LARVAL SALINITY TOLERANCES OF THE SIBLING SPECIES OF *ANOPHELES FARAUTI*

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ABSTRACT. Experiments conducted with laboratory colonies of the sibling species of *Anopheles farauti* showed larvae of *An. farauti* No. 1 had a higher salinity tolerance than larvae of *An. farauti* No. 2 and *An. farauti* No. 3. The salinity response of field-collected larvae of *An. farauti* No. 1 from Cowley Beach, Queensland, Australia was similar to that of larvae from two colonies of this species which originated from Papua New Guinea. These results indicate that *An. farauti* No. 1 is the species which is likely to be found breeding in brackish water whereas the other species may be restricted to freshwater habitats. Laboratory experiments conducted with the colonies and with specimens collected from three localities in northern Queensland indicated that a simple test, based on exposure of first-instar larvae to sea water for 1 hr, should enable identification of *An. farauti* No. 1 in the field.

INTRODUCTION

Anopheles farauti Laveran, the most important malaria vector in coastal areas of the South West Pacific Region, has been shown to consist of a group of three sibling species designated *An. farauti* No. 1, *An. farauti* No. 2, and *An. farauti* No. 3 (Bryan 1973, Mahon and Miethke 1982). The distribution of *An. farauti* s.l. extends from the Moluccas in the west, through Irian Jaya, Papua New Guinea, the Solomon Islands and Vanuatu in the east as well as the tropical north of Australia but the sibling species are known from only a few localities (Mahon and Miethke 1982).

At the present time the relative importance of the members of the *An. farauti* complex as vectors of malaria is not known. Similarly, little is known of their adult and larval biology though this is clearly of significance to malaria epidemiology. *Anopheles farauti* s.l. breeds in a wide range of freshwater habitats and is often found in brackish water up to 70% salinity of sea water near the coast (Daggy 1945, Belkin 1962) but the salinity tolerances of the individual species has not been documented. This paper reports laboratory observations on the effects of rearing larvae of the three sibling species in water of various salinities. The mosquitoes used for these experiments were obtained from laboratory colonies of each species as well as field collected larvae of *An. farauti* No. 1 collected from Cowley Beach, Queensland. Observations were also made with larval progenies of field-collected females of the three sibling species.

MATERIALS AND METHODS

Reference colonies of the *An. farauti* sibling species are maintained in our laboratory at Ingleburn. They include: two colonies of *An. farauti* No. 1 (one from Rabaul, Papua New Guinea established by J. H. Bryan in 1968 and the other from Port Moresby, Papua New Guinea established by the author in 1974); a colony of *An. farauti* No. 2 from Russell River, Queensland, Australia, (established by R. J. Mahon in 1980); and a colony of *An. farauti* No. 3 from Etty Bay, Queensland (established by the author in 1982).

Larvae of these colonies are routinely reared in our laboratory in water from O'Hares Creek, a freshwater stream near Appin, New South Wales. For these experiments larvae of each colony were hatched in this water and after one day they were placed into plastic trays (18x12x5 cm) with each tray containing 20 larvae and 200 ml of water of the desired test salinity. Twenty trays of larvae from the four colonies were reared in the following salinities obtained by diluting O'Hares Creek water with sea water: freshwater (0% salinity); 10% sea water (0.3% salinity); 30% sea water (0.9% salinity); 50% sea water (1.7% salinity); 75% sea water (2.6% salinity); 90% sea water (3.1% salinity); 100% sea water (3.4% salinity). Salinity percentages were determined with a YSI model 33 salinity meter. Plastic lids were fitted to each tray to prevent evaporation and they were maintained at a temperature of 22–25°C. The larvae were fed daily with powdered larval food (Kanda 1979). For all test trays records were made of larval survival after 7 days and survival to the pupal stage. The numbers of pupae surviving to adults were also determined.

