to salt may have decreased through time. Even though slight recovery is indicated after only five generations of selection, definite conclusions cannot be drawn because of the variation within generations. Selection for salt preference through many generations, requiring several years, would be necessary to show conclusively that such selection could occur.

EFFECT OF AMBIENT RELATIVE HU-

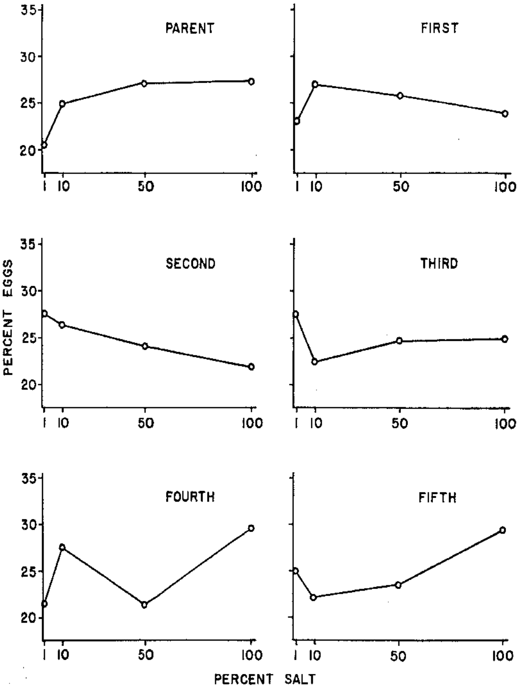

Fig. 5.—Combined oviposition response to marine salt by females selected for salt preference through five generations maintained in tap water (salt concentrations are expressed as dilutions of a concentration equivalent to 19% sea water).

midity. Throughout this work considerable variation was noted between responses of mosquitoes handled and tested in the same manner. Even mosquitoes tested on the same day frequently responded differently. Therefore, extensive replication was sometimes necessary to obtain valid results when the dose-response relationship was not striking. McGaughey and Knight (1967) found that ambient relative humidity affected the response to moist versus dry substrates. Since the experiments reported here were conducted in cages with no humidity control at the time of testing, other than being at an initial level above 85 percent, the humidity could be expected to drop from the initial high level at differing rates in different cages and possibly exert some influence on the response of the mosquitoes. Therefore, this experiment was conducted to determine the effect of three ambient relative humidity levels at the time of testing on the response to three concentrations of marine salt.

Three relative humidity levels were used (high, 82.5±5 percent; medium, 76±4 percent; low, 65±5 percent). The relative humidity levels were tested simultaneously in separate cages and were rotated among the cages to insure the removal of any differences due to the cages.

The results are plotted in Figure 7. Examination of the figure reveals two effects of ambient relative humidity. When the responses are plotted as eggs per female on each salt concentration, egg production appears markedly higher at the low humidity level. When the responses are plotted as the percentages of eggs laid on each salt concentration, the dose-response

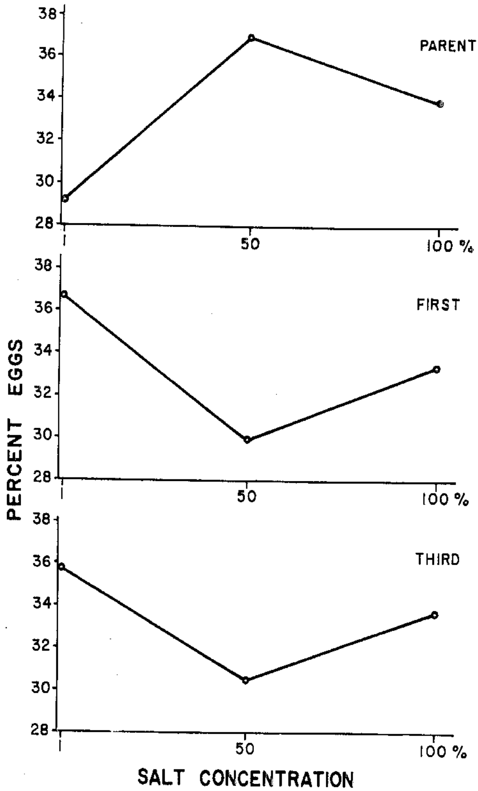

FIG. 6.—Oviposition response to marine salt by females selected for salt preference through three generations maintained in salt water (salt concentrations are expressed as dilutions of a concentration equivalent to 19% sea water).

relationship appears slightly amplified at the low and medium relative humidity levels, although the salt concentration preferred remains unchanged. These results suggest that ambient relative humidities above about 80 percent may be higher than optimal for oviposition. Relative humidity measurements made in 13 randomly selected cages of mosquitoes used in subsequent tests revealed that the mean relative humidity during 5-hr test periods ranged from 60 percent to 88 percent. The median of these tests was 81 percent. Thus, more than half the tests were performed at higher-than-optimal relative humidities.

DISCUSSION

The data reported here indicate that *A. taeniorhynchus* responds to sulfates in solution on the oviposition substrate. Sodium chloride appeared repellent except when combined with sulfuric acid.

