SELECTIVE OVIPOSITION RESPONSE OF *AEDES DORSALIS* AND *AEDES NIGROMACULIS* TO SOIL SALINITY[1]

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INTRODUCTION. *Aedes dorsalis* (Meigen) and *Aedes nigromaculis* (Ludlow) are known as floodwater breeding mosquitoes. In Utah, both species commonly co-inhabit the same larval habitats, and in most instances *A. dorsalis* is the most common larval associate of *A. nigromaculis*. In California (Telford, 1958) and Nevada (Chapman, 1960), *Aedes melanimon* Dyar was found to be the major associate species of *A. nigromaculis*.

As a result of observations made in Utah, it is apparent that *A. nigromaculis* is much more restricted in larval habitat than *A. dorsalis*. This restriction is apparent in the highly alkaline marshes bordering the Great Salt Lake where *A. dorsalis* larvae are present in great numbers without the larvae of *A. nigromaculis*, while the larvae of both species are found in great numbers in irrigated pastures and alfalfa fields adjacent to these marshes. More striking, however, is the fact that during this study from 1961 to 1965 inclusive, it was found that in many irrigated pastures and fields known to produce larvae of both species, there were areas which produced only *A. dorsalis*.

Variation in distribution and the fact that chemical control of these two species is becoming more difficult with the restrictions on the use of insecticides in pastures and alfalfa fields, established the need for this study. In this study an attempt is made to determine the effects of indigenous inorganic salts on the selection of oviposition sites by the females of these two species.

Previous investigations of the effects of inorganic salts on the habitat preferences of *A. dorsalis* and *A. nigromaculis* have dealt with the variations in chemistry of the water rather than of the soil taken from the larval habitats. Chapman (1960) in Nevada reported that *A. dorsalis* were present in water ranging in pH from 6.0 to 10.1, with a chloride range of 14 to 42,778 p.p.m. (0.4 to 1208 meq.), while *A. nigromaculis* were found inhabiting waters with a pH from 4.7 to 9.2 and 18 to 391 p.p.m. (0.5 to 11 meq.) chloride ion.

Kleiwer *et al.*, (1964) in working with temperature and salinity effects found that *A. nigromaculis* occurred in water ranging in chloride content from 12 to 260 mg./liter (= p.p.m.) while *A. melanimon* larvae occurred in waters ranging from 12 to 1485 mg./liter.

It is reasonable to conclude that if a factor, or a combination of factors, has an attracting or repelling effect on ovipositing females of these species, this

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factor or combination of factors must be present in the soil of the oviposition site on which the eggs are deposited. This conclusion was also stated by Knight (1965) in his work with soil chemistries underlying breeding sites of *Aedes sollicitans* and *Aedes taeniorhynchus*.

The primary objective of this study was to determine if there were any differences in the salinity of the soils of the oviposition sites selected by these two species and if so, which ions appear to be the determining factor in this selection. The secondary objective was to determine if there were distinguishable differences in the salinity of the water taken from the larval habitats of these two species.

MATERIALS AND METHODS. Samples were collected during this study from Salt Lake, Davis and Tooele counties. The majority of the collections were made in northern Salt Lake and southern Davis counties from larval sites in (1) marshes bordering the Great Salt Lake, and (2) pastures, alfalfa fields and associated areas adjacent to these marshes. The presence of larvae was used as evidence that the site had been selected by the females of the given species for oviposition. Larval samples were taken from each location following each successive flooding in an attempt to determine if any changes occurred in the species inhabiting a given site. When possible, fifty or more larvae were collected from each site and all specimens were identified to species in the laboratory.

The soil samples were taken at random from areas known to produce large numbers of both species and from those known to produce only *A. dorsalis*. The samples were analyzed and compared using soluble salt content. Soil samples were obtained by removing the vegetation and then taking the top half-inch of soil from a square-foot plot. The samples were placed in plastic bags and stored until chemical analysis could be made. The water samples were taken only when fourth instar larvae or pupae were present.

