

## Salinity tolerance of *Anopheles farauti* Laveran sensu stricto

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### SUMMARY

To assess the salt tolerance of the malaria vector *Anopheles farauti* sensu stricto, larvae were collected from a freshwater environment on the outskirts of Honiara, Solomon Islands and placed in trays containing water with salinity varying from freshwater to seawater. Dead larvae and pupae and emerged adults were recorded and preserved. Most adults and nearly half of the larvae and pupae were then subjected to DNA analysis for species identification. No adult *An. farauti* emerged after prolonged immersion of larvae in undiluted seawater (3.5% salinity), although temporary immersion before pupation was compatible with survival. Salinities of up to 2.2% to 2.5% were compatible with good survival and adult emergence, at least from fourth instars. The results suggest that higher salinities may slow larval development and show that mortality at a given salinity is not uniform.

### Introduction

Malaria is a major health problem in Honiara, the capital of the Solomon Islands, where members of the punctulatus group act as vectors. The predominant vector within the capital is *Anopheles farauti* Laveran sensu stricto (s.s.), formerly designated *An. farauti* No 1. *An. punctulatus* occurs only rarely (1). Two other members of the farauti complex, *An. farauti* No 2 and *An. farauti* No 7, have been recorded in the Solomon Islands (2). *An. farauti* sensu lato has long been known to tolerate brackish water, having been recorded in coastal pools elsewhere in the Solomon Islands in salinities above that of seawater (3). In Honiara *An. farauti* can be found in habitats ranging from fresh, running streams to brackish pools close to beaches (1). Using larvae from colonies established from specimens collected in Papua New Guinea and northern Queensland, Sweeney (4) demonstrated that more than 70% of larvae survived in salinities of 25% of seawater (0.9% salinity) and 32-53% survived for a week in salinities of 75% of seawater. The LC<sub>50</sub>s for larvae from the colony and from wild-caught

females ranged from 2.3% to 2.8% salinity (ie, 68-82% seawater).

To overcome the problem of *An. farauti* breeding near the mouth of the Mataniko River within Honiara and in other obstructed streams in the vicinity, efforts have been made to increase the salinities in these sites by constructing pipelines through the sandbars at the stream mouths (5). The following research was carried out to assess the salinity tolerance of the local *An. farauti* s.s. and thus its susceptibility to such high-cost environmental modification.

### Methods

Anopheline larvae were collected on three occasions from freshwater in rice paddies and ditches at an experimental rice farm on the eastern outskirts of Honiara. Larvae were separated into late (fourth) instars (n = 224) and earlier instars (n = 474) and placed in plastic trays (10 cm x 12 cm x 5 cm) containing 400 ml of water of different salinities. The required salinity was obtained by diluting freshly collected seawater with

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rainwater. Salinities were checked regularly using an ATAGO S/MILL hand refractometer and the required salinity was maintained by adding rainwater to replace water lost by evaporation. Salinity tolerance was determined in three batches, salinities of batch 'a' being 0% (freshwater), 0.9% (25% seawater), 1.8% (50% seawater) and 3.5% (seawater); batch 'b' 0%, 2.2%, 2.6% and 3.1%; and batch 'c' 0%, 2.1% and 2.5%. Pupae were transferred to containers of identical salinity until adult emergence. Early instar trays of batch 'c' (29 larvae, 15 adults) were excluded from analysis due to inconsistencies in the maintenance of correct salinity. Fourth instar larvae which pupated within 1 hour of immersion (22 larvae) were also excluded from analysis, as Sweeney (4) concluded that pupal survival was similar across differing salinities and early pupation could therefore bias results.

All trays contained a small amount of vegetation and the experiments were carried out at ambient conditions at the Solomon Islands Training and Research Institute. Larvae were fed twice a day, at which time dead larvae and pupae were removed and preserved in 100% alcohol. Specimens were identified using DNA probes (6,7) and by PCR-RFLP where DNA probing was inconclusive (8).

## Results

### DNA analysis

DNA probing was performed on 194 of the 202 adults which emerged. The remaining 8 individuals were from salinities ranging from 0% to 2.2%, including 1 escapee and 7 in condition too poor for analysis. All 194 were *An. farauti* s.s. (Table 1).

Deaths during adult emergence were included with pupal deaths. Many dead larvae, particularly the early instars, were not retrieved in suitable condition for preservation and analysis. A further 15 specimens, predominantly early instars, could not be identified. All but 3 of the 134 early instars to undergo analysis were *A. farauti* s.s. The remaining 3 were not of the punctulatus group and were excluded from survival statistics.

### Salinity tolerance

The mortality of larvae and pupae at 24 and 48 hours, and the numbers of adults emerging, are shown in Table 2. Most mortality occurred at the larval stage, with relatively few pupal deaths at any salinity.

Mortality rates of early instar larvae and pupae showed a clear trend with greater than 95% survival up to 2.2% salinity (63% seawater). Of the 6 adults to emerge from 3.1% salinity (88% seawater), 2 had survived for 8 days as larvae in the test tray and 2 for 4 days. Nearly all of the mortality at this concentration occurred in the first 24 hours. There was no survival beyond 24 hours in 3.5% salinity (seawater).

