

a 62160



WHO/Mal/322 ✓
7 December 1961

ORIGINAL: ENGLISH

BIONOMICS OF SALT-WATER ANOPHELES GAMBIAE
IN EAST AFRICA

by

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Introduction

The breeding of Anopheles gambiae in salt-water in East Africa has been reported by several authors. Gebert (1936) recorded their presence in Mauritius, while Wilson (1936) & Mackay (1938) found it breeding in brackish water around Dar es Salaam, but recorded the form with the extra dark band on the palps as A. gambiae var melas. Jepson et al. (1947) confirmed Gebert's findings but reported that all melanic larvae gave typical three-banded A. gambiae adults, whereas Halcrow (1957) found that 92% of the adults bred from larvae possessed an additional dark band on the palps. That melanism was not always associated with salt-water was shown by Gibbins (1936) in Uganda. Salt-water A. gambiae has also been found on the East Coast as far south as Natal (Muspratt, quoted by de Meillon, 1947) and at Pebane, Mozambique, in 1938 (de Meillon, personal communication). Muirhead-Thomson (1951) in a study of salt-water A. gambiae in Dar es Salaam has shown that these salt-water forms were not the same as A. melas found in West Africa. More recently, Maffi (1960) recorded salt-water A. gambiae in Somalia.

The object of this paper is to collate the available information on the bionomics of salt-water A. gambiae in East Africa and to present results obtained in our studies in Pemba Island.

Identification of salt-water A. gambiae

The fact that females bred from salt-water A. gambiae larvae in East Africa may either possess three-banded palps (as in typical A. gambiae) or four-banded ones (as in A. melas in West Africa) has, as just mentioned, been observed previously

by several workers. While the four-banded forms could easily be distinguished from fresh water A. gambiae, the three-banded adults are identical with the latter in all morphological characters. A similar situation exists in West Africa with regard to A. melas, but there the three-banded A. melas can be distinguished from A. gambiae to a certain degree by morphological differences in egg (Muirhead-Thomson, 1945) and larval characters (Ribbands, 1944), and in differences in the physiological reactions of larvae of the two forms to saline water (Ribbands, loc. cit.). It was on these differences that the two authors raised A. melas to specific status. How far these differences, both morphological and physiological, are constant to enable retention of A. melas as a distinct species has been questioned by Bruce-Chwatt (1945) & Fox (1958). In East Africa, on the other hand, although the four-banded adults of salt-water A. gambiae could be distinguished from fresh water A. gambiae, Muirhead-Thomson (1951) has shown that there is no morphological character by which the three-banded salt-water A. gambiae could be distinguished from the fresh water form either in the egg, larval or adult stage. He then employed the physiological test as a means of identifying the two forms. By this test eggs from A. gambiae adults were put into separate bowls of fresh water and after hatching, the first stage larvae were transferred to another bowl containing 75% sea-water. Larvae of fresh water A. gambiae died within two hours while those of salt-water A. gambiae survived for at least six hours.

During our investigations, in an area in Zanzibar where A. gambiae larvae were found breeding in large numbers in fresh water, adults were caught in houses near by and isolated for egg laying. 226 egg batches from these adults were bred out and the first stage larvae tested to 75% sea-water. It was observed that the majority of the larvae died within an hour but in all cases none survived for more than 1-1/2 hours. Similar tests were carried out in an area in the southern part of Pemba Island where larvae of A. gambiae were found breeding in lakes into which sea-water entered during the higher spring tides. First stage larvae from 1551 egg batches (1307 from three-banded adults and 244 from four-banded forms) were tested with 75% sea-water and it was noticed that all survived for six hours and sometimes even for twenty-four hours. From these results it was considered that all, or nearly all, A. gambiae present in this area were of the salt-water form. From these two sets of tests it will be seen that the difference in the reaction of first stage

larvae of the two forms to 75% sea-water is quite distinct and it appears that, at present, the only method of identifying the two forms of A. gambiae in East Africa is by this physiological method. It is still possible, however, that a detailed study of egg, larval and adult characters may reveal some morphological differences.

Halcrow (1957) has named the salt-water form of A. gambiae from Mauritius A. gambiae subspecies litoralis mainly by reason of its physiological differences from fresh water A. gambiae. How far this would be justifiable is open to question. Moreover the name litoralis has already been designated to another anopheline from the Philippines (King, 1932).

House catches

Working in Dar es Salaam, Muirhead-Thomson (1951) observed that salt-water A. gambiae was the dominant form during the dry months of September 1947 and August 1948, but the reverse was the case during the wet months of May and June 1948 when the fresh water form was dominant. Using experimental huts fitted with window traps, he also studied the proportion of females of the two forms leaving a hut after feeding. He found that fresh water A. gambiae showed little tendency to leave the hut after feeding, only about 2% being found in the window traps, as compared with 35-40% of the salt-water form.

