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EFFECTS OF SALINITY ON *ANOPHELES ALBIMANUS* OVIPOSITIONAL BEHAVIOR, IMMATURE DEVELOPMENT, AND POPULATION DYNAMICS

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ABSTRACT. Laboratory tests showed that 2 strains of *Anopheles albimanus* Wiedemann. when given a choice of oviposition medium of 0, 25, 50, or 100% sea water, laid ca. 40% of their eggs in fresh water, but laid ca. 10% in pure sea water. Subsequent laboratory rearing showed that higher salt content of the rearing medium definitely reduced the development and survival of both strains in the immature stages, but 1 strain produced pupae in water with as high as 15.075 parts per thousand (ppt) salt. The salinity of the medium did not significantly affect percentage emergence in either strain of those insects that reached the pupal stage. Since high salinity affects larval development in the laboratory, we could expect the same to be true in estuarine habitats. However, field observations suggest that breeding in brackish water probably is associated with the presence of favorable breeding sites in areas with much vegetation, rather than with the reduced salinity of the water.

INTRODUCTION

Many species of mosquito are capable of larval development in salt water, and several species prefer oviposition sites that contain relatively high levels of salt (Wallis 1955). In general, these species inhabit estuaries or salt marshes near sea coasts, salt lakes, or other inland bodies of salt water. Other species, such as *Anopheles albimanus* Wiedemann, typically are fresh water breeders, but can tolerate various levels of salt (Nicolson 1972), and thus can be found in salty or brackish water. *An. albimanus* is the primary vector of malaria in El Salvador, Central America, and high populations of this species occur there, especially along the Pacific coastal plain. The larval habitat of *An. albimanus* is extremely varied, but generally it is fresh water, e.g., river pools separated from the main river current, within river currents where vegetation is present, flooded fields, marshes, or in irrigation or

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drainage ditches. However, it may also be found in estuaries that are periodically flooded with salt water from tidal action.

During 1969–70 scientists at the Central America Research Station (CARS) Laboratory studied the presence of *An. albimanus* larvae in the San Diego estuary in El Salvador in relation to salt content of the water during different times of the year (Central America Malaria Research Station Report, 1970). During the dry season in El Salvador, water flow in all the rivers is greatly diminished, and many of them become completely dry. The estuaries then become closed to the Pacific Ocean by sand deposited by wave action from the sea. The CARS study showed that from July 1969 to June 1970 *An. albimanus* larvae were present in the San Diego estuary only during March, April, May, and June, when the estuary was closed. The average salt content of the estuary during that time was 12.35±8.00 parts per thousand (ppt) and larvae were found in water with a range of 0.2-23.6 ppt. During the other 8 months larvae were absent. During 7 of those months the average salinity was high (23.36±6.3 ppt), but in many water samples it was well within the range at which larvae were collected. Salinity for the other month (September 1969) averaged only 4.5±2.15 ppt, but all larval collections were negative. The CARS researchers concluded that the absence of larvae during the wet season was due to the constant flushing of the estuary with sea water by tidal action. However, the salinity during this time (mean = 21.05±7.1 ppt) was not statistically different ($P = 0.05$) from that observed for the closed estuary.

The combined observations presented a question as to whether the presence of larvae was limited by salt content or by other factors. Thus, we designed a laboratory experiment to determine the selectiveness of *An. albimanus* females to oviposit and the ability of immature stages of this species to develop in various concentrations of sea water. We also assessed the breeding habits in a closed estuary containing brackish water and the effects of draining the brackish water.

METHODS AND MATERIALS

Sea water was collected from the Pacific Ocean near La Libertad, El Salvador, from a pier extending ca. 200 meters from the shoreline, and brought to the laboratory in 19 liter glass carboys. The salt content of the sea water (ppt) was determined by density measurements after correcting for temperature. The sea water used in this experiment, with a specific gravity of 1.025, was calculated to contain 33.5 ppt of salt. Dilutions were then made of the sea water with fresh water and various concentrations were used as oviposition media, or as water for rearing larvae of *An. albimanus;* salt content of the dilutions was calculated in ppt.

Two strains of *An. albimanus,* designated MACHO and CAMPO, were tested. The MACHO strain was being mass reared to supply sterile males for a study of the effects of releasing sterile males of *An. albimanus* on the natural populations in a coastal area of El Salvador. MACHO was developed from stock that had been reared in the laboratory for more than 4 years. The CAMPO strain had been recently colonized from adults collected throughout the sterile-male study area, and was in the F_3 generation when tested.

