OVIPOSITION RESPONSE OF *AEDES SOLLICITANS, AEDES TAENIORHYNCHUS,* AND *PSOROPHORA CONFINNIS* TO SEVEN INORGANIC SALTS[1]

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INTRODUCTION. Selection by ovipositing females is considered by most investigators to be the primary factor determining the distribution of larval habitats of a given species of mosquito. Therefore, it is reasonable that factors affecting this selection are found in the substrate available for oviposition.

In southwestern Louisiana, the salt-marsh mosquito species, *Aedes sollicitans* (Walker) and *Aedes taeniorhynchus* (Wiedemann) are often found developing in marginal salt-marsh habitats that also contain *Psorophora confinnis* (Lynch-Arribálzaga). In the salt marshes, however, where the two *Aedes* spp. are most often found, *P. confinnis* rarely occurs and in rice fields and other fresh water habitats where *P. confinnis* breeds in large numbers, the salt marsh species are seldom found. The salinity of the water and soil of habitats producing these three species of mosquitoes has been studied (Darsie and Springer, 1957; Chapman, 1959; Micks and McNeill, 1963; Knight, 1965), but no tests have been attempted to demonstrate oviposition preference due to the differences in the salinity of the oviposition substrate. Since the factors affecting oviposition preference are important in the control of mosquitoes, the study was made at the Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory in an attempt to determine the importance of inorganic salts and concentrations of such salts on the oviposition preferences of salt-marsh *Aedes* spp. and *P. confinnis.*

MATERIALS AND METHODS. The tests were conducted with field-collected *A. sollicitans* and *P. confinnis,* and with *A. taeniorhynchus* taken from a laboratory colony. Approximately 200 freshly engorged females of each species were placed in test cages (21 x 11 x 11 inches) and held for 4 days. The mosquitoes were then given a choice of oviposition sites consisting of white paper toweling placed in the lid portion of a petri dish so as to provide a protruding oviposition surface of approximately 40 cm.[2] Forty milliliters of the appropriate salt solution were added and the bottom portion of the petri dish was then inserted into the lid. The insert helped to reduce evaporation, mortality of the mosquitoes by drowning, and also helped support the wet toweling in an erect position. All test cages contained duplicate sets of five different dilutions of the salt (10 sites). Each test consisted of a series of replications in which gravid females were exposed to a set of oviposition sites for 48-hour periods. At the end of each replication the sites were removed and replaced with fresh ones. This procedure was followed until excessive mortality terminated the test. All tests were conducted in an insectary under conditions of controlled temperature and humidity and all salt solutions were made by volumetric dilutions of 1-normal stock solutions made from reagent grade salts.

Since the three species of mosquitoes lay their eggs singly and may use several oviposition sites before finding one on which they will lay the majority of their eggs, exact counts were made of the eggs laid on each site. Eggs failing to undergo the normal darkening process were noted on certain salt solutions and separate counts were made of these eggs.

RESULTS AND DISCUSSION. One test was conducted with each of the three species

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TABLE 1.—Oviposition of *Psorophora confinnis, Aedes taenionhynchus* and *Aedes sollicitans* on sites treated with various concentrations of sodium chloride (3–4 replications).

Concentrations of sodium chloride (Normal)	Number and percentage of eggs laid by the three species					
	P. confinnis		*A. taeniorhynchus*		*A. sollicitans*	
	No.	%	No.	%	No.	%
0.0	1326	43	1815	17	2077	25
0.03	641	21	2116	20	988	12
0.06	571	19	2533	23	1991	24
0.09	224	7	2487	23	1767	21
0.12	328	11	1807	17	1591	19
Totals	3090		10,758		8414	

using oviposition sites treated with dilutions of sodium chloride (NaCl) ranging from 0.0 (distilled water) to 0.12 N (Table 1). *Psorophora confinnis* exhibited a definite preference for sites treated with distilled water and used sites treated with 0.03 and 0.06 N NaCl to a lesser extent; *A. taeniorhynchus* and *A. sollicitans* exhibited little or no preference at these concentrations. As a result, subsequent tests (Figs. 1–3) were conducted with concentrations of 0.0 (distilled water), 0.15 N, 0.30 N, and 0.45 N, and 0.60 N.