Emergence rates of early instars were low at all salinities, probably reflecting suboptimal growth conditions. Maturation in higher salinities was relatively slow, the last of 7 adults from 1.8% salinity (batch 'a') emerging after 21 days, 2 having emerged at 20 days and 1 each at 18 and 19 days. Emergence from 2.6% and 3.1% salinities (batch 'b') occurred up to 13 days and 10 days after immersion respectively. All emergence from control trays of these batches occurred before 18 and 9 days respectively.

Of the 224 fourth instar larvae, 22 pupated within 1 hour of immersion. Fourth instar larval survival to 48 hours was little affected by salinities up to 2.5%, but with higher salinities 48-hour survival declined. There was high survival at 24 hours at 3.5% salinity (seawater) but only a small number of larvae were involved. However, this illustrates the high salt tolerance of some individuals.

Adult emergence from fourth instar larvae varied from 75% to 95% across salinities up to 2.1%, dropping from 90% at this salinity to 61% at 2.2% salinity, 53% at 2.5% salinity, 21% at 2.6% salinity and 3% at 3.1% salinity. No adults emerged from 3.5% salinity (seawater) after exclusion of pupation within the first hour. 3 individuals which pupated within 1 hour of immersion at this salinity did successfully emerge as adults. The lone survivor from 3.1% salinity did not pupate until 4 days after immersion.

**TABLE 1**

DNA PROBE AND PCR IDENTIFICATION OF LARVAE AND PUPAE WHICH DIED, AND EMERGED ADULTS FROM EARLY AND LATE INSTARS, ALL SALINITIES COMBINED

Specimens	Total	DNA analysis		
		<i>An. farauti</i> s.s.	Other spp.	No reaction
First, second, third instar larvae/pupae*	375	131	3	13
Adults from first, second, third instar larvae**	70	65	0	0
Fourth instar larvae/pupae	92	81	0	2
Adults from fourth instar larvae	132	129	0	0

PCR was performed on those which were not shown to be *An. farauti* s.s. on DNA probing

Adults include those which pupated less than 1 hour after immersion

\* excludes batch 'c' (22 specimens)

\*\* includes batch 'c'

**TABLE 2**

*AN. FARAUTI* S.S. LARVAL AND PUPAL SURVIVAL AT 24 AND 48 HOURS AND ADULT EMERGENCE AFTER IMMERSION OF EARLY AND FOURTH INSTAR LARVAE IN WATER OF VARIOUS SALINITIES\*

Salinity %	Batch	N	Early instar Larval/pupal mortality		N	Fourth instar Larval/pupal mortality		Adult emergence
			24 hours	48 hours		24 hours	48 hours	
3.5	a	64	59	64	7	0	4	0
3.1	b	40	32	32	30	6	28	1
2.6	b	40	9	10	34	10	13	7
2.5	c				15		0	8
2.2	b	25	0	0	33	1	3	20
2.1	c				10		0	9
1.8	a	112	0	1	8	8	0	6
0.9	a	66	0	0	6	10	0	5
0.0	a	56	2	2	6	17	0	5
0.0	b	27	0	1	38	3	0	36
0.0	c				15		0	14
Total control	a,b,c	83	2	3	59	20	0	55

\* Individuals that pupated within one hour are excluded

All adults were identified by DNA analysis as *An. farauti* s.s. except from one early instar from 2.2% salinity, one early instar from 1.8% salinity, one late instar from 2.1% salinity, one fourth instar from control 'b' which was not tested and one fourth instar (escaper) from 1.8% salinity

## Discussion

This study indicates that considerable salt water tolerance occurs amongst *An. farauti* s.s. larvae in the Solomon Islands. While adult emergence from early instars was difficult to interpret due to the poor emergence across all salinities (probably due to difficulties in maintaining good growth conditions) there was still some emergence from early instars at close to 90% seawater and little effect on 48-hour mortality at 2.2% to 2.5% salinity (63% to 72% seawater). These tolerances fit fairly well with the LC<sub>50</sub>s of 2.3% to 2.8% obtained by Sweeney (4) with larvae derived from Papua New Guinea and northern Australia.

Of interest is the high mortality in the first 24 hours at 3.1% salinity amongst both early and late instar larvae, with all the survivors then surviving the next 24 hours. This early mortality could not be related to handling as control larvae showed good early survival, suggesting either that there is variation in salt tolerance within the population, or increased sensitivity to rapid changes in salinity at certain stages in larval development (eg, just before or after moulting). The larvae involved were all derived from freshwater, but *An. farauti* larvae have been found in brackish water collections behind beaches near the collection site (D. Bell et al., unpublished data). The presence of populations of varying salinity tolerance could be clarified by observation of oviposition and larval growth in saline water of mosquitoes derived from freshwater and brackish water.

While long-term immersion in full seawater was uniformly fatal, temporary immersion was tolerated, since all 3 larvae which pupated within 1 hour of immersion in seawater developed into adults. Larvae immersed in seawater for longer periods prior to pupation failed to reach adulthood. Sweeney (4) found larvae in a tidal lagoon in Queensland with widely varying salinity, in concordance with these findings. It may be that the high salinity of pools in which Maffi and Taylor (3) found larvae was the product of very recent evaporation and the larvae may not have been viable. This experiment was unable to confirm whether adults which emerged from higher salinities were of normal vigour.

The results suggest that although survival is high at moderate salinities, growth rates may be reduced. This could not be confirmed without knowing the ages of larvae more precisely than was possible in this study.

Modifications to river estuaries to increase salinity may only be of significant benefit in the control of *An. farauti* if salinities above 2.6%, or about 75% seawater, can be maintained for prolonