THE EFFECT OF POPULATION DENSITY AND OF NaCl CONCENTRATIONS IN TEST SERIES IN LABORATORY EXPERIMENTS WITH OVIPOSITING *AEDES AEGYPTI* [1]

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During past years variable results have been reported from laboratory experiments concerning the preference or selection among saline oviposition sites by *Aedes aegypti*. Two factors that have been variable in works reported to the present time are the density of the adult population in the cage at time of testing, and the types of solutions used in experiments. The purpose of this study was to determine the effect which variations of these two factors might have on egg distribution among saline oviposition sites.

As early as 1919, Fielding reported that *Aedes aegypti* in the laboratory would oviposit on water containing as high as two per cent NaCl if fresh water was removed from the cage. Later, Macfie (1921) reported that this species would not lay eggs on sea water. Woodhill (1946) in experiments with *A. aegypti*, used dilutions of sea water in test series and found a distinct preference for distilled water over dilutions representing .5 to 3.5% salinity in his test series of oviposition sites. In his observations, a mean of 43.1% of eggs were deposited on distilled water, 33.4% of eggs on .5% saline concentration, 18.2% of eggs on 1.0% concentration, 3.2% of the eggs on 1.75% concentration, and no eggs were oviposited on 3.5% solution. This work of Woodhill's has been the most complete study reported up to the present time concerning the response of *Aedes aegypti* to salinity in oviposition water. There have been no studies of the effects of population densities on the oviposition response or of the ranges of salinity that would be preferred or tolerated if more choices of salinity concentrations were available in test series.

MATERIALS AND METHODS. The strain of *A. aegypti* used in these experiments has been maintained in the Johns Hopkins Insectory since before 1949, and originated from eggs obtained from the Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland. From the stock colony, gravid females were transferred to experimental cages, 3 x 3 x 2.5 feet in size. All tests were run at room temperature of 27 ±3 degrees C. In testing procedures, a series of test sites was placed in the mosquito cage during an oviposition period of 24 hours. During this period the locations of test sites were changed around to prevent a "position preference," and nearly saturated humidity conditions were maintained in the cages to reduce evaporation of test solutions. Each oviposition site was a 65 sq. cm. petri dish containing 80 ml. of test solution. Strips of white filter paper 3 x 16 cm. were placed around the inner side of the dishes in such a manner that 2 cm. of moist paper extended above the water surface providing oviposition areas of 32 sq. cm. each. To reduce the amount of salt concentration by capillarity in the paper, these strips were inverted at the end of twelve hours.

Saline solutions were made up by volumetric dilution with distilled water of stock six per cent (by weight) sodium chloride solutions. Sodium chloride was of Reagent grade, molecular weight 58.45, commercially produced by Merck & Company, Rahway, New Jersey. In preparing

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the stock solutions, dry, crystalline NaCl from moisture-proof sealed bottles was weighed out on a chemical balance to within one milligram. Six per cent stock solutions were stored in clean brown glass bottles. Saline dilutions of required concentrations were kept in tightly stoppered one-liter flasks for everyday use. Solutions of over three per cent were made up at the time they were needed.

Even though an attempt was made to minimize concentration of the test solutions due to evaporation by maintaining as nearly saturated conditions of humidity as possible in the cages, in a series of control tests it was found that under the conditions in which the experiments were carried out there was a volumetric loss of fluid from the 80 ml. of solutions used that ranged as high as 12 ml. in a 24-hour period. This resulted in a concentration of the test solution; therefore, dilutions of fractions of a per cent were not used in testing below the 0.25 level; furthermore, all values of saline concentrations in test dishes refer to the per cent of NaCl at the beginning of an experiment, and there was sufficient difference allowed in a test series so that while the actual mean per cent of NaCl concentration for the 24-hour test period was somewhat higher than that at the beginning of an experiment, there was actually no overlapping of concentrations in a test series of saline solutions. For example, 80 ml. of a test solution of 0.25 per cent saline contained 0.20 gms. of NaCl at the beginning of an experiment. Under the mean range of conditions of low humidity, 6 ml. of solvent was lost by evaporation, leaving 0.20 gms. of solution in 74 ml. of solvent at a concentration of 0.27 per cent saline.