Aedes sollicitans laid 84,082 eggs in 14 tests, two each with sodium chloride (Na Cl), potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), sodium sulfate (Na_2SO_4), sodium bicarbonate ($NaHCO_3$), and sodium carbonate (Na_2CO_3) (see Fig. 1, a and b). In tests with NaCl, this species showed about equal preference for distilled water and concentrations of 0.15 N NaCl, while in tests with each of the other six salts this species exhibited a slight to marked preference for the distilled water over the available salt concentrations. Ninety-one percent of the eggs in the sodium chloride tests were laid on concentrations of 0.30 N and below and concentrations of KCl and Na_2SO_4 above 0.30 N were also avoided. A slight preference was shown for the distilled water sites in tests with $CaCl_2$ and $MgCl_2$, but a strong avoidance was not evident at the higher concentrations (especially with $MgCl_2$). *Aedes sollicitans* avoided all salt concentrations in tests with $NaHCO_3$ and Na_2CO_3, by laying 71 and 82 percent, respectively, of their eggs on the sites treated with distilled water.

Aedes taeniorhynchus laid 52,802 eggs in 12 tests with the seven salts as selecting factors (see Fig. 2, a and b). The responses to NaCl and Na_2SO_4 were similar to those of *A. sollicitans;* in both tests, nearly half the eggs were laid on the sites treated with distilled water and concentrations of 0.45 N and above were avoided. *Aedes taeniorhynchus* displayed equal preferences for distilled water and 0.15 N KCl with 78 percent of the eggs laid on these two sites. A response similar to that of *A. sollicitans* was also shown for concentrations of Ca Cl_2 and $MgCl_2$, as no sharp avoidance was evident though preference declined slightly as the concentrations were increased. *Aedes taeniorhynchus* avoided $NaHCO_3$ and Na_2CO_3 even more markedly than did *A. sollicitans.* Ninety and 95 percent of the eggs, respectively, were laid on the distilled water sites.

In 12 tests with *P. confinnis* (see Fig. 3, a and b) only 27,914 eggs were obtained because of the high mortality of the test insects. In the tests with NaCl, KCl, Na_2SO_4, $NaHCO_3$, and Na_2CO_3, *P. confinnis,* displayed a strong preference for sites treated with distilled water. In each of these tests, 70 percent or more of the eggs were laid on the salt-free sites. Also, *P. confinnis* avoided any concentrations of NaCl, $NaHCO_3$ and Na_2CO_3 and

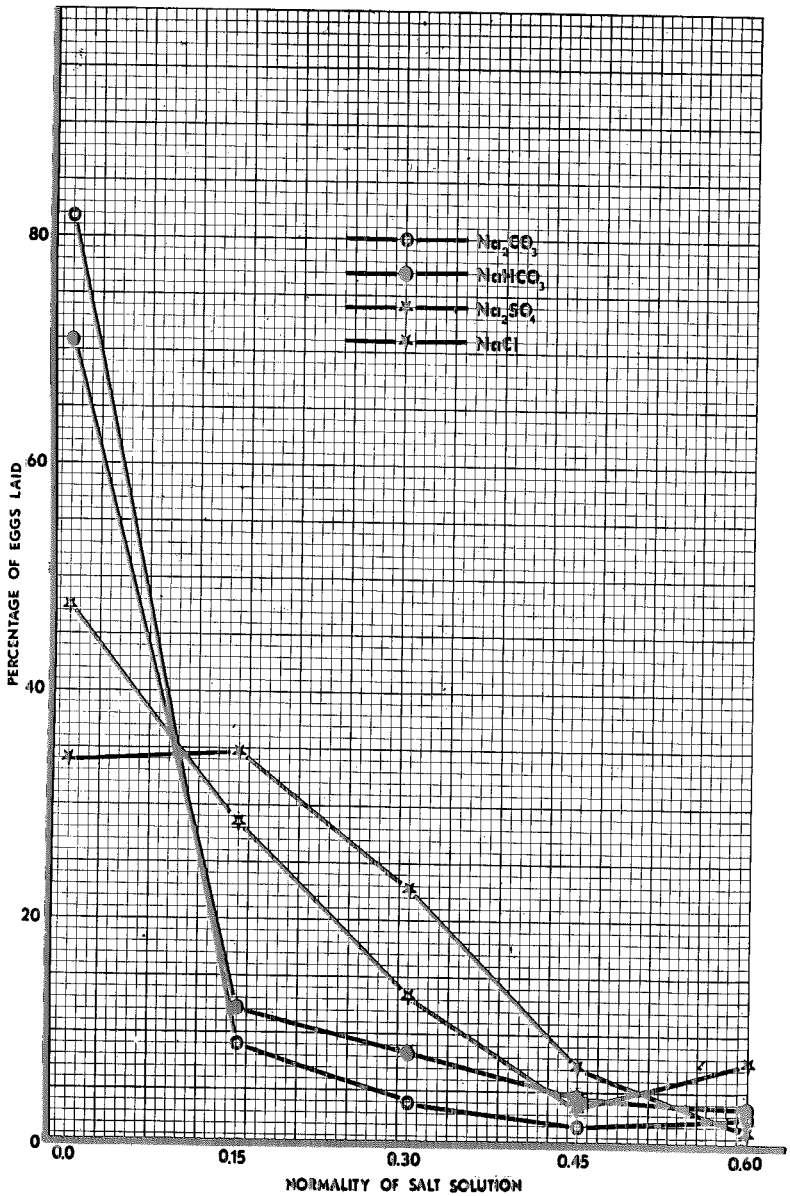

FIG. 1(a)—Percentage of eggs laid by *Aedes sollicitans* on five concentrations of four sodium salts.