LABORATORY OVIPOSITION TESTS. To determine whether the oviposition rate of *An. albimanus* females was a function of salt content, we measured 250 ml samples of sea water at 4 percentages (0, 25, 50, and 100%) into plastic pans (15 cm diameter and 8 cm deep). Then 4 pans, each containing a different dilution, were randomly placed in each of 4 MACHO and 4 CAMPO colony cages (61 x 61 x 61 cm), each of which contained ca. 10,000 adults. The next day the pans were removed from the cages, and the contents were poured through standard window screen to remove dead adults and other debris. The strained water was then held for 24 hr. The eggs from each pan were then dried (Dame et al. 1978) and measured volumetrically to determine egg production. The test was replicated 4 times on different days.

Laboratory Rearing Tests. Twenty concentrations from 0 to 100% sea water (0 to 33.5 ppt salt) were tested to determine how egg hatch, larval development and adult survival of *An. albimanus* varied as a function of salt concentration.

Each of the 20 concentrations of sea water was placed in a white plastic rearing tray (56 x 43 x 7.5 cm high), with a total of 3 liters of water per tray. The trays were placed on shelves equipped with controlled electrical heating tapes (Dame et al. 1978) for 24 hr for the water temperature to equilibrate at 29±0.5°C. At the same time the sea water dilutions were made, eggs that had been collected from colony cages and incubated for 24 hr at 25±0.5°C in the various concentrations of sea water were dried and volumetrically measured. From the CAMPO strain 0.085-ml samples of eggs were used, but the MACHO strain was 50% sterile, due to genetic alteration (Seawright et al. 1978) so 0.17-ml samples were required. These samples were placed in separate styrofoam plastic cups containing appropriate dilutions of sea water and 1.5 ml of a 2% liver and yeast suspension (1:1). These were held for 24 hr at 29±0.5°C for the eggs to hatch. Then a 100-egg sample from each hatch was examined microscopically to determine hatch. Each hatch cup was emptied into a rearing tray containing the same concentration of sea water as the hatch cup. The larvae were then reared inside plastic enclosures to minimize evaporation, according to the techniques of Bailey et al. (1980a). The larvae remaining after 2 pupal harvests on day 6 and 7 were counted and discarded. The pupae were counted, held for 48 hr, and the percentage of adult emergence was recorded. The test was replicated 4 times on different days.

Field Breeding Studies. In another experiment, we determined the effects of draining an estuary after it had been closed to the sea and high *An. albimanus* populations had appeared. The mouth of the Ticuiziapa estuary closed to the Pacific Ocean due to tidal action shortly after the cessation of heavy rains in mid-October of 1978. The San Antonio river, which empties into the Ticuiziapa estuary, although diminished, was still flowing and the estuary soon began to flood the surrounding pastures. Adult collections in 2 calf-baited traps and 2 stables near the estuary and larval collections from the estuary indicated that the population of *An. albimanus* was steadily rising.

The estuary was opened by bulldozer on January 6, 1979. It remained open for less than 48 hr, but the water level receded to the natural boundaries of the estuary, and the adjacent flooded areas dried. To insure that the estuary water level would remain low in the ensuing months, we reopened the estuary to the ocean with a bulldozer daily for 5 days. The estuary was opened in the morning following high tide and closed in the evening before the next high tide. This procedure allowed drainage for ca. 9 hr per day and prohibited salt water intrusion.

RESULTS AND DISCUSSION

Oviposition Tests. The regressions of sea water concentration (ppt) on volume of eggs recovered (Fig. 1a) indicate that oviposition may be deterred in media with high salt levels but can occur in 100% sea water. When females were offered the

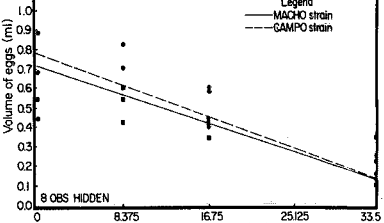

Figure 1a. Oviposition rates into various concentrations of sea water.

different concentrations of sea water ca. 40% of all eggs were recovered from fresh water, ca. 20% from 16.8 ppt salt, and ca. 10% from 33.5 ppt salt (100% sea water). The regression lines for the 2 strains were not significantly different (P = 0.05).

Eggs were deposited in concentrations of salt that were higher than many of those samples taken in the CARS study during July to February when no *An. albimanus* larvae were found in the San Diego estuary. The fact that this species did oviposit in 100% sea water when lower concentrations also were available does not support the notion that estuarine breeding is limited by salt content of the water.

Rearing Tests. High salt content of the rearing media definitely affected development of immature stages of both strains of *An. albimanus* tested; the CAMPO strain was affected more adversely by increased salt concentrations than the MACHO strain. All of the regressions presented in Fig. 1b-1d are significantly different (P<0.001), but not those in Fig. 1e.