Soil samples were allowed to dry at room temperature and then sifted through three screens with respective openings of 16, 64, and 196 per square inch. This produced homogeneous, finely divided samples free of most extraneous debris. From each sample a sufficient amount to weigh 100 grams dry weight was placed in porcelain evaporating dishes and oven-dried for 24 hours at 105° C. to 115° C. After cooling, 100 grams of dry weight soil from each sample were placed into 500 ml. ground glass stoppered flasks. Two hundred milliliters of distilled water were added to each flask at room temperature. The flasks were agitated for two minutes and allowed to stand the remainder of a 30-minute period. The process was repeated five times for each sample; thus at the end of 2½ hours each sample had been leached by one liter of distilled water. The extraction water was allowed to stand in closed containers until the solution became clear. Following clearing, samples were decanted and the clear solution filtered to remove floating and suspended materials.

The first test run on each sample was made to determine the specific electrical conductance. Results of this test are indicative of the total concentration of the ionized constituents in the sample. It is closely related to the sum of the cations or anions present in the sample and usually correlates closely with the total dissolved solids. This test enabled estimates to be made on the ion concentrations in the solution. These governed the preparation of proper dilutions to be used in the analysis of other samples which saved time and large quantities of chemical reagents.

The electrical conductivity was measured by a "Rd Solu Bridge" conductivity meter and was read directly in micromhos per centimeter at 25° C. ($EC \times 10^6$).

Hydrogen ion concentration (pH), carbonates (CO_3) and bicarbonates (HCO_3) were determined by an electric pH meter and titration with N/50 sulfuric acid. Results were determined in milliequivalents per liter for carbonates and bicarbonates. Calcium-magnesium hardness, calcium ion and magnesium ions were determined by the ethylenediaminetetracetate (versenate) titration procedure and determined directly in milliequivalents per liter. Sodium and potassium analyses were accomplished by flame spectrophotometry. Chloride determinations were made by silver nitrate titration and sulfates by the barium sulfate turbimetric method using a spectrophotometer. The results presented in parts per million (p.p.m.) were converted to milliequivalents per liter (meq.) by dividing the value in p.p.m. for a given ion by the equivalent weight of the ion. Nitrates, nitrites and phosphates were not determined for any of the samples.

Water samples were analyzed for conductivity, pH, chlorides and total calcium-magnesium hardness in a manner similar to that used on the soil.

RESULTS AND DISCUSSION. During this study 107 surface soil samples were analyzed from areas known to produce *A. dorsalis, A. nigromaculis* and *A. vexans* (Meigen). Ninety-seven of these samples were used in tabulating the results summarized in Table 1.

There was a high correlation between the variation in the concentration of salt in the soil of a habitat and the presence or absence of *A. nigromaculis*. The sodium and chloride ions appeared to exhibit the highest correlation between habitat preferences of *A. dorsalis* and *A. nigromaculis*. (See Figures 1 and 2.) In 63 habitats sampled in which *A. nigromaculis* larvae developed, the chloride ion content was from 0.1 to 10.7 meq. with an average of 1.9 meq. In 33 samples taken from areas known to produce *A. dorsalis* exclusive of *A. nigromaculis,* the chloride ion content ranged from 2.9 to 108.0 meq., except for one sample at 0.6 meq., with an average meq. content of 22.7.

The range of electrical conductivity in micromhos/cm. for habitats occupied by *A. nigromaculis* was 250 to 2700, with an average of 654, compared with "*A. dorsalis* only" habitats ranging from 566 to 14,000 with an average of 3151. Specific electrical conductance exhibited about the same correlation between breeding sites

TABLE 1.—Summary of chemical analysis of soil extracts.

