ARTICLES

SOME PHYSICAL AND CHEMICAL CHARACTERISTICS OF COASTAL SOILS UNDERLYING MOSQUITO BREEDING AREAS

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The salt marsh mosquito species, *Aedes sollicitans* (Walker) and *A. taeniorhynchus* (Wiedemann), are characterized by the fact that their larvae develop in saline aquatic sites in the rather limited coastal area lying between mean high tide level and the extreme inland edge of the zone affected by sea water or its derivatives. Wherever larvae have been collected outside this zone, (for example, Barber 1939, Carpenter 1941, Rozeboom 1942, Fellton 1944, Ross 1947, Dixon 1955, and Horsfall 1956), they are nonetheless found to be developing in waters richer than normal in soluble inorganic salts.

It is reasonable to expect that the soils underlying these saline waters also have higher than normal amounts of soluble inorganic salts. Since these mosquito species in nature lay eggs only on moist soils, it can be inferred that a primary environmental factor attracting the ovipositing females must be either the presence in the soil of a certain minimal amount of one or more of the soluble inorganic salts, or the occurrence there of one or more factors found only in association with such salts.

Past efforts to elucidate this problem (reviewed by Clements 1963, 306), so far concerned with mosquito species other than *A. sollicitans* and *A. taeniorhynchus,* have been unsuccessful. When brought into the laboratory, such mosquitoes lay readily on non-saline oviposition substrata (in some cases even in preference to saline substrata) and furthermore are perfectly capable of accomplishing normal development in non-saline water.

Since a full knowledge of the factors influencing oviposition site selection is obviously important to mosquito control efforts, a field and laboratory study of this problem as it relates to *A. sollicitans* and *A. taeniorhynchus* has been instituted.

A preliminary phase of the study dealt with the determination of soil moisture levels required by these species for optimum oviposition and has been reported (Knight and Baker 1962).

Another portion of the study has been concerned with the characterization of the physical and chemical (inorganic) features of soils known either to have been used or rejected for oviposition by *A. sollicitans* and *A. taeniorhynchus.* The results of this portion of the work are reported here.

MATERIALS AND METHODS. All field work for this project was done in the environs of the inlet of New River, Camp Lejeune, North Carolina. The presence of larvae in a breeding site was used as evidence that the female had selected that site for oviposition.

Following the occurrence of rains sufficient to produce a brood of salt-marsh *Aedes,* a close watch on potential breeding areas was kept until larvae were large enough to permit easy discovery. At that time, larval and soil samples were collected

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from each distinct breeding site found. Additionally, to serve as controls, a number of mosquito-breeding areas lying adjacent to but outside the tidally-affected zone were also included in the survey.

In sampling for larvae, no records were taken until the first larva was found in a breeding site. Beginning with that dip, 10 dips were taken, and the total number of larvae and pupae captured was determined. All specimens collected in these 10 dips were brought into the laboratory and identified to species.

Soil samples were taken with a hollow metal cylinder and were 23 x 77 mm. Twenty of these cores were taken just above, along, and in the margin of the water of a breeding site so as to sample a cross section of the area in which the eggs hatched by the flooding might have been laid. These 20 soil cores were combined in a plastic bag and brought into the laboratory.

There, each soil sample was spread out on paper to become air-dry. When this had occurred, the samples were broken down with a wooden rolling pin and put through a sieve with round holes 2 mm. in diameter. Coarse material was worked over again with the rolling pin. Screening and crushing was continued until all aggregate particles were fine enough to pass the sieve, and only stones and heavier organic residues remained behind. The samples were then stored in cardboard cartons.