Fig. 1b presents egg hatch as a function of salt water concentration of the rearing media for the 2 strains. Because the genetic alterations to the MACHO strain involved a sex-linked, propoxur-resistant gene that allowed exclusive production of males, the expected hatch was 50% in fresh water. The ca. 60% hatch for the CAMPO strain in fresh water was typical of newly colonized *An. albimanus* from El

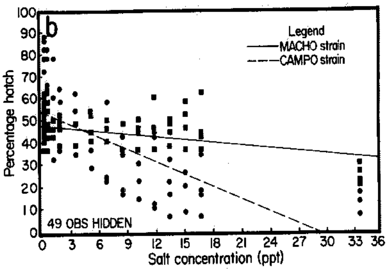

Figure 1b. Percentage hatch as a function of salt concentration in the incubation media.

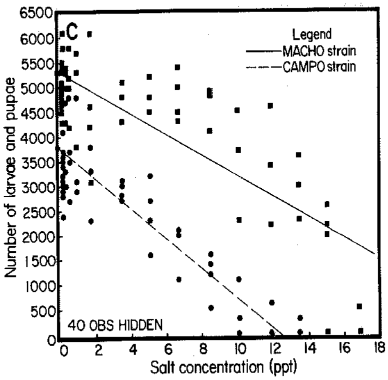

Figure 1c. Number of larvae and pupae surviving after 7 days.

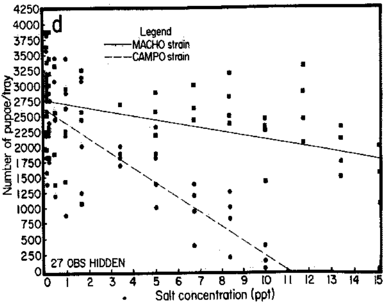

Figure 1d. Number of pupae produced per tray.

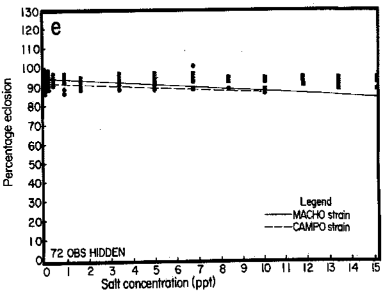

Figure 1e. Percentage adult eclosion.

*"OBS HIDDEN" refer to observations falling on the same point as another observation.

Salvador (Bailey et al. 1980b). In MACHO, hatch was not significantly affected until the salt concentration was 33.5 ppt; at this level, hatch was ca. 50% of that in fresh water. For CAMPO, hatch was markedly depressed at the higher concentrations; levels were only 18.3 and 12.5% at 13.4 and 33.5 ppt, respectively. The slopes of the 2 lines in Fig. 1b are significantly different ($P<0.0001$).

Because our colonization procedures tend to select a strain amenable to tray rearing, the MACHO strain, as expected, produced more larvae and pupae per tray (at 7 days posthatch) than the newly colonized CAMPO strain (Fig. 1c). Salt concentrations of 16.75 and 13.9 ppt were the highest that still allowed larval growth for the MACHO and CAMPO strains, respectively. (Salt concentrations above these levels are not shown in the figures because the larvae did not survive at the higher levels.) The slopes of the 2 lines were statistically different ($P<0.05$). A 2nd measure of the variation of immature survival and development as a function of sea water concentration was the total number of pupae produced per tray (Fig. 1d). Again, both strains were adversely affected by increasing salt but pupal production declined at a faster rate for CAMPO than for MACHO ($P<0.001$). CAMPO failed to produce any pupae above 10.025 ppt while MACHO produced pupae in water with as high as 15.075 ppt salt.

Increasing salt concentrations did not significantly reduce an adult's chances of emergence once pupation occurred (Fig. 1e). Adult emergence for the 2 strains was ca. 90% or higher from all salt concentrations in which pupation occurred.

The difference in salt tolerance between the 2 strains is difficult to explain. The CAMPO strain was probably more heterogenous in terms of genetic variability, and its ability to utilize different breeding habitats, including brackish water, indicates a high degree of adaptability. Although it is not known whether this ability to breed in different habitats is due to simple Mendelian inheritable traits, it would certainly make an interesting study. Despite the presence of a heterogenous genome, wild *An. albimanus* have been difficult to colonize, and the CAMPO strain was certainly not well conditioned to our laboratory rearing procedures (Bailey et al. 1980b). On the other hand, the MACHO strain had been selected for successful laboratory rearing for more than 4 years. The rearing water was chlorinated with sodium hypochlorite, although the degree of chlorination varied throughout the year, and it is possible that we were selecting a strain that would do well in association with the residual sodium chloride left in the rearing trays. This would help explain the adaptiveness to sea water. Or, if specific genes were involved, they could have been inadvertently selected for our overall rearing procedures, or included in the chromosomal inversion that is a part of the genetic sexing mechanism of MACHO.