		A. nigromaculis and *A. dorsalis*	*A. dorsalis* only	*A. vexans*
Specific Cond. (micromhos/cm)				
	range	250–2700	566–14,000	340–2400
	average	654	3151	980
pH	range	6.0–7.9	5.6–8.2	7.1–7.9
	average	...	...	...
Na^+	range	0.35–16.1	1.61–173.9	0.50–15.6
	average	2.85	26.7	4.44
K^+	range	0.31–2.70	0.46–6.90	0.23–1.30
	average	0.83	1.77	0.64
Ca^{++}	range	0.76–22.4	1.08–105.4	1.08–10.4
	average	3.89	14.6	4.14
Mg^{++}	range	0.04–8.25	0.08–19.6	0.08–9.32
	average	1.24	4.67	2.12
$SO_4^=$	range	0.17–41.6	2.12–229.0	0.42–18.1
	average	3.72	28.4	4.60
Cl^-	range	0.1–10.7	0.6–108.0	0.4–11.3
	average	1.9	22.7	3.4
No. of samples		63	33	9

Hydrogen ion concentration values (pH) ranged from pH 5.6 to 8.2. No correlation was detected between the pH and habitat preference for these three mosquitoes.

as did chloride ion values. Since electrical conductivity is commonly used for indicating the total concentrations of ionized constituents in solution, and is closely related to the sum of the cations, or anions,

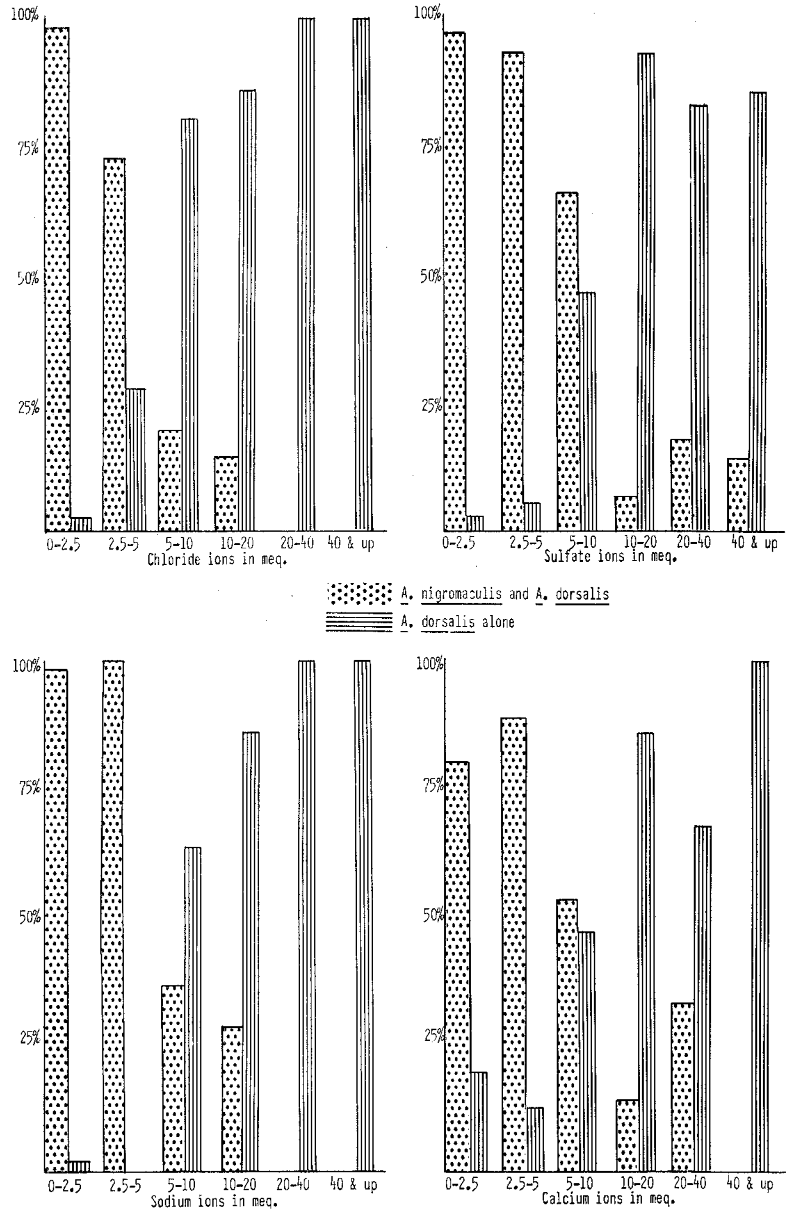

FIG. 1.—Comparison of habitats known to produce *A. nigromaculis* and to produce *A. dorsalis* only, at various ion concentrations for four inorganic ions.

