of this species was (for example) 20 or 200. The fact remains that, in aggregate, Graham found mites on less than 5 percent of females and on only ⅓rd of the species he examined. So (except possibly for *Aedes excrucians*) the practical usefulness of mites for age-grading at George Lake, Alberta is, as he concludes, very small.

CONCLUSIONS. Graham (1969) provides no incisive evidence regarding the reliability of parasitic water-mites as indicators of the nulliparous condition of mosquitoes in Alberta. On the contrary, it remains probable that, had the relevant facts been recorded, mites *would* have constituted reliable indicators in at least some species. On the other hand, Graham's work leaves little doubt that, whether they be reliable indicators or not, mites occur on too few females at George Lake for them to be of practical use for age-grading. This serves to emphasize a point that has been made before (Corbet, 1963): that mites are only likely to be useful for age-grading common species on which they have a fairly high incidence. This is because, to check the mites' reliability, extensive trials must be undertaken to determine patterns of infestation before the method can be used with assurance. Such heavy investment of effort is seldom justified except as a prelude to a long-term investigation or to one involving an abundant species of mosquito.

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OVIPOSITION RESPONSES OF *CULEX PIPIENS QUINQUEFASCIATUS* AND *CULEX SALINARIUS* IN THE LABORATORY

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Culex pipiens quinquefasciatus Say commonly breeds in foul water in street gutters, catch basins, cesspools, open septic tanks, artificial water containers, and polluted ground pools (King *et al.*, 1960; Carpenter and LaCasse, 1955). *Culex salinarius* Coquillett generally breeds in unpolluted (fresh or brackish) water, and the larvae are found in grassy pools, ditches, marshy places, and occasionally in water barrels (King *et al.*, 1960), and treeholes (Petersen and Chapman, 1969). The literature contains inconsistent reports about the preference of *C. salinarius* for fresh or brackish waters, but Williams (1956), Darsie and Springer (1957) and Chapman (1959) all established that the species breeds in both fresh water and in water that has relatively high salinity.

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Numerous studies have been made to determine the oviposition preference of *C. salinarius* and *C. p. quinquefasciatus* for water containing organic chemicals (Gjullin and Johnson, 1965), bacteria (Hazard *et al.*, 1967), or odors (Gjullin *et al.*, 1965) and for natural breeding water and extracts of these waters (Ikeshoji, 1966). Few studies, however, have been concerned with concentrations of inorganic salts as a limiting factor in the oviposition behavior of these two species. Woodhill (1941) reported that *Culex fatigans* (= *p. quinquefasciatus*) was able to distinguish between distilled water and salt solutions containing 0.5 percent (0.09N) sodium chloride (NaCl) and would not deposit eggs on solutions containing 1.0 percent (0.17N) salt when distilled water was available. Wallis (1954) also reported that *C. p. quinquefasciatus* restricted oviposition to concentrations of 0.5 percent NaCl and below and preferred distilled water; in addition, he found that *C. salinarius* would oviposit on concentrations as high as 1.5 percent (0.26N) and that its preference for distilled water was not as marked.

The present study was made at the Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory at Lake Charles, Louisiana in 1968 to determine the effects of inorganic salts and concentrations of these salts on the oviposition behavior of *C. p. quinquefasciatus* and *C. salinarius*.

MATERIALS AND METHODS. Four days after a blood meal, populations (about 200 adult females from the laboratory colonies) of each species in test cages (53 x 28 x 28 cm.) were offered five concentrations of a given salt (40 ml) in open petri dishes. In each test, the cages contained duplicate sets of the five solutions (10 sites). Replications of each test were run nightly until oviposition ceased; usually 3 to 4 nights. The egg rafts were removed and counted after each replication, and the oviposition sites were rearranged randomly for the next replication. Each test was repeated one or more times depending on the number of egg rafts obtained. All tests were made in an insectary with controlled temperature and humidity.

The salt solutions of the eight inorganic salts were derived by volumetric dilutions of 1-normal stock solutions made from reagent grade salts; dilutions are reported as normal (N) concentrations.