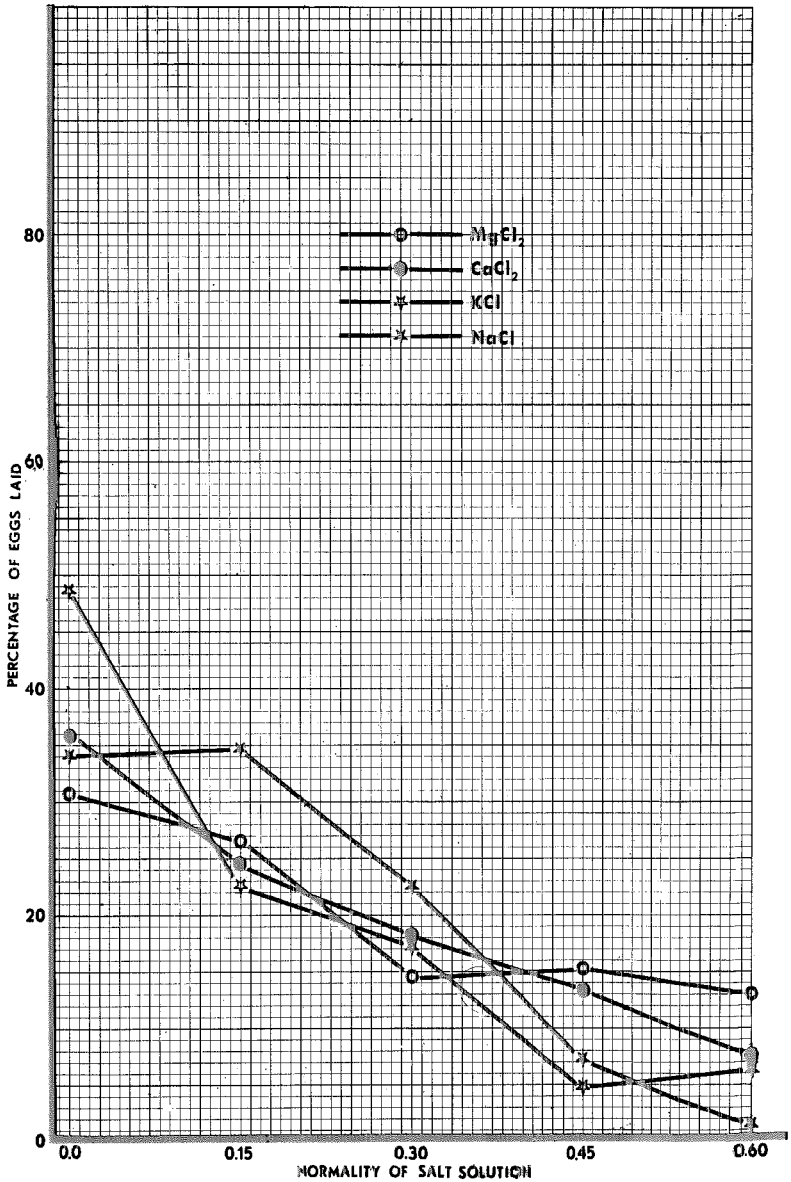

FIG. 1(b)—Percentage of eggs laid by *Aedes sollicitans* on five concentrations of four chloride salts.