The first step in the soil analysis was the determination of the soil saturation moisture content. The soil saturation moisture content is defined "as the maximum amount of water held in the puddled soil without free water collecting in a depression made in the soil mass" (Jackson 1958, 240) and provides an estimate of the water-absorbing capacity of the soil. To make this determination a portion of the soil sample, usually about 200 gms., was placed in a 1000 ml. beaker. Distilled water was used, and the first half or two-thirds of the estimated needed amount was added down the side of the beaker. The soil was not disturbed during this process because water movement through puddled soil is very slow. Increments of water were added until the soil mass was rather fully wetted by capillarity, time being allowed for each increment to be fully absorbed before the next was added. The soil was then stirred with a glass rod, and more water was added to give the final adjustment of water content. The water content was considered right when the soil barely flowed together into a hole made with a stirring rod, the mixture slid off the rod, and the soil surface was wet enough to glisten; free water should not collect in the depressions on the surface when the sample is left for a few minutes. If free water stands on the surface, too much water has been used and just sufficient additional soil should be added to attain the desired end point. With practice, the characteristic saturation moisture content can be adequately reproduced. Greater accuracy can be obtained by deliberately going by the end point and then adding just sufficient soil to regain it. The soil saturation moisture content was determined in percent (weight of water/weight of wet soil) by taking 10 or 15 grams of this moistened soil, weighing it, oven-drying it to a constant weight, and determining the amount of water contained in the sample by subtracting the dry weight from the original saturated weight.

As a rough measure of the weight per unit volume (gms/ml) of the soil, the weight of the amount of soil that could be placed in a volume of 240 ml., settled by gently tapping the container against the table top, was determined.

The soil extract required for determination of soluble inorganic salts was obtained by first allowing the remainder of the saturated soil sample to equilibrate for 5 hours. Then, the sample was placed in a suitable size of buchner funnel on a tightly seated filter paper, and the filtrate was removed by suction. The collected sample was then centrifuged at 5000 r.p.m. for 30 minutes to remove as much turbidity as possible. The samples were stored in a refrigerator at 5° C. in polyethylene bottles until analysis could be accomplished.

Analyses for sodium and potassium were accomplished by emission spectrophotometry and, for chlorides, by silver nitrate titration. Calcium, magnesium, and sulfate were determined by use of a titrimetric procedure based on the Versene chelation of the excess barium remaining after barium sulfate precipitation (Jackson 1958, 263). Values were obtained in milliequivalents per liter (meq/l). Assuming that one gram equals one milliliter, these values can be converted into parts-per-million by multiplying by the equivalent weight of the involved ion. Organic matter was determined in terms of percentage of carbon oxidized by chromic acid with sulfuric acid heat of dilution. The procedure used was that of Peech, Alexander, Dean, and Reed (1947), except that end points were read by potentiometric methods rather than by colorimetric. The pH was determined for each extract with a Beckman Model G pH meter.

During the latter portion of the work, total soluble salts were also determined. This was accomplished by use of a conductance bridge (Type RC, Industrial Instruments, Inc., Jersey City 5, N. J.) and values were obtained in millimhos/centimeter (mmhos/cm). A linear relationship exists between the specific electrical conductance of a water extract of soils and the concentration of salts as found by analysis. The biological effects of salts are apparently more closely related to the equivalents of salts per million parts of solution than gravimetric weight units per million. However, using specific conductance figures, the latter can be estimated for waters, at least of alkaline soils, as follows (Jackson 1958, 245): p.p.m. of salts $= 640 \times$ mmhos/cm and percent salts in solution $= 0.064 \times$ mmhos/cm.

RESULTS. In all, 60 combined soil-mosquito larval collections were made. Because of progressive refinement, elaboration, or reduction of procedures, only 41 of the collections were sufficiently complete to permit adequate comparison of the observed factors.

The correlation between the concentrations of soluble inorganic salts occurring in dried and subsequently re-saturated soils and the mosquito species found breeding over these soils when flooded is shown in Table 1. It can be seen that *A. sollicitans*, *A. taeniorhynchus*, and *Anopheles bradleyi* (King) all occurred over soils rich in soluble inorganic salts. In each case, the collections containing these three species were from soils having at least intermittent contact with tidally-derived waters. Conversely, *Psorophora confinnis* (Lynch-Arribalzaga) was taken only from waters overlying soils with comparatively small amounts of the soluble inorganic salts, and in each case these breeding areas were well removed from direct contact with tidally-derived waters. *Aedes vexans* (Meigen) occurred over both non-saline (seven instances) and saline soils (four instances). In no case, however, was it found to occur abundantly in the saline soil areas.