Personal observations during the preparatory phase (May - December 1958) of the Malaria Eradication Project now in operation in Pemba Island, showed A. gambiae in large numbers in houses (13 different catching stations) treated in rice-growing areas. Unfortunately no work was carried out on the proportions of the two forms of A. gambiae as the possible presence of the salt-water form did not then occur to us. The average monthly day-resting density per hut of A. gambiae from these catching stations together with the sporozoite rates for this period is furnished in Table 1.

TABLE 1. A. GAMBIAE: AVERAGE MONTHLY DENSITY
PER HUT AND SPOROZOITE RATES FROM CATCHING STATIONS
FOR THE PERIOD MAY - DECEMBER 1958

	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Average density per hut	38.2	29.7	6.3	2.6	0.7	0.8	1.2	9.4
Sporozoite rate (%)	10.5	4.9	7.6	3.5	7.0	7.6	5.4	1.6

Residual spraying of catching stations was begun in December 1958 and by the middle of February 1959 all catching stations in the island had been sprayed. These stations have since been residually treated twice at intervals of 9-10 months. The insecticide used was dieldrin 50% w.d.p. at a nozzle dosage of 80 mg/sq.ft. (0.8 g/m^2). No adult of A. gambiae has been caught in any of the stations since the first spraying cycle. Outdoor resting and night outdoor biting catches have been carried out but with negative results. On the other hand, outdoor resting and night biting catches in the salt-water areas revealed large numbers of adult A. gambiae. Salinity tests on first-stage larvae bred out from eggs obtained from these adults showed that they were entirely of the salt-water form. As will be seen later, salivary gland dissections failed to reveal a single positive. It is believed possible from these findings that the fresh water form of A. gambiae has been eradicated from Pemba Island, and the existing A. gambiae population appears to consist only of the salt-water form.

House catches in the salt-water areas of Pemba have shown that salt-water A. gambiae enter houses as early as 14 days after residual treatment, and in May 1960, four months after residual treatment, 107 adults were caught in 35 huts. Such adults, however, when hand caught do not survive to deposit eggs, indicating that they are, as will be seen later, still susceptible to dieldrin. Experimental huts made of coconut thatch and each fitted with a window trap were constructed to study the movement of salt-water A. gambiae in and out of houses. Results obtained indicate that nearly 45% of adult A. gambiae leaving unsprayed huts in the morning are blood fed, which is similar to the results obtained by Muirhead-Thomson in Dar es Salaam.

Role in malaria transmission

Very little is known about the role of salt-water A. gambiae in malaria transmission. Mackay (1938) mentioned that no "var. melas" adults were found infected in Dar es Salaam, whereas Muirhead-Thomson (1951) records sporozoite rates of 0.8% and 9.4% in the salt-water and fresh water forms of A. gambiae respectively in a village near Dar es Salaam where both forms occurred together in the same houses for several months of the year.

In the area of Pemba where salt-water A. gambiae is prolific, 1775 salivary gland dissections failed to reveal a single positive (Table 2). It should be noted here that the area had been residually treated with dieldrin twice before. That these

A. gambiae were of the salt-water form was shown by physiological tests of their first stage larvae to 75% sea-water. The mosquitos used for these dissections were caught in houses, resting outdoors or attempting to bite man out of doors at night.

TABLE 2. RESULTS OF SALIVARY GLAND
DISSECTIONS OF SALT-WATER A. GAMBIAE

Type of habitat	No. examined	No. positive
Inside houses	535	0
Outdoor resting	636	0
Biting man at night	604	0

In a malaria parasite survey carried out in this area in June 1960, no infant under one year of age was found with malaria parasites in 86 blood examinations (Stoker and Shute, personal communication).

Outdoor catches

Information available on the outdoor resting habits of salt-water A. gambiae in East Africa is scanty. The only previous record is that of Muirhead-Thomson (1951) from Dar es Salaam where he found four blood fed and two gravid females resting in a large pit.

Our searches in the salt-water areas of Pemba have revealed a large outdoor resting population. Adults have been found resting (i) at the base of heavily shaded mangrove and mango trees; (ii) on the pneumatophoric stalks of Avicennia; (iii) on shaded termite mounds; (iv) under coralline rocks; (v) under fallen leaves and (vi) in crab holes. During the period September 1959 - September 1960, 5963 adult females were collected. These adults were caught with great ease and during the period of heavy rains between April and June it was quite possible for an experienced mosquito searcher to collect as many as 50 adults in an hour. Of these, 1754 were classified according to their abdominal stages and the results showed that 25% were unfed, 63.9% were blood fed and 11.1% were gravid. The fact that these catches were carried out in natural shelters situated near houses and the presence of cattle tethered outside at night would explain the high incidence of freshly fed females. On the other hand, if collections had been carried out in areas situated

away from both houses and cattle and nearer the breeding sites, an entirely different picture would probably have been obtained with the unfed and gravid predominating as was shown by Gillies (1954) with fresh water A. gambiae.

Results of precipitin tests

Results of precipitin tests on A. gambiae caught in the salt-water areas of Pemba are presented in Table 3.