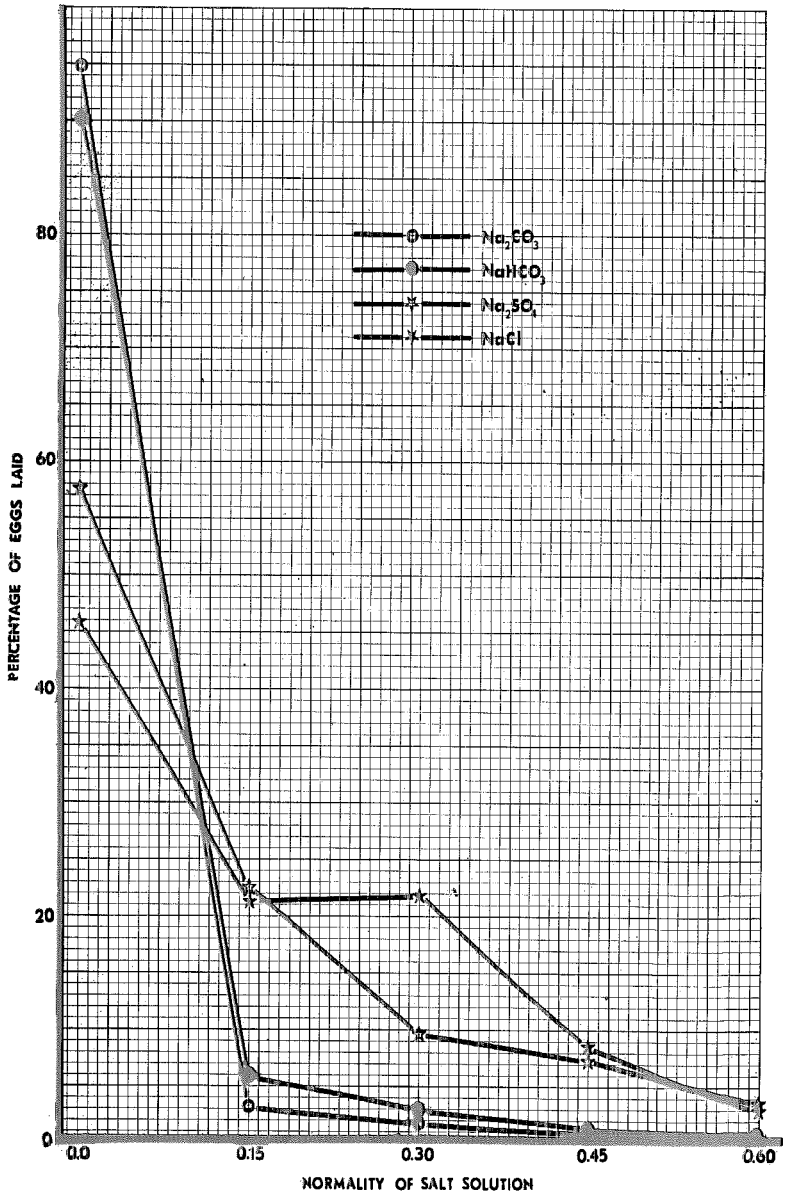

FIG. 2(a)—Percentage of eggs laid by *Aedes taeniorhynchus* on five concentrations of four sodium salts.