Seven hundred first-instar larvae of *An. farauti* s.l were collected in a brackish lagoon adjacent to Cowley Beach, Queensland in November 1982. The salinity of the lagoon was influenced by the tide and varied from 0.05 to 0.35% salinity during the collecting period which extended over 3 days. Five trays of these larvae (20 larvae/tray) were reared at each of the test salinities and under the same conditions as those of the laboratory colonies and their survival was recorded after 7 days. Thirty-one of these mosquitoes (selected at random) were reared to the adult stage and subjected to the isoenzyme elec-

trophoresis technique of Mahon (1984) to determine their specific identity. All were found to be *An. farauti* No. 1. The 7 day survival data for the two *An. farauti* colonies and for the Cowley Beach larvae were converted to percentage mortality at each salinity and were corrected for natural mortality by Abbott's formula using the mortality in fresh water as a control. These data were then subjected to probit analysis by the techniques of Finney (1971) to determine the LC_{50}s for salinity.

For another experiment 400 one-day-old first-instar larvae from each colony were placed in batches of 20 into plastic trays containing 200 ml of full-strength sea water. Larval food was added and after 1 hr the mortality of larvae in each tray was recorded.

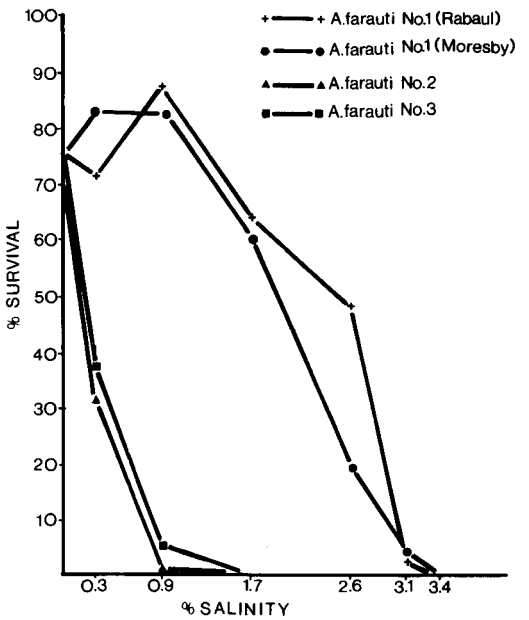

Fig. 1. Survival to pupation of larvae from laboratory colonies of the *Anopheles farauti* sibling species reared from the first instar in water of various salinities.

Fifty-three engorged female *An. farauti* were collected in biting catches from three localities in northern Queensland: Etty Bay on the east coast (17° 25'S, 126° 5'E), Weipa (12° 40'S, 141° 51'E) and Ina Creek (13° 10'S, 141° 38'E). The latter two sites are on the west coast of Cape York Peninsula adjacent to the Gulf of Carpentaria. Each female was placed into a separate gauze-covered cup containing 100 ml of freshwater for oviposition. The eggs were allowed to hatch in these cups and the larvae were then removed within 24 hr and placed in full-strength sea water for 1 hr after which the numbers alive and dead were recorded. The females were killed; stored in liquid nitrogen; and later identified electrophoretically (Mahon 1984).

RESULTS

The percentages of larvae from the four colonies which survived to the pupal stage in various test salinities are shown in Fig. 1. Both the Rabaul and Port Moresby colonies of *An. farauti* No. 1 showed a similar trend in their response to increasing salinity. More than 70% of larvae from these two colonies survived to pupation when reared in freshwater as well as in water of 0.3% salinity and 3.1% salinity. As the concentration of sea water increased above these levels the survival of larvae from these colonies decreased progressively from approximately 60–65% in water of 1.7% salinity to less than 10% in water of 0.9% salinity. None of the larvae from the *An. farauti* No. 2 and *An. farauti* No. 3 colonies survived to the pupal stage when reared in water of 1.7% salinity or above. There was no apparent adverse effect of salinity on pupal survival as adult emergence for all the colonies was more than 80% of the larvae which reached the pupal stage.