RESULTS AND DISCUSSION. In two preliminary tests, each species was given a choice of five concentrations of NaCl ranging from 0.00 (distilled water) to 0.40N. *C. p. quinquefasciatus* laid 87 percent of their egg rafts on the distilled water and none on concentrations above 0.10N. *C. salinarius* similarly avoided sites containing concentrations of 0.20N and above but did not show the strong preference for distilled water; they laid 37 percent of their egg rafts on the 0.10N concentrations. Since concentrations of 0.20N NaCl and above were inhibitory to both species, all subsequent tests were conducted with concentrations of salts of 0.10N and below.

In a second series of tests the oviposition preferences of *C. p. quinquefasciatus* and *C. salinarius* were tested against eight inorganic salts. *C. p. quinquefasciatus* exhibited a significant difference in response (chi square, 0.05 percent level of confidence) to the concentrations of all eight salts. Also, the species showed slight to marked preference for distilled water over the lowest concentrations available (0.025N) in tests with calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), potassium chloride (KCl), sodium carbonate (Na_2CO_3), and potassium carbonate (K_2CO_3). In tests with NaCl and sodium sulfate (Na_2SO_4), the 0.025N concentration was chosen most often; in tests with sodium bicarbonate ($NaHCO_3$), the 0.075N concentration was most often selected (Fig. 1). Also, responses were similar to NaCl, KCl, $MgCl_2$ and K_2CO_3: 84 to 90 percent of the egg rafts were laid on concentrations of 0.050N and below. However, with $NaHCO_3$, more than 50 percent of the egg rafts were laid on concentrations of 0.050N and above, and