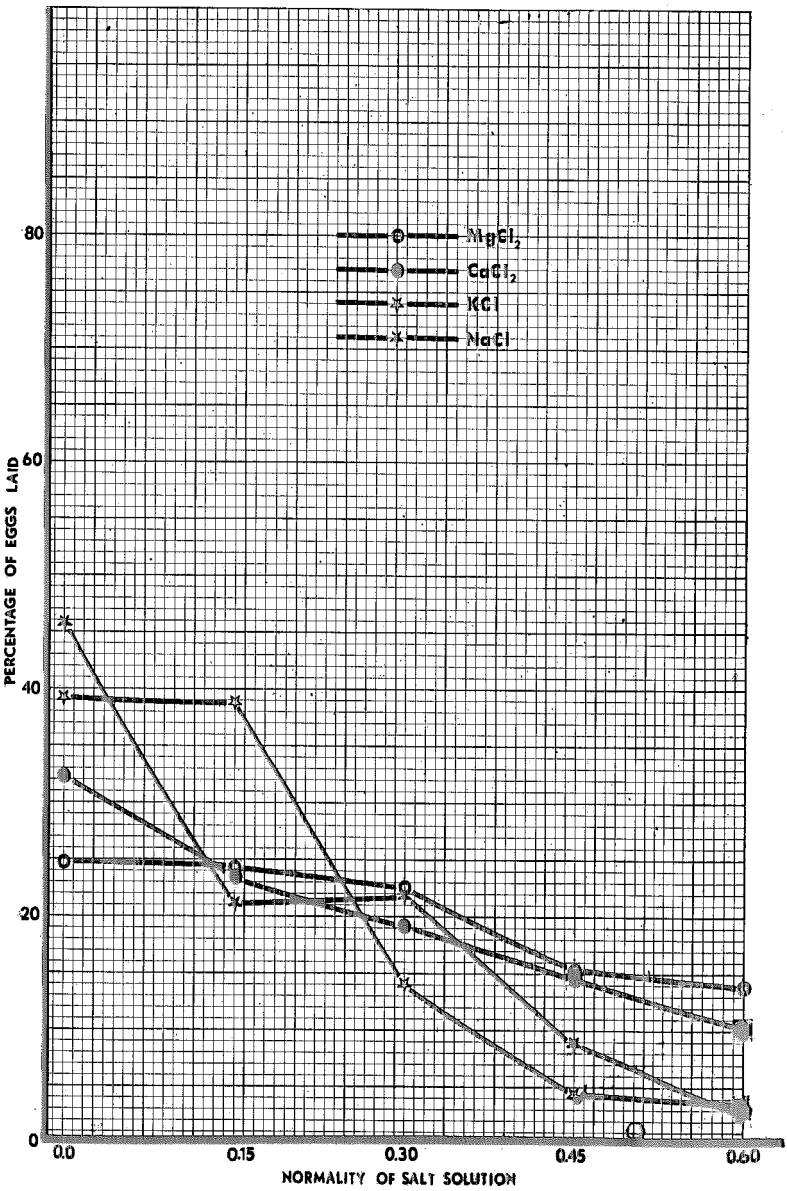

FIG. 2(b)—Percentage of eggs laid by *Aedes taeniorhynchus* on five concentrations of four chloride salts.

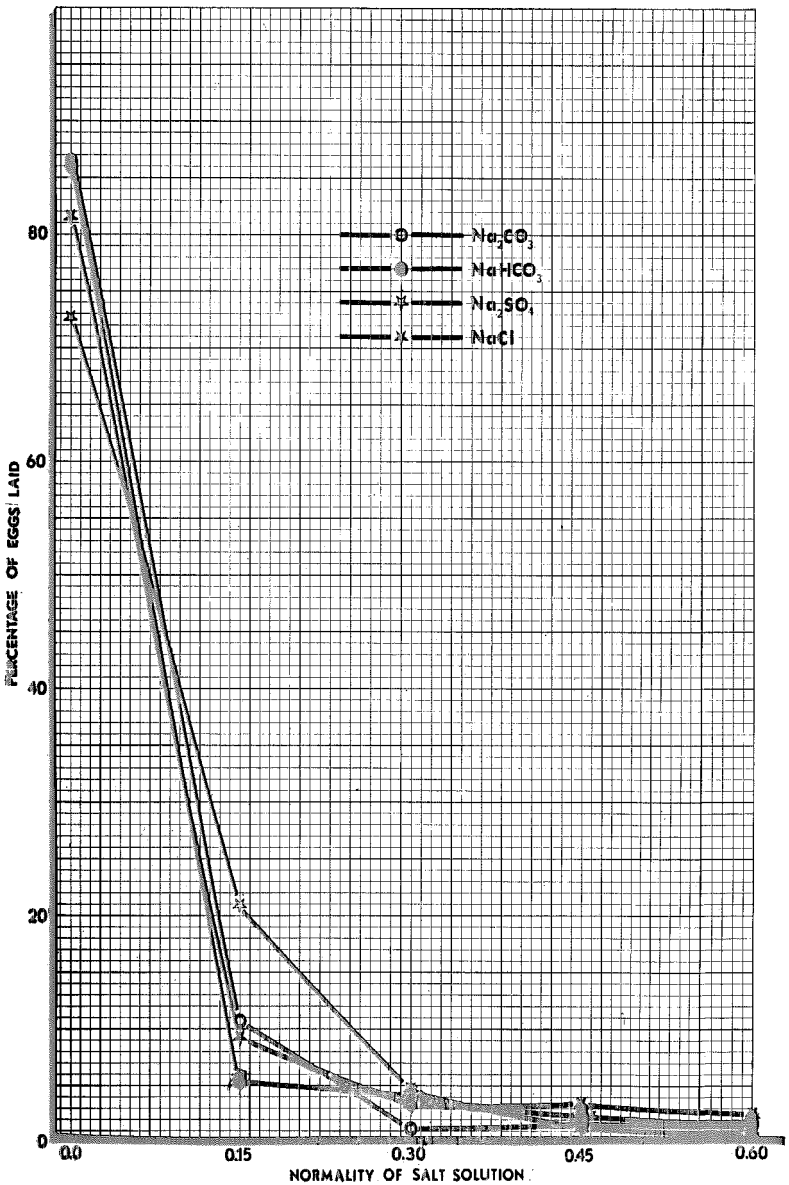

FIG. 3(a)—Percentage of eggs laid by *Psorophora confinnis* on five concentrations of four sodium salts.