The effects of salinity on the seven-day survival of larvae from the laboratory colonies as well as the larvae from Cowley Beach are shown in Table 1. Ten percent of the larvae collected

Table 1. Percentage survival for 7 days of first-instar larvae from laboratory colonies of *Anopheles farauti* sibling species compared with that of field collected first-instar larvae of *An. farauti* No. 1 from Cowley Beach, Queensland.

Percent salinity	*An. farauti* No. 1 Cowley Beach field-collected	*An. farauti* No. 1 Rabaul colony	*An. farauti* No. 1 Port Moresby colony	*An. farauti* No. 2 Russell River colony	*An. farauti* No. 3 Etty Bay colony
0	96.0	88.5	90.0	81.3	96.3
0.3	96.0	89.0	91.4	87.5	63.1
0.9	92.0	90.0	95.0	15.0	5.6
1.7	92.0	92.5	73.1	0	0
2.6	64.0	53.0	32.5	0	0
3.1	41.0	2.5	6.3	0	0
3.4	10.0	0	0	0	0

at Cowley Beach survived in water of 3.4% salinity for a week whereas none of the larvae from the *An. farauti* colonies survived this treatment. This might suggest that the former larvae were more salinity tolerant. However, the estimated LC_{50}s were 2.8% salinity (fiducial limits 2.4–3.3%) for Cowley Beach larvae and 2.3% salinity (fiducial limits 2.0–2.5%) for larvae from the Port Moresby colony. The over-lapping confidence limits indicate that there was no significant difference between these two LC_{50}s at the 5% level of probability. The LC_{50} of larvae from the Rabaul colony was intermediate between the other 2 and was estimated as 2.6% salinity. In the latter case it was not possible to calculate meaningful fiducial limits as there were insufficient data points between 0% and 100% mortality. These results show that the salinity response of field collected *An. farauti* No. 1 from Australia agrees closely with that of laboratory-reared colonies of this species which originated from Papua New Guinea. All the test larvae from the *An. farauti* No. 2 and *An. farauti* No. 3 colonies exposed to 1.7% salinity or above died within 7 days.

All of the first-instar larvae from the Port Moresby and Rabaul colonies of *An. farauti* No. 1 survived 1 hr exposure to full-strength sea water whereas all of the *An. farauti* No. 2 larvae and all of the *An. farauti* No. 3 larvae died under these conditions. The results of 1 hr exposure to sea water of newly hatched larval progeny of 53 field-collected female *An. farauti* are shown in Table 2. Twenty-six batches were identified by electrophoresis as *An. farauti* No. 1 (19 from Weipa, 2 from Etty Bay and 5 from Ina Creek). Almost all of the larvae from these batches survived with only one or two individuals dying within the exposure period. In another 26 batches virtually all of the larvae died when placed in sea water with only one specimen from each of three batches surviving for 1 hr. Thirteen of these batches were identified by electrophoresis as *An. farauti* No. 2 (8 from Weipa, 4 from Etty Bay and 1 from Ina Creek), and the other 13 batches (all from Etty Bay) were identified as *An. farauti* No. 3. However, in contrast to the above results one batch from Weipa was identified as *An. farauti* No. 2 with 48/50 larvae surviving exposure to sea water for 1 hr.

DISCUSSION

These experiments indicate that *An. farauti* No. 1 is the species which is sometimes found in brackish water as the other two species are adversely affected by even small concentrations of salinity. However, this is not to imply that only the former species has a coastal distribution and that the other species are restricted to inland areas, as all three species have been found sympatrically within 1 km of the coast in north Queensland at Etty Bay (Mahon and Miethke 1982) and at Cowley Beach (Sweeney, Cooper and Medcraft; unpublished data). Moreover, a colony of *An. farauti* No. 2 was established from larvae breeding in a temporary pool within 100 m of the beach at Red Island Point near Bamaga, Queensland (Sweeney 1980).