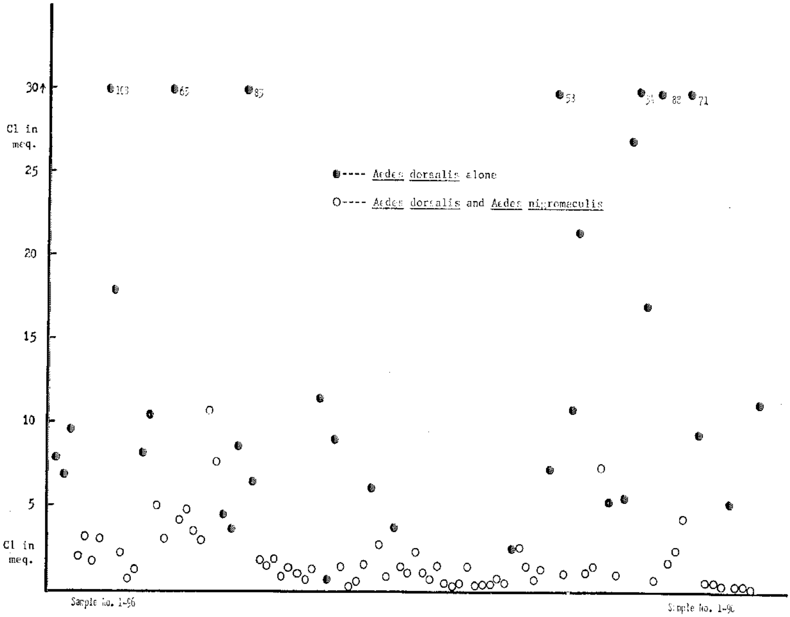

FIG. 2.—Chloride ion content in milliequivalents of soils from breeding sites of *A. dorsalis* and *A. nigromaculis*.

as determined chemically, the authors agree with Knight (1965), that the measurement of dissolved salts by specific electrical conductance is sufficient in itself to conduct salinity comparison studies between oviposition habitats of mosquitoes in studies of this type.

It was observed during this study that *A. nigromaculis* appeared to select soils with lower salinity for oviposition when these soils were readily available. As an example, in a 90-acre pasture known to produce large numbers of *A. nigromaculis*, there were two known areas which produced only *A. dorsalis*. The chloride ion content of soil samples taken from these two sites was 3.9 and 6.1 meq. Cl., which is within the upper limits of the range of *A. nigromaculis*. Samples from the pasture areas known to produce *A. nigromaculis* had concentrations of 1.0, 1.7 and 2.8 meq.

To determine if the variations in the salt concentrations between the two habitats were due to chance, a group comparison test and "t" distribution tests were made. All results were significant beyond the 0.001 level.

Only ten of the samples analyzed were known to be from habitats containing *A. vexans* larvae. All values for this species averaged higher than those for *A. nigromaculis* but lower than for *A. dorsalis*.

The correlation between salt content in surface soil samples of larval sites and the species inhabiting them is not as evident when soil samples are taken at a depth of 6 inches. Of 49 samples taken at this depth, 72 percent of the "*A. dorsalis* only" sites and 79 percent of the *A. nigromaculis* sites had conductivity readings of less than 500 micromhos/cm. These results emphasize the necessity of using surface soil samples only in determining salt concentrations.

Table 2 summarizes the results of 61 determinations made on water samples taken from breeding areas of these two species. As expected, some correlation existed between the salinities of the water in

TABLE 2.—Summary of chemical analysis of water samples.