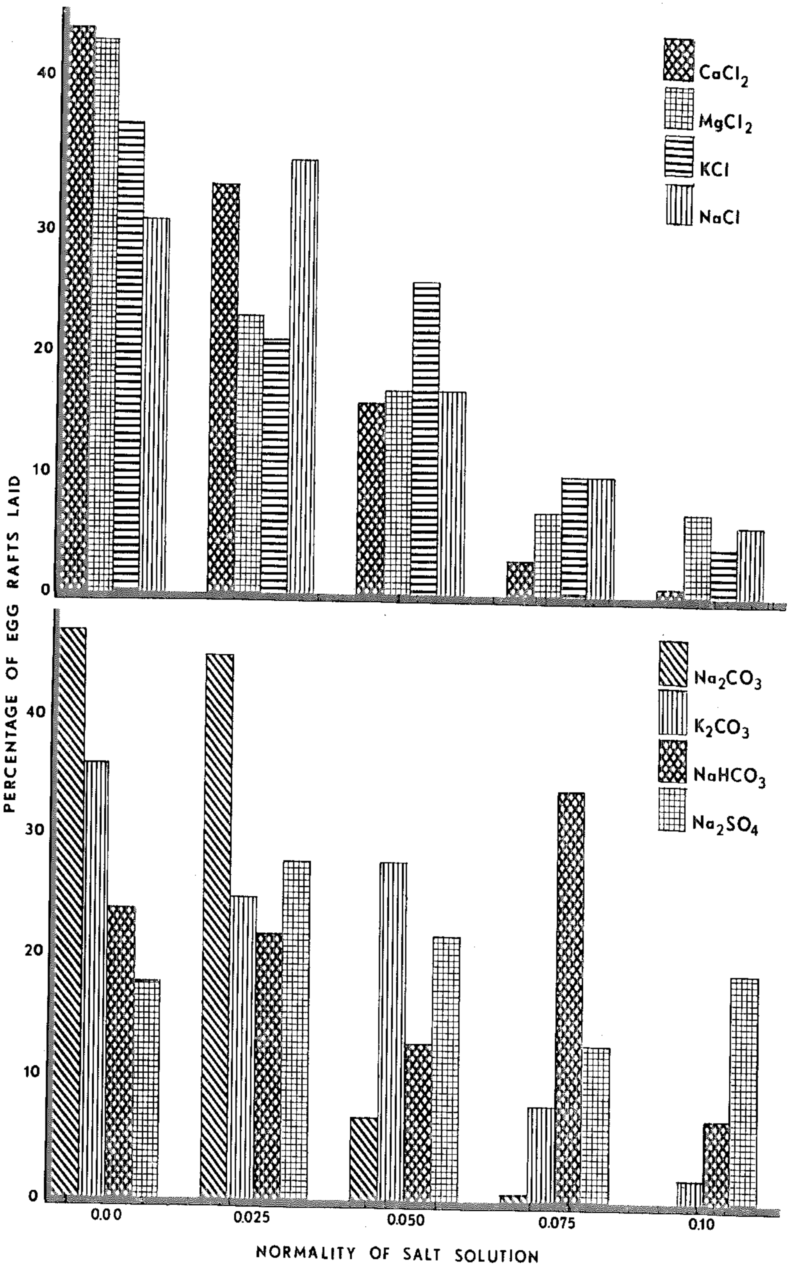

FIG. 1.—Oviposition response of *Culex pipiens quinquefasciatus* to five dilutions of eight inorganic salts (each test replicated twice).