The results of this study demonstrated that *An. albimanus* can be reared in relatively high concentrations of sea water in the laboratory. Other workers (Central America Malaria Research Station Report, 1970) reported the presence of larvae of this species in even higher concentrations (23-24 ppt) of salt in natural habitat, but did not prove survival to the adult stage. This might help explain why in estuaries in El Salvador higher densities of *An. albimanus* larvae are found when the estuaries are closed than when they are open to the sea.

In the estuaries salt content is highest immediately before the estuary is closed, as shown by the CARS study. Because of the diminished river flow and the more or less constant influx of sea water into the estuaries the salinity increases until they are finally closed by sand deposits. When sea water ceases to enter the estuaries, the salt content begins to be diluted by the diminished, but continual, flow of fresh river water. As the estuaries fill, extensive flooding of surrounding grassland and mangrove occurs, providing excellent larval habitat for *An. albimanus.* Bailey et al. (1980c) showed that the larval population was much lower at the beginning (November) than it was toward the end (February) of the dry season. Early in the dry season water in the estuaries is not only moderately saline, but also is confined to definite boundaries, and contains little vegetation. Later, with the flooding of grassland, water around the periphery of the estuaries contains extensive vegetation, thus providing more attractive oviposition sites for this species. This condition is prevalent for the San Diego estuary only during the dry season. Three rivers flow into this estuary, and it remains open several months following the cessation of rains. After the mouth does close, flooding soon occurs and continues until the onset of rains. However, 2 such floodings occur in the Toluca and Ticuiziapa estuaries. These estuaries close to the ocean much sooner than the San Diego estuary, flood into the surrounding pastures, slowly dry up as the dry season continues, and then flood again at the initiation of the rainy season. As might be expected, this results in 2 seasonal population peaks in these estuaries. Adult collections confirm these observations for the San Diego estuary (Breeland 1972) and the Toluca and Ticuiziapa estuaries (Hobbs 1973). Therefore, the estuaries are generally void of larvae during much of the rainy season (March–October) because of the flushing effect of heavy rains, and because of a general lack of standing water in areas of heavy vegetation near the estuaries during this time, and not necessarily because of the salt content of the estuaries.

The effect of draining the Ticuiziapa estuary on the adult and larval collections of *An. albimanus* is clearly demonstrated in Table 1. The estuary was opened at the end of wk 1 and again during wk 2, and adult collections were clearly reduced in the wk following the openings (wk 3) in the calf-traps (#1 and #2) and in the 2 stables (El Coco and Chansenora). The adult collections used for controls were from a calf-trap (#3) and a stable (Santa Lucia) 5 km and 13 km, respectively, from the Ticuiziapa estuary. At the time of the opening, other estuaries in the area were closed, and breeding persisted around them. However, as is shown in the

Table 1. Trends in population densities of adult and larval *An. albimanus* before and after draining an estuary to remove potential mosquito breeding habitats (the estuary was drained during wks 1 and 2 of 1979).

	Population estimates at indicated wk in 1978–79							
Location	51	52	1	2	3	4	5	6
	Adults from calf-traps[a]							
#1	247	76	128	57	20	4	5	4
#2	545	251	204	117	97	7	13	17
#3 (control)[d]	22	9	52	24	157	75	186	140
	Adults from stables[b]							
El Coco	96	71	35	56	27	12	5	5
Chanesenora	52	36	14	16	12	9	2	1
Santa Lucia (control)[d]	66	257	231	564	475	370	332	272
	Larvae from estuary[c]							
Ticuiziapa estuary	130	45	40	55	0	15	0	0

[a] No. adult females/trap-night.

[b] No. adult females/man-hr.

[c] No. third- and fourth-instar larvae/100 dips.

[d] Controls were located more than 5 km from the Ticuiziapa estuary.

table, larval collections near Ticuiziapa estuary declined sharply during wk 3 and remained low through wk 6. Thus, draining the Ticuiziapa estuary caused a definite reduction in the population of *An. albimanus* in the area at a time when the salt content of water in the estuary should have remained relatively constant. Also, the laboratory data showed that *An. albimanus* are able to survive and develop in water with salt content as high as would be expected in the estuary during that time. Thus, we think that the population was reduced because the water in the estuary receded to its normal boundaries, eliminating flooding in areas with extensive vegetation. Possibly under these ecological conditions the larvae do tolerate the existing level of salinity, and the population thrives only if adequate protective habitat is abundant.

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