In view of the reports of *A. sollicitans* breeding in Kentucky and Illinois in mine drainage and similar situations with high sulfate contents, but with varying concentrations of other salts such as chlorides (Fellton 1944, Dixon 1957), ferrous or ferric sulfates and sulfuric acid may be preferred. Dixon (1957) indicated that, in natural situations, ferrous sulfate is

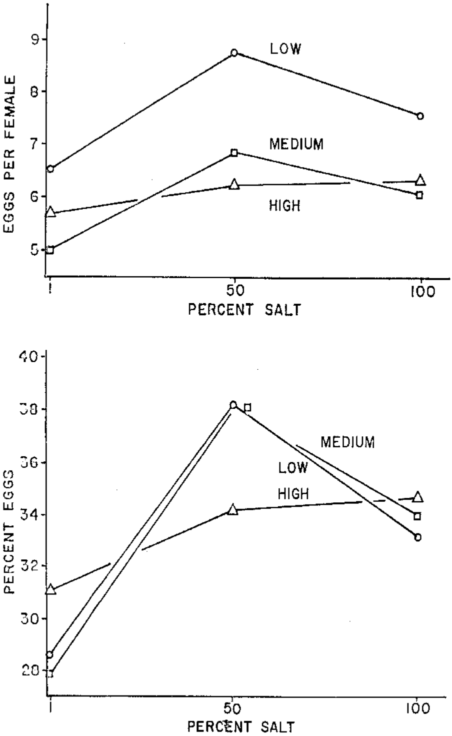

FIG. 7.—Effect of low (65%), medium (76%) and high (82.5%) ambient relative humidities on the oviposition response to marine salt (salt concentrations are expressed as dilutions of a concentration equivalent to 19% sea water).

oxidized to ferric sulfate, which is then hydrolyzed to ferric hydroxide. This material is hydrated to form iron oxide and is precipitated out. Sulfuric acid remains in solution and should be available to ovipositing mosquitoes. Oviposition sites having a low pH could be attractive in this way.

In coastal marshes where the pH is in the range of 4.6 to 7.6 (Knight 1965), sulfuric acid and magnesium and sodium sulfates may function in combination. The levels of sodium and magnesium sulfate found attractive in the work reported here (0.025–0.050 M or 50–100 meq/1) correspond closely with the levels of sulfate reported by Knight (1965) as occurring in such areas (average, 62 meq/1; range, 4–285 meq/1).

Hudson (1956) found a correlation between the osmotic pressures of various salt solutions and the responses of two fresh-water mosquito species. However, the extremely low concentration of sulfuric acid found attractive in this work indicates that osmotic pressure may only be critical for brackish-water species at the upper levels.

The degree of response obtained toward a salt solution seems quite dependent upon the laboratory technique. McGaughey and Knight (1967) found that acceleration of the oviposition response by manipulation of the photoperiod decreased the selectiveness of ovipositing females. This work suggests that ambient relative humidity may affect the response also. Above 80 percent relative humidity, egg production is reduced, and, although the preference remains unchanged, the degree of preference exhibited by the mosquitoes may be reduced to a level such that no preference is evident. Bar-Zeev (1960) reported a similar situation in which *A. aegypti* avoided wet substrates at high relative humidities.

The number of substrate choices offered at one time and the type control used may also be important factors. Distilled water, when used as a control, seems to offer an unnatural situation and will sometimes receive more eggs than the lower salt concentrations with which it is compared (Table 1, Na_2SO_4). The mosquitoes evidently oviposit on any nonrepellent material under laboratory conditions. Also, the dose-response relationship assumes different forms, depending upon the number and spacing of the salt concentrations over a given range (Figures 1, 2, and 3). Wallis (1954) observed similar differences with *A. aegypti*. A higher degree of preference seems to be exhibited when the number of choices is minimized.

The possibility that a decrease in the salt preference of the laboratory colony has occurred as a result of continuous laboratory rearing seems likely. Selection for salt preference through five generations indicated a slight increase in salt preference. However, selection through many generations, requiring several years, would be necessary to show conclusively that this was a factor in the laboratory response. The use of salt water in larval rearing had no apparent selective effect.

Further studies concerning the role of salts in oviposition site selection by *A. taeniorhynchus* probably should be conducted in the field. Careful refinement of the experimental technique does enable the demonstration of preferences for appropriate concentrations of sulfates in the laboratory. However, the demonstration of such a response to salts does not preclude the possibility of other, perhaps olfactory, materials being functional in oviposition site selection under natural conditions.

SUMMARY

Aedes taeniorhynchus, a salt-marsh mosquito, responded to sulfates in pure solution and in combination with other salts on the oviposition substrate. The response appeared to be due to the chemical and was independent of osmotic pressure except at higher levels where such solutions may be repellent. Normal oviposition occurred on distilled water because of its nonrepellent nature.

Ambient relative humidities above 80 percent caused a reduction in egg production that may have made detection of an ovipositional preference more difficult. Also, the possibility that the salt preference of the laboratory colony had decreased as a result of being maintained in the laboratory was investigated by attempting to select from the colony a strain with a higher degree of preference for salt. Selection through five generations produced only slight recovery. The use of salt water in larval rearing had no obvious effect on the selectiveness of the adults.

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