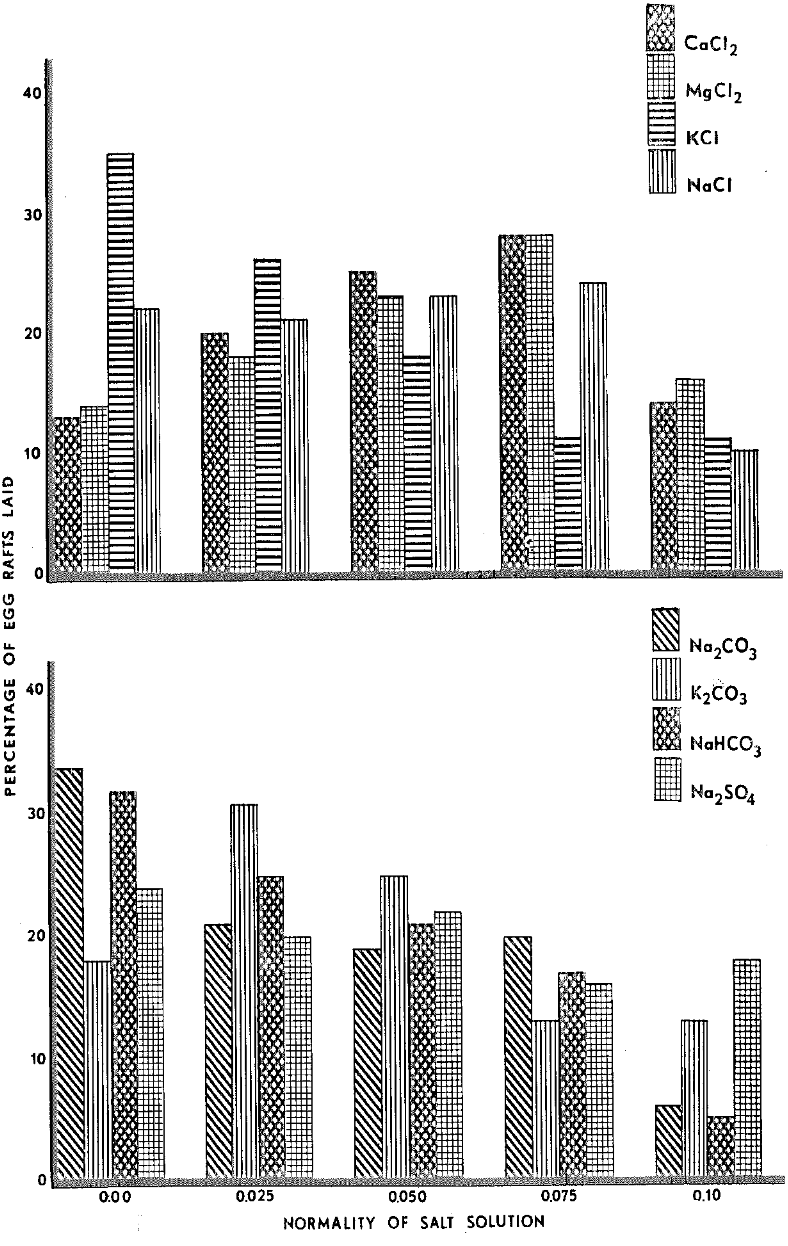

FIG. 2.—Oviposition response of *Culex salinarius* to five dilutions of eight inorganic salts (each test replicated twice or more).

with Na_2SO_4 concentrations of 0.10N had little effect on oviposition preferences. *C. p. quinquefasciatus* avoided the higher concentrations of $CaCl_2$ and Na_2CO_3 more than they avoided any other salts and laid 81 and 92 percent respectively, of their egg rafts on concentrations below 0.050N.

In a similar series of tests with *C. salinarius,* oviposition was not restricted to the lower concentrations (Fig. 2). This species showed little or no preference among concentrations of Na_2SO_4 (not significant, 0.05 level) though preferences were significant (0.05 level) for the other seven salts. *C. salinarius* laid equally on all concentrations of NaCl, up to 0.75N. Also, they responded similarly to $CaCl_2$ and $MgCl_2$ and oviposited most often on 0.075N concentrations of these salts. Also, the 0.025N concentration of K_2CO_3 were oviposited on more often than either distilled water or the higher concentrations of this salt. With KCl, $NaHCO_3$, and Na_2CO_3, the species laid about one-third of its egg rafts on distilled water and avoided the higher concentrations of these salts more than they did the higher concentrations of the other five salts.

Therefore, the two species responded differently to the salts and concentrations of salts. Except with Na_2SO_4 and $NaHCO_3$, *C. p. quinquefasciatus* never laid more than 16 percent of their egg rafts on concentrations of 0.075N and above when lower concentrations were available. However, *C. salinarius* laid 22 percent or more of their egg rafts on the higher concentrations (0.075N and above) in all tests with all eight inorganic salts. Also, *C. salinarius* tolerated higher concentrations of all salts (especially NaCl, $CaCl_2$, $MgCl_2$, and Na_2CO_3) except $NaHCO_3$; both species responded similarly to this salt.

The greatest difference in the oviposition response of the two species was to $CaCl_2$. *C. p. quinquefasciatus* laid 81 percent of their egg rafts on distilled or 0.025N concentrations and 97 percent on concentrations of 0.050N and below. *C. salinarius* laid 67 percent of their egg rafts on concentrations of 0.05N and above and 42 percent on concentrations of 0.075N and above.

In a third series of tests, both species were given a choice of the four chloride salts and of a combination of the same four salts at concentrations of 0.05N and 0.10N (Table 1). *C. p. quinquefasciatus* showed a significantly different response (0.05 level) to the chloride salts at both concentrations: at concentrations of 0.05N, the species laid most often on sites containing KCl and oviposited least on $CaCl_2$; however, at concentrations of 0.10N, $MgCl_2$ was selected twice as often as any other salts, and the other three were oviposited on with equal frequency. *C. salinarius* laid about equal numbers of rafts on the four salts and combinations at concentrations of 0.05N (not significant) but showed a significant preference for concentrations of 0.10N; at this con-

Table 1.—Average oviposition preference of *Culex p. quinquefasciatus* and *Culex salinarius* to 0.05 and 0.10 N concentrations of four chloride salts and a combination of the four salts (3–5 replications).

Concentration of salt	Total egg rafts laid in all replications	Percentage of total egg rafts laid on—				
		NaCl	KCl	$CaCl_2$	$MgCl_2$	NaCl, KCl $CaCl_2$ $MgCl_2$
			C. p. quinquefasciatus			
0.05 N	425	21	30	9	23	17
0.10 N	269	15	16	16	38	14
			C. salinarius			
0.05 N	212	21	18	20	22	19
0.10 N	323	22	20	16	27	15

centration, $MgCl_2$ was selected most often and $CaCl_2$ least often.