Attempts to correlate the presence of the salt-marsh mosquito species with that of specific ions were unsuccessful. In general, all six considered ions varied in unison. Because of this, it would seem that a measure of total soluble salts would indicate adequately whether a soil was sufficiently saline to support the breeding of salt-marsh mosquito species. A fairly quantitative estimate of the total salt content of solutions extracted from soils can be made from their electrical conductance (Jackson 1958, 234). Accordingly, the specific conductance of each extract was determined (Table 2). In general, the results obtained were in full agreement with those secured through the more time-consuming process of analyzing for each ion separately. As can be seen from the pH data, most of the soil extracts were acid. No correlation between the pH and the salinity of the extracts was noted.

Incidental to the collection of the other data, the saturation moisture content, the organic matter content, and the weight per unit volume were determined for each soil sample. These relate directly to the water-holding capacity of the soil; since the lighter the soil, the more organic matter is present, and the more organic matter present, the more water that can be absorbed.

TABLE 1.—Milli-equivalents per liter of soluble inorganic salts extracts from soils underlying mosquito breeding areas.

Species	No. of Samples	Na^+ Ave.	Na^+ Range	K^+ Ave.	K^+ Range	Ca^{++} and Mg^{++} Ave.	Ca^{++} and Mg^{++} Range	Cl^- Ave.	Cl^- Range	$SO_4^=$ Ave.	$SO_4^=$ Range
Aedes sollicitans	22	217	13–503	5	1–11	81	11–171	200	10–765	60	4–285
Aedes taeniorhynchus	15	195	13–503	4	1–11	68	11–171	184	10–765	62	4–285
Anopheles bradleyi	6	283	123–503	6	3–9	112	62–164	246	123–407	71	36–113
Aedes vexans	11	21	2–119	1	0.5–2	10	2–42	21	0.2–126	5	2–11
Psorophora confinnis	11	2	1–7	0.7	0.5–1	5	2–14	1	0.2–6	5	2–14

TABLE 2.—Organic matter content, saturation moisture content, and weight per unit volume of soils underlying mosquito breeding areas; and specific conductance and pH of aqueous extracts from these same soils.

Species	No. of Samples	% Organic Matter Ave.	% Organic Matter Range	Saturation % Moisture Content Ave.	Saturation % Moisture Content Range	Weight (gms) per ml. Ave.	Weight (gms) per ml. Range	pH Ave.	pH Range	Specific Conductance (mmhos/cm) Ave.	Specific Conductance (mmhos/cm) Range
Aedes sollicitans	22	26	0.4–69	31	23–76	0.74	0.48–1.38	5.6	4.2–7.6	12.7	2.6–26.2
Aedes taeniorhynchus	15	21	0.4–69	35	7–76	0.88	0.29–1.38	6.0	4.6–7.6	11.8	2.6–26.2
Anopheles bradleyi	6	48	18–69	62	50–76	0.48	0.29–0.75	4.9	4.2–5.6	18.0	12.8–24.3
Aedes vexans	11	5	1–9	29	25–32	1.13	0.99–1.30	6.4	4.4–7.9	2.5	0.4–9.3
Psorophora confinnis	11	3	1–6	24	15–30	1.21	0.99–1.40	6.0	5.1–7.6	0.7	0.2–1.3

Since marsh soils would conceivably have a greater proportional volume of organic matter than non-marsh soils, it follows that the salt-marsh species (*Aedes sollicitans, A. taeniorhynchus,* and *Anopheles bradleyi*) would generally be associated with soils light in weight, rich in organic matter, and having a high saturation moisture content. This was borne out by the data (Table 2). Although no fresh-water marsh habitats were sampled, it seems obvious that they would not differ from salt marshes with regard to these particular factors.

Aedes mitchellae (Dyar) occurred in four collections, *A. canadensis* (Theobald) in two, and *Culex territans* (Walker) in one. These were all from fresh-water habitats, and the specific conductance of the corresponding soil extracts ranged from 0.4 to 0.7 mmhos/cm.