At the present time there are no known reliable morphological characters to identify the *An. farauti* sibling species. Larvae can be identified by differences in banding patterns of polytene chromosomes (Mahon 1983) and adults can be distinguished by isoenzyme electrophoresis (Mahon 1984). The only other method of identification is by laboratory hybridization experiments using the forced-mating technique. All three methods cannot be readily applied in the field as they require the use of sophisticated apparatus or laboratory facilities. It is possible that detailed study of field-collected and laboratory-reared material may provide reliable taxonomic characters to permit accurate identification of the member species of the complex but, in the meantime, any simple methods which might aid identification would be useful for field use.

Table 2. Effects of 1 hr exposure to sea water of first-instar larval progeny obtained from 53 wild caught *Anopheles farauti* females collected from three localities in northern Queensland.

Electrophoretic identification of female parent	Locality	No. of egg batches	Exposure of larvae to sea water	
			No. dead after 1 hr	Total exposed
An. farauti No. 1	Weipa	19	3	679
An. farauti No. 2	Weipa	9	234*	284
An. farauti No. 1	Etty Bay	2	1	38
An. farauti No. 2	Etty Bay	4	75	75
An. farauti No. 3	Etty Bay	13	367	367
An. farauti No. 1	Ina Creek	5	1	249
An. farauti No. 2	Ina Creek	1	29	30

* Egg batch No. 4 from Weipa had only 2 dead larvae from 50 exposed.

Saltwater tolerance forms the basis of tests to distinguish larvae of the saltwater species from the freshwater species of *An. gambiae* s.l. In West Africa, Ribbands (1944) showed that exposure of larvae to 37½% sea water for 1 day followed by exposure to 75% sea water for a second day permitted the survival of *An. melas* Theobald but led to death of freshwater *An. gambiae* Giles. It was later found by Muirhead Thomson (1951) that, in East Africa, newly hatched larvae of *An. merus* Dönitz survived exposure to 75% sea water for 2 hr whereas larvae of freshwater *An. gambiae* died under these conditions. It would appear from the present study that a similar simple test could be used to distinguish *An. farauti* No. 1 from the other two known species of the *An. farauti* complex.

Experiments with laboratory colonies of the three species suggested that the exposure to sea water of first-instar larvae for 1 hr should result in the survival of *An. farauti* No. 1 and death of *An. farauti* No. 2 and *An. farauti* No. 3. The application of this test with field-collected mosquitoes showed that the identification of larvae based on this hypothesis agreed with electrophoretic identification of the female parent in 52 out of 53 cases. The correct identification of the aberrant egg batch from Weipa (identified as *An. farauti* No. 2 by electrophoresis but with a high survival following sea water exposure) is not known but it may be that the electrophoresis result was not correctly interpreted in this instance. It could be argued that the salinity tolerances of the individual species may not be constant throughout their respective distribution ranges. Nevertheless, the close agreement between the larval salinity tolerances of the two colonies of *An. farauti* No. 1 established from widely separated localities in Papua New Guinea compared with that of field-collected larvae of *An. farauti* No. 1 from Australia implies that this is a constant characteristic for this species. The other two species have only been reported from northern Australia and the agreement between the results of the salinity test and electrophoretic identification from individuals in this area indicates that the test is valid on the basis of the known distribution of these species. It would still not be possible to distinguish between the latter 2 species by this technique. Nevertheless, the field application of this method should reveal useful information as *An. farauti* No. 1 is the only species of the group which has been recorded outside Australia. A preliminary indication of the presence of the other species in areas such as Papua New Guinea and the Solomon Islands could be obtained by 1 hr exposure to sea water of first-instar larvae collected from field breeding sites or obtained as progeny from wild caught females. This technique has the advantage that it can be carried out by field entomology teams without the need for special equipment or the application of complex techniques. Any indication of the presence of species other than *An. farauti* No. 1 in an area could be followed up by further observations using either electrophoretic, cytological or hybridization techniques.

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