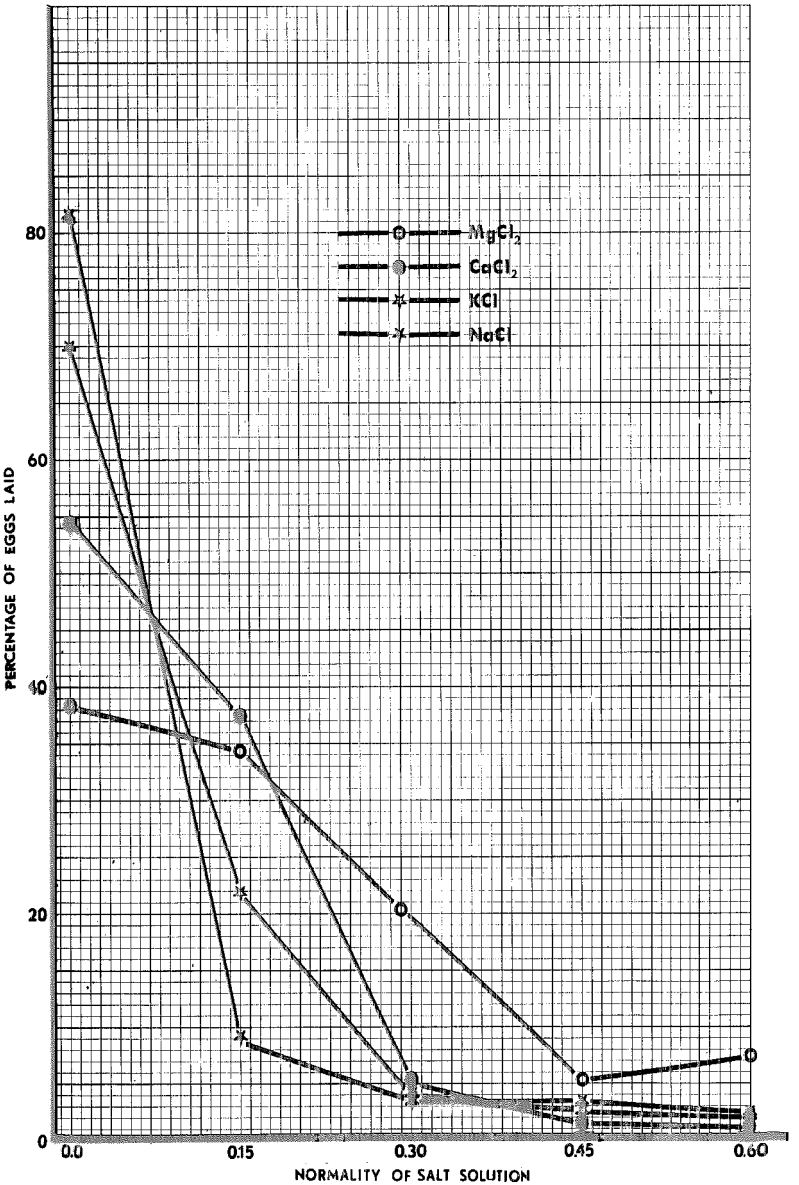

FIG. 3(b)—Percentage of eggs laid by *Psorophora confinnis* on five concentrations of four chloride salts.

all concentrations of KCl, $CaCl_2$ and Na_2SO_4 of 0.30 N and above. *P. confinnis* displayed a greater tolerance for concentrations of $MgCl_2$ than it did to any of the other salts tested.

In another series of tests, gravid females of *A. sollicitans* and *A. taeniorhynchus* were given a choice of the various individual salts at equivalent normal concentrations and certain combinations of salts. Two tests were conducted with each species with four chloride salts and the same four salts combined at concentrations of 0.15 N and 0.45 N (Table 2), and one test was made with each species with four sodium salts and the same four salts combined at concentrations of 0.15 N (Table 3). *Aedes sollicitans* showed no particular preference for any chloride salt at concentrations of 0.15 N; however, a few more eggs were laid on sites treated with $MgCl_2$ than on the others. At 0.45 N, this species showed a decided preference for $MgCl_2$ and for the combination of four chloride salts; more than twice as many eggs were laid on these two sites than on the other three choices available. These results compare closely with those obtained in the previous tests (Fig. 1). *Aedes taeniorhynchus* exhibited little or no preference for a particular chloride salt at concentrations of 0.15 N but, like *A. sollicitans*, markedly preferred sites treated with $MgCl_2$ at the 0.45 N level. Also, *A. taeniorhynchus* appeared to avoid sites treated with 0.45 N concentrations of KCl.

TABLE 2.—Oviposition preferences of *Aedes sollicitans* and *Aedes taeniorhynchus* for four chloride salts.

Salt concentration	Replications	Total no. of eggs laid on various chloride salts (Percentage in parentheses)					
		NaCl, KCl $MgCl_2$, $CaCl_2$	NaCl	KCl	$MgCl_2$	$CaCl_2$	Total
				Aedes sollicitans			
0.15 N	5	1289 (16.3)	1602 (20.2)	1407 (17.8)	2048 (25.9)	1562 (19.8)	7908
0.45 N	2	620 (30.1)	163 (7.9)	214 (10.4)	749 (36.3)	316 (15.6)	2062
				Aedes taeniorhynchus			
0.15 N	4	538 (17.6)	496 (16.2)	574 (18.8)	741 (24.3)	705 (23.1)	3054
0.45 N	4	286 (17.2)	445 (26.7)	55 (3.3)	534 (32.0)	347 (20.8)	1667

TABLE 3.—Oviposition preference of *Aedes sollicitans* and *Aedes taeniorhynchus* for four sodium salts.