The abundance of larvae of *A. taeniorhynchus* and *A. sollicitans* in each breeding site was not correlated with the specific conductance of the soil extract from that site, large numbers of larvae being found associated with both low and high specific conductances. *Anopheles bradleyi* larvae were never found in more than relatively small numbers.

DISCUSSION. Except for Micks and McNeil (1963), no data have been published on the physical and chemical characteristics of coastal soils underlying known salt-marsh mosquito-breeding areas. In contrast, there are a number of references reporting analyses of water supporting the breeding of salt-marsh mosquitoes. The data from some of these are compared in Table 3 with the soil extract salinities reported by Micks and McNeil (1963) and by this paper. Although a wide range of variation exists, some measure of correlation can be seen.

In themselves, salinity figures for breeding area waters have little validity as indicators of sites selected by ovipositing females, since it is not unusual to have

TABLE 3.—Comparison of chloride concentrations (p.p.m.) of mosquito breeding waters with extracts from soils underlying breeding areas.

	Breeding area water					Extracts from breeding area soils	
Species	Cory and Crosthwait (1939)* Maryland	Vogt (1947) Maryland	Darsie and Springer (1957)* Delaware	Dixon (1957) Kentucky	Chapman 1959 New Jersey	Micks and McNeil 1963 Texas	Present project North Carolina
Aedes sollicitans	11,992		2,545 20,937 (11,992**)	9,400	3,543 44,128 (15,944)	7,384 22,791	355 27,127 (7,092)
Aedes taeniorhynchus	9,885						355 27,127 (6,525)
Anopheles bradleyi	9,064 (as *crucians*)	1,500 6,100 (3,383)	2,705 18,360 (12,562)		1,482 17,973 (9,019)		4,361 14,432 (8,723)
Aedes vexans			515 1,707 (999)		483 8,858 (3,028)		7 4,468 (745)

* Conversions from percent of sea water by multiplication by 32,210 (mean p.p.m. of chlorides in Atlantic Ocean, Darsie and Springer 1957, 43).
** Figures in parentheses are averages.

salt-marsh mosquito broods produced by tidal flooding, by rains, or by combinations of the two. As shown by Richards (1938), the salinities of waters found containing the larvae of *A. sollicitans* can vary from zero to nearly that of sea water. The salinity of extracted soil water should, however, provide a more reliable measure of the suitability of an area for producing salt-marsh mosquitoes, since this more nearly approximates the conditions existing at the time of the oviposition.

An unexpected finding in this study was the extremely low minimal level of soluble salts characterizing the soils of *A. sollicitans* and *A. taeniorhynchus* larval habitats. In terms of specific conductance, the minimal value obtained was 2.6 mmhos/cm (1664 p.p.m. total soluble salts). This is only very slightly above the maximum figure of 1.3 mmhos/cm (832 p.p.m. total soluble salts) obtained for a typical fresh water breeder, *P. confinnis*. Yet the slight difference between these two figures appears to have great biological significance for it quite distinctly separates the salt-marsh and fresh-water biotas.

A. vexans was found living in both these two major habitats but was never found numerically abundant in habitats having contact with seawater, i.e., with a specific conductance of 2.6 mmhos/cm or above.

SUMMARY. The characteristic larval habitat of *A. sollicitans* and *A. taeniorhynchus* is in the limited coastal area between mean high tide level and the extreme inland edge of the zone of brackish water. As part of an effort to understand this rather striking habitat restriction, soluble inorganic salt contents were determined for aqueous extracts taken from soils underlying breeding areas of these two species and compared with similar data for the fresh-water species *A. vexans* and *P. confinnis*. All soils from larval habitats of *A. sollicitans*, *A. taeniorhynchus*, and *Anopheles bradleyi* had total soluble salt contents of 1644 p.p.m. or more. On the other hand, *P. confinnis* larvae were never found in habitats having in excess of 832 p.p.m. of total soluble salts. *A. vexans*, although abundant only in fresh-water habitats (below 1,000 p.p.m.), was present in habitats having as much as 5,952 p.p.m. of total soluble salts.

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