In the last series of tests, *C. p. quinquefasciatus* and *C. salinarius* were offered the four sodium salts and a combination of the four at the same concentrations (Table 2). *C. p. quinquefasciatus* showed a significant preference among the salts (0.05 level) at both concentrations, but *C. salinarius* did not. Thus, *C. p. quinquefasciatus* oviposited most often on $NaHCO_3$ at both concentrations and least often on Na_2CO_3 and the combined salts; also, the species avoided NaCl at the 0.1N concentration.

TABLE 2.—Average oviposition preference of *Culex p. quinquefasciatus* and *Culex salinarius* to 0.05 and 0.10 N concentrations of four sodium salts and a combination of the four salts (2–4 replications).

Concentration of salt	Total egg rafts laid in all replications	Percentage of total egg rafts laid on—				
		NaCl	Na_2SO_4	$NaHCO_3$	Na_2CO_3	NaCl, Na_2SO_4 $NaHCO_3$, Na_2CO_3
		C. p. quinquefasciatus				
0.05 N	402	21	18	30	13	18
0.10 N	469	9	37	48	0.6	4
		C. salinarius				
0.05 N	205	22	19	19	20	19
0.10 N	277	14	20	23	21	22

C. p. quinquefasciatus and *C. salinarius* therefore can detect differences in concentrations of salt as small as 0.025N. Wallis (1954) found that *Aedes aegypti* (L.) also was able to detect differences in concentrations of NaCl as slight as 0.02N and suggested that mosquitoes distinguished chemical differences by the contact chemoreceptive spines located on their tarsi.

Petersen (1969) reported that *Psorophora confinnis* (Lynch-Arribálzaga), *Aedes sollicitans* (Walker), and *Aedes taeniorhynchus* (Wiedemann) tolerated much higher concentrations of salt, especially $MgCl_2$ and $CaCl_2$, than the two *Culex* spp. However, these three floodwater species were strongly repelled by $NaHCO_3$ and Na_2CO_3 whereas the *Culex* spp. were not noticeably affected. The narrower tolerances of the *Culex* spp. were expected since they lay their eggs on the surface of water and normally do not encounter the higher concentrations of salts that occur on most soil surfaces where *Aedes* and *Psorophora* oviposit.

Inorganic salts therefore appear to be restrictive in nature rather than attractive to ovipositing females of the two *Culex*. The gravid females are probably attracted to a potential habitat by a combination of environmental factors (temperature, odors, vegetation). The inorganic salts present in the water then repel certain species while other species find the habitat suitable for oviposition.

SUMMARY. The oviposition responses of *C. p. quinquefasciatus* and *C. salinarius* to concentrations of eight inorganic salts were studied. *C. p. quinquefasciatus* avoided sites containing higher concentrations of NaCl, KCl, $MgCl_2$, and K_2CO_3 and laid rafts more often on the higher concentrations of Na_2SO_4 and $NaHCO_3$. They showed the strongest avoidance to concentrations of $CaCl_2$ and Na_2CO_3. The preference of *C. salinarius* was not restricted primarily to the lower concentrations of salts as was *C. p. quinquefasciatus*. However, this species laid fewer eggs on the higher concentrations of KCl, $NaHCO_3$, and Na_2CO_3 than on any of the other five salts. The two species therefore detected differences in concentrations as small as 0.025N and responded differently to the salts and to the concentrations of these salts. The greatest difference in response between the two species was to $CaCl_2$: *C. p. quinquefasciatus* laid the majority of their egg rafts on the lower

concentrations; *C. salinarius* laid the majority of their egg rafts on the higher concentrations. Inorganic salts therefore appear to be restrictive in nature rather than acting as an attractant to the ovipositing female of these species of *Culex.*

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