Salt concentration	Replications	Total no. of eggs laid on various sodium salts (percentage in parentheses)					
		NaCl, Na_2SO_4 $NaHCO_3$, Na_2CO_3	NaCl	Na_2SO_4	$NaHCO_3$	Na_2CO_3	Total
				Aedes sollicitans			
0.15 N	2	97 (3.1)	1760 (57.0)	832 (27.0)	267 (8.6)	131 (4.2)	3087
				Aedes taeniorhynchus			
0.15 N	3	288 (21.4)	391 (29.1)	408 (30.3)	146 (10.8)	112 (8.3)	1345

In the tests with 0.15 N sodium salts, *A. sollicitans* and *A. taeniorhynchus* responded similarly as in the previous tests (Figs. 1 and 2) with *A. sollicitans* showing a strong preference for NaCl, a lesser preference for Na_2SO_4 and avoiding sites containing $NaHCO_3$ and Na_2CO_3. *Aedes taeniorhynchus* displayed about equal preferences for 0.15 N concentrations of NaCl and Na_2SO_4, and tended to avoid sites treated with $NaHCO_3$ and Na_2CO_3. The combination of the four sodium salts was less attractive than any single salt.

All three species similarly avoided any of the available concentrations of $NaHCO_3$ and Na_2CO_3 probably due in part to the very high pH values resulting from the concentrations of these salts. *Aedes sollicitans* and *A. taeniorhynchus* responded similarly to each of the salts tested, especially $MgCl_2$, $CaCl_2$, KCl, NaCl and Na_2SO_4. In contrast, *P. confinnis* strongly avoided even 0.15 N concentrations of Na_2SO_4, NaCl and KCl and thus had a markedly different response than the two *Aedes* spp. *Psorophora confinnis* avoided $CaCl_2$ and especially $MgCl_2$ less than any other salts. This was true of both *A. sollicitans* and *A. taeniorhynchus*, especially at the highest concentrations.

Also noted during the study were the differential effects the various salts had on the eggs of the mosquitoes and the differences in tolerances of the eggs between species (Table 4). Eggs of all three species laid on sites treated with the higher concentrations of some salts failed to undergo the normal darkening process. For example, $NaHCO_3$ and Na_2CO_3 markedly affected the newly laid eggs of all three species. Essentially all of the eggs of *P. confinnis* which were laid on these two salts at concentrations of 0.15 N and above, were affected. About 30 percent of the

TABLE 4.—Percentage of eggs of *Psorophora confinnis*, *Aedes sollicitans*, and *Aedes taeniorhynchus* remaining white after exposure to various concentrations of salt.

Salt	Total no. of eggs laid	Percentage of eggs remaining white after exposure to indicated concentrations				
		0.0 N	0.15 N	0.30 N	0.45 N	0.60 N
			P. confinnis			
$MgCl_2$	4361	0	0	0	0	0
$CaCl_2$	3424	0.3	1.5	2.2	10	11
Na_2SO_4	3261	0	0	16	20	95
NaCl	5651	0	7.7	81	78	70
KCl	5257	0.4	4.0	81	94	79
$NaHCO_3$	3266	0	94	98	100	96
Na_2CO_3	2694	1.3	90	97	96	86
			A. taeniorhynchus			
$MgCl_2$	10,995	0	0	0	0	0
$CaCl_2$	7287	0	0	0	0	0
Na_2SO_4	7582	0	0	0	0	0
NaCl	10,959	0	0	0	1.4	6.6
KCl	8346	0	0	0	6.7	10
$NaHCO_3$	2933	0	32	65	38	73
Na_2CO_3	4700	0	13	40	38	75
			A. sollicitans			
$MgCl_2$	15,203	0	0	0	0	0
$CaCl_2$	13,924	0	0	0	0	0
Na_2SO_4	16,184	0	0	0	0	0
NaCl	8679	0	0	0	0	0
KCl	13,753	0	1.9	0	11	12
$NaHCO_3$	8647	1.2	24	54	71	70
Na_2CO_3	7692	0	37	58	66	70

eggs of the *Aedes* spp. were affected at 0.15 N, and about 75 percent of the eggs of the *Aedes* spp. were affected at concentrations of 0.60 N. *Aedes taeniorhynchus* eggs were slightly affected at 0.45 N and 0.60 N concentrations of NaCl and KCl and the other salts had no effects at concentrations of 0.60 N. The higher concentrations of KCl had slight effects on the eggs of *A. sollicitans,* but no effects were observed with $MgCl_2$, $CaCl_2$, Na_2SO_4, and NaCl. The eggs of *P. confinnis,* however, showed some effects at the lowest concentrations of NaCl and KCl and were markedly affected at concentrations of 0.30 N. At 0.60 N, Na_2SO_4 also markedly affected the darkening process of the eggs of *P. confinnis.* Only slight effects were evident with $CaCl_2$ and none with $MgCl_2$ at concentrations as high as 0.60 N.