		A. nigromaculis and *A. dorsalis*	*A. dorsalis* only	*A. vexans*
Specific Cond. (micromhos/cm)				
	range	380–7250	1500–24,000	500–4800
	average	1991	5751	2260
pH	range	7.2–8.7	7.7–9.1	7.7–9.1
	average	. . .	. . .	. . .
Mg+Ca	range	0.9–40.8	3.4–120.0	3.8–40.8
	average	11.3	30.2	12.3
Cl^-	range	0.4–62.0	6.0–164.0	0.6–62.0
	average	10.0	36.2	14.5
No. of samples		42	19	14

the breeding sites and the species of larvae inhabiting them. The correlation is not nearly so evident as that of soil extracts. This correlation in water salinity was probably due to some degree to the salt content of the soils over which the water sample was taken. The salinity of the water had a greater variance than the soil due to the chemical nature of the water which flooded the area, as well as the amount of water remaining in the breeding site at the time the samples are taken. Many of the marsh areas known to produce only *A. dorsalis* upon flooding, averaged water conductivities of about 2,000 micromhos/cm. (Rees *et al.,* 1965), which is only slightly higher than the average conductivity of the *A. nigromaculis* habitats sampled. It is evident that water salinity exerts a direct effect on the salt concentrations present in the soils of these breeding sites, but the salt content of the water in itself is obviously not the factor preventing *A. nigromaculis* larvae from appearing in the marsh areas.

The results of the analysis of the soil salinity and those of the water salinity can not be compared directly, as the values obtained for the soil salinity are at least ten times lower than the natural content of the soil in the oviposition site due to the dilution factor of the extraction process used in the analysis.

If a particular salt, or combination of salt concentrations, is the factor that limits *A. nigromaculis* to a habitat, then it is logical to assume that the inhibiting responses must be working on either the ovipositing females, or upon the maturing eggs, or on both. As limiting factors, it can be assumed that both are definite possibilities. Since ovipositing females of many species of mosquitoes have shown an ability to select oviposition sites according to variations in salt concentrations, this would appear to be the major limiting factor. It must not be ignored that numerous biological organisms do not exhibit complete "all or none" responses, therefore, it would seem that site selection by ovipositing females cannot be the sole solution to this marked habitat difference as some authors have indicated may be the case (Bates, 1940 and De Zuleta, 1950). Other factors such as variations in tolerance of eggs of different species to such factors as salt concentrations may be involved.

SUMMARY. A study was conducted in Utah from 1961 to 1965 inclusive to determine if there are distinguishable differences in the salinity of the soil upon which *A. dorsalis* and *A. nigromaculis* deposited their eggs. The study was made in the marshes bordering the Great Salt Lake and in irrigated lands adjacent to these marshes. During the study, the water and the soil beneath the water in which the larvae of these species were found were chemically analyzed for pH and specific ion concentrations.

There was no apparent correlation between pH and the presence or absence of the larvae in a given habitat. *A. nigromac-*

ulis was found to be much more restricted in its selection of breeding sites than *A. dorsalis,* with soil salinity apparently being the limiting factor. In the determinations made, sodium and chloride ion concentrations exhibited the highest degree of correlation between the presence or absence of *A. nigromaculis* in a particular breeding site. Ranges in chloride and sodium ions for areas known to produce only *A. dorsalis* were ten times higher than they were in areas containing *A. nigromaculis.* This correlation did not extend to any appreciable depth in the soil.

Some correlation exists between the salt concentration of the water and the presence of larvae, but these concentrations do not appear to be the factor limiting *A. nigromaculis* to its selected breeding habitat in the study area.

Selection preferences exhibited by ovipositing *A. nigromaculis* females to salt concentrations in the soil upon which the eggs are laid appear to be a major factor determining the habitat range of the larvae of this species; however, there appear to be other factors that may influence the larval distribution of this species.

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