TABLE 3. RESULTS OF PRECIPITIN TESTS
OF A. GAMBIAE OF THE SALT-WATER AREA, PEMBA

Resting place	Man	Bovid	Sheep/ goat	Dog	Other mammal	Negative	Total tested
Sleeping quarters	59.2%	19.7%	0.7%	2.1%	12.2%	6.1%	147
Outdoor resting	1.6%	71.4%	0.2%	0.2%	11.9%	14.7%	872

It will be seen that 59.2% of those caught in houses were positive for man. On the other hand if it is considered that the outdoor resting population would give a truer picture of the feeding habits of a species and where the bias of an indoor collection is eliminated, it will be seen that only 1.6% had fed on human blood, while 71.4% had fed on cattle. If the results obtained from the experimental huts are taken into consideration, one would expect a higher percentage positive for human blood than that obtained. As noted above, in the areas where these studies were carried out, large numbers of cattle are kept outside houses at night and the high incidence of females showing a positive reaction for cattle blood may be regarded as falling into the group described by Gillies (1956) as exhibiting facultative exophily. It is considered probable that a better method of sampling combined with other experimental techniques may give more precise information on the feeding preferences of this form of A. gambiae.

Susceptibility tests

Susceptibility tests using the WHO test kit were carried out with adults of A. gambiae caught in salt-water areas of Pemba which had been residually treated with dieldrin thrice before. Adults were exposed for an hour to 0.4% dieldrin impregnated papers supplied by WHO. Results show that the salt-water form of A. gambiae is still susceptible to dieldrin (Table 4).

TABLE 4. SUSCEPTIBILITY TESTS ON ADULT
A. GAMBIAE FROM SALT-WATER AREAS OF PEMBA

Concentration	No. tested	No. dead after 24 hours	Mortality (%)
0.4% Dieldrin	549	549	100
Control	394	75	19

Larval habitat and water salinity

In East Africa, larvae of salt-water A. gambiae have been found mainly in pools which are formed in the interior by the high spring tides and which are constantly diluted by rainfall or seepage from the land. These pools are usually fringed with Paspalum grass and slight growth of Avicennia. It has been noticed that dense growth of Avicennia and Rhizophora is not very conducive to larval breeding.

Almost all previous workers studying the salt-water form of A. gambiae in East Africa have, quite understandably, dealt with the salinity of the water sample where larvae of this form have been found. Gebert (1936) found that larvae in Mauritius underwent complete development in water which at the beginning of the experiments contained 24.55 g NaCl per litre (i.e. about 76% sea-water) and, by the time the adults had emerged, had a salinity of 46.77 g NaCl per litre. Jepson et al. (1947) report that A. gambiae larvae will pass through several stages in sea-water, and that in one salt pan, larvae were found in water containing 65 g NaCl per litre. Muirhead-Thomson (1951) found evidence that in Dar es Salaam, continuous breeding of salt-water gambiae was no longer possible when the salinity of the water reached 26.4 g NaCl per litre (i.e. about 83% sea-water). Other records of salt-water A. gambiae breeding in salinities varying between 14.6 g NaCl per litre and sea-water have come from Mackay (1938), Halcrow (1957) & Maffi (1960).

Estimation of the sodium chloride content of water in which A. gambiae larvae were found, was also carried out in Pemba. It was noticed that larvae occurred in salinities as high as 59.0 g NaCl per litre (which is nearly twice that of sea-water), but the maximum salinity in which complete development took place was found to be 42.0 g NaCl per litre, a figure much higher than that in Dar es Salaam.

Summary

1. In East Africa, the salt-water form of Anopheles gambiae has been reported from various parts of the East Coast from Somalia to Natal, and from the islands of Mauritius and Pemba.
2. Adults possess either three-banded palps as in typical A. gambiae or four-banded ones as in A. melas.
3. No morphological character exists for the separation of East African salt-water and fresh water forms of A. gambiae either in the egg, larval or adult stage. The two forms can only be distinguished by the physiological reactions of their first-stage larvae to 75% sea-water.
4. Adults of the salt-water form if they enter huts, tend to leave soon after feeding.
5. Results obtained from precipitin tests, experimental huts and outdoor collections show that this form of A. gambiae is mainly exophilic with a preference for feeding on cattle.
6. Susceptibility tests show that they are susceptible to dieldrin, three spraying cycles having been carried out in the area.
7. Larvae have been found in salinities varying from 14.6 g NaCl per litre to a concentration higher than sea-water. The maximum salinity in which complete development took place was found to be 42.0 g NaCl per litre.

Acknowledgements

I would like to express my gratitude to Dr W. J. Stoker, WHO, Project Leader, Zanzibar, and to Dr B. de Meillon, WHO Regional Entomological Adviser, for their helpful criticism and guidance. I should like to thank also Mr J. Storey and Mr A. Paggott, WHO Technicians, for their valuable assistance during the course of these studies. Precipitin tests reported in this paper were carried out by Dr B. Weitz of the Lister Institute of Preventive Medicine to whom I am much indebted. This study was made possible by funds allocated from the Malaria Eradication Special Account for which I am grateful to both the Regional Office and the Headquarters of the World Health Organization. My thanks are also due to the Director of Medical Services, Zanzibar, for permission to publish this paper.

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