The way in which the various salts affect the response of gravid mosquitoes is not clear. Hudson (1956) found that the number of rafts of *Culex pipiens molestus* Forskal laid on different solutions was more closely related to their osmotic pressures than to their molarities, but when the mosquitoes were offered different concentrations of glucose they laid almost at random. However, osmotic pressure is a function of ionic activity, and it is likely that the responses of the chemoreceptors of the insect would be directly proportional to the ionic activity (Clements, 1963).

Conflicting reports have been particularly evident in studies involving salinities as a factor in the oviposition response of mosquitoes. For example, Kligler and Theodor (1925) found that the preference for fresh water habitats was no more pronounced in *Anopheles superpictus* Grassi, a species that breeds in fresh water, than in *Anopheles sacharovi* Favre., a species that breeds in brackish marshes. De Buck *et al.* (1932) reported that the brackish water mosquito *Anopheles atroparvus* Van Thiel avoided even weak solutions of sodium chloride though a fresh water species *Anopheles messeae* Falleroni appeared indifferent to salt. Also, Wallis (1954) working with three species of *Anopheles,* three species of *Aedes* and four species of *Culex,* found that all ten species preferred sites treated with distilled water over those treated with concentrations of sodium chloride (from 1 to 6 percent) and that all the *Aedes* and the *Culex* species were repelled by concentrations of 2 percent or more NaCl. He also found that the fresh water species *Aedes aegypti* (L) preferred dilute solutions of NaCl (0.25–0.75%) to distilled water. These studies clearly demonstrate that mosquitoes can detect differences in the salinity of the oviposition site. Moreover, most results obtained with salt-marsh species, especially *Culex* and *Aedes,* compare favorably with those observed in the present study: species demonstrated an equal or slight preference for distilled water over saline oviposition sites (Wallis, 1954; O'Gower, 1958; Woodhill, 1941).

The definite differences in the oviposition response between the fresh water species *P. confinnis* and the two species of salt-marsh *Aedes* in the present study (especially with NaCl, KCl, and Na_2SO_4) indicate that salinity is probably an important factor in the distribution of the larval habitats of these species. It appears, however, that salinity is not an attraction factor in itself. It seems rather to contribute to other ecological factors that do attract a particular species to the brackish water habitats. Salinity is probably more important as a repellent factor to ovipositing mosquitoes; those species that are more tolerant to salt find such habitats suitable for oviposition, while others seek less saline environments. The differences in the tolerance of the freshly laid eggs to salinity may also be important to distribution. Downs (1951) reported that the sensitivity of freshly laid eggs of anopheline mosquitoes to relatively low saline concentrations may explain the failure of many anopheline species to succeed in brackish water. Also, Petersen and Rees (1967) observed a marked difference in tolerance to salinity between the eggs of *Aedes dorsalis* (Meigen) and *Aedes nigromaculis* (Ludlow) that was similar to those observed in the present study between *P. confinnis* and the two *Aedes* spp. The lack of tolerance in the eggs of *P.*

confinnis to salt may be an additional factor in preventing this species from breeding in many of the more saline habitats.

SUMMARY. Oviposition preference tests were conducted with *Aedes sollicitans, Aedes taeniorhynchus,* and *Psorophora confinnis* by using dilutions of NaCl, KCl, $CaCl_2$ $MgCl_2$ Na_2SO_4, $NaHCO_3$, and Na_2CO_3. All three species similarly avoided any concentrations of $NaHCO_3$ and Na_2CO_3, and the two *Aedes* species responded similarly to all salts tested. *Psorophora confinnis* avoided even the lowest concentrations of Na_2SO_4, NaCl, and KCl and responded differently to these three salts than the two *Aedes* spp. Also, *P. confinnis,* and to a lesser extent the *Aedes* spp., showed less avoidance to Ca Cl_2 and especially $MgCl_2$ than to the other salts.

The various salts had differing effects on the eggs of the mosquitoes, and differences between species were evident in the tolerance of the eggs. Those of the *Aedes* spp. showed little or no effects when they were exposed to 0.6 N concentrations of NaCl or KCl, but those of *P. confinnis* were markedly affected at 0.30 N concentrations. It appears that salinity may be more important as a repelling factor than as an attracting factor in the location of suitable oviposition sites by mosquitoes.

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