In the first experimental procedure, in order to determine the effect of different numbers of mosquitoes used in oviposition experiments, a number of tests were made in which the order and magnitude of the saline series and the cage size did not vary, but the numbers of females ovipositing were varied in successive trials. In these, eight test oviposition sites were placed in the cage for the 24-hour oviposition period. These sites were dishes containing solutions of 0 (distilled water), 0.25, 0.5, 0.75, 1, 1.5, 2, and 3 per cent saline for each oviposition period. During the first period of egg-laying, two females were allowed to oviposit. Resulting eggs were counted and their distributions recorded. Subsequent groups of 10, 20, 40, 100, 200, and 350 were allowed to oviposit on the same kind of series. The results were tabulated as in Table 1 in order to determine the approximate point at which the range of spread of egg-laying on the saline series remained constant.

In the second experimental procedure, a series of tests were run to determine the effect of different ranges of salinity concentrations used in oviposition tests and to determine the sensitivity of ovipositing females to salinity in the water. In these tests, the standard cages were used, the adult population (200) kept constant, the combinations of solutions comprising the test series varied, and the number of choices of sites in the series was varied in successive trials.

Saline series of the following combinations were used:

Series A. 0, 0, 0, 0.125 percent
Series B. .0, 0.125, 0.25, 0.5, 1, 2, 2.5, 3 percent
Series C. 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 percent
Series D. 0, 0.5, 0.75, 1, 1.5, 2, 2.5, 3 percent
Series E. 0, 1, 2, 3 percent

The number of eggs on the dishes in each series was determined and the percentage of eggs for each dish within a series recorded as shown in Table 2.

RESULTS. Table 1 represents a summary of the results of the tests with various adult densities of ovipositing females. The percentage of eggs deposited on each concentration of saline is given as well as the number of eggs upon which the percentage is based. These results show that when two mosquitoes oviposited, as in the first test, the distribution of eggs extended to only the 0.5 per cent concentration. In the second test, 10 mosquitoes oviposited, distributing their eggs in a range up to 0.75 per cent. In the third, 20 oviposited with a range to 1 per cent. In the fourth, 40 oviposited with no extension of the

TABLE 1.—The effect of different numbers of ovipositing females on distribution of eggs of *Aedes aegypti* on a saline series

No. of females ovipositing—		2	10	20	40	100	200	350
Dish No.	% NaCl	Percentage of eggs oviposited on each dish						
1.	0*	45.4	16.6	11.4	15.5	13.2	13.4	15.2
2.	0.25	51.3	40.7	48.9	50.8	51.5	45.1	42.1
3.	0.5	*3.3*	25.5	16.8	14.5	19.6	22.7	20.7
4.	0.75	0	7.2	18.7	12.9	10.7	14.2	19.4
5.	1	0	0	*4.7*	*8.4*	5.2	4.6	1.9
6.	1.5	0	0	0	0	0.5	0.2	0.6
7.	2	0	0	0	0	0	*0.1*	*0.1*
8.	3	0	0	0	0	0	0	0
Total eggs		172	580	1592	2861	4204	9882	12,063

* Distilled water.

TABLE 2—The percentage distribution of eggs deposited by *Aedes aegypti* on saline solutions in test series with different concentrations of NaCl solutions

Conc., % NaCl	0	0	0	0.125	0.25	0.5	0.75	1	1.5	2	2.5	3	Total No. eggs
Series A	23.9	24.3	24.4	26.7	..	..	..	..	..	..	..	..	1617
Series B	..	..	24.1	46.4	21.4	7.3	0.8	0	0	0	..	..	5489
Series C	..	..	12.2	..	50.2	19.8	11.8	5.1	0.6	0	..	0	1686
Series D	..	..	57.0	..	..	27.0	14.0	2.0	0	0	0	0	7058
Series E	..	..	94.3	..	..	..	..	5.4	..	0.3	..	0	4140

ange. In the fifth, 100 oviposited with a ange to 1.5 per cent. Tests 6 and 7 howed that doubling and more than ripling the number of mosquitoes re- ulted in extending this range of distribu- ion to no more than 2 per cent, and then nly 0.1 per cent of the eggs were on this oncentration. These trials showed that he distribution of eggs on a series of aline sites was affected very little by in- reasing the number of ovipositing females n the test. The proportion, or percentage f eggs deposited on the various concen- rations, was very consistent when between o and 350 mosquitoes were used. There vas some extension of range of distribu- ion among the larger populations, but vhen their numbers were increased be- ond 20, the extension of range of the listribution involved not more than 0.5 er cent of the eggs deposited.

Table 2 shows the percentage distribu- ion of eggs deposited on each concentra- ion of solution and the numbers of eggs n the entire series upon which the per- entage was based. The results demon- trated the marked variation in the dis- ribution of eggs that could be obtained rom different numbers of concentrations n the test series. In series A it was in- ended to determine only whether the .125 per cent saline would be differen- iated from distilled water; such a distinc- ion was not made. In series E, 94.3 per ent of the eggs were deposited on the listilled water when no other choice was iven in the series below the one per cent oncentration. Series D indicated that the najority of eggs were again deposited on listilled water when no other concentra- ion was available below 0.5 per cent. It ould be concluded from these results that listilled water was preferred for ovi- osition. However, if another concentra- ion amounting to one-half of the 0.5 solu- ion was added as in series C, the majority f eggs were deposited on the saline con- entration rather than on distilled water. n this series the range of distribution ex- ended to 1.5 per cent, and at the one per ent concentration was comparable to the ne per cent distribution in results of Series E. Series B indicated the same peak of distribution above the distilled water level, but in this series the range extended to only 0.75 per cent, or one-half as high as in Series C. Series A shows a possible reason for this, in that when the 0.125 concentration was tested in a series of distilled water sites no differentiation was made, and egg deposition occurred in comparable amounts.

Discussion. The results of the first procedure of varying the population in oviposition selection tests show that during the testing period eggs will be deposited on a series of test solutions in a representative distribution regardless of the numbers of adults in the test as long as a reasonable number is used. This may be from 10 to 350, and the only change in distribution with the larger numbers will be a slight extension of the range of saline concentrations utilized by the mosquito.

In the first series of tests the distribution of eggs among the test sites did not coincide with the results obtained by Woodhill (1946) in his tests using dilutions of sea water. Throughout the replications in the first tests, more eggs were always deposited on the 0.25 per cent NaCl solution than on the distilled water although it was expected, following Woodhill's distribution curve, that there would be more on the distilled water. However in the second series of tests, in which the number of choices and the saline concentrations in the test series were varied, and the conditions were comparable to those used by Woodhill, the results coincided very closely with his results. When only concentrations of 0.5 per cent or higher were available in the series, the mosquito deposited more eggs on the distilled water than on any saline solution. When lower concentrations were available in the test series more eggs were deposited on the low saline concentration than on the distilled water, and the mosquito actually preferred 0.25 per cent NaCl solution over distilled wated. Therefore, low concentrations of saline are preferred by *Aedes aegypti* and high concentrations are repellent.

The results shown in Table 2 give an

indication of the chemosensitivity of the mosquito when NaCl is used in the test solution. No differentiation was made between 0.125 per cent NaCl and distilled water, but when a geometric series of concentrations was used in the test in which distilled water, 0.125, 0.5 and higher percentages of NaCl solution were available, the 0.25 per cent solution was obviously selected. This indicates that in this range of concentrations the mosquito can distinguish between NaCl solutions of at least 0.02 (M) difference. The ability of the mosquito to detect this slight difference indicates a degree of sensitivity definitely associated with contact chemosensitivity rather than a general chemical sensitivity. It must be remembered, however, that these demonstrated differences do not represent the smallest that the mosquito can detect, but rather the smallest that have been demonstrated for it; and they represent those involved with the oviposition response of the female. It is quite possible that much smaller differences can be detected, but are not sufficiently repellent to cause the mosquito to seek other oviposition sites.

SUMMARY AND CONCLUSIONS. Testing procedures were carried out to determine the effect of varying the density of adult populations as well as the nature of the saline test series in oviposition experiments with *Aedes aegypti*. The distribution of eggs among saline test sites was affected very little by increasing the population of ovipositing females from 10 to 350 except for a slight extension of the range of salinity concentrations used for egg deposition. On the contrary, varying the composition of the series of saline test sites caused considerable difference in the distribution of eggs among the sites. In low concentrations, saline solutions are acceptable to *Aedes aegypti* for oviposition (0.25 per cent NaCl solution is actually preferred over distilled water), but are repellent in high concentrations. In the low range of concentrations, the mosquito differentiated between saline solutions in a geometric series which varied by only 0.02 M. differences in concentrations—indicating a degree of sensitivity associated with contact chemoreceptors rather than a general chemical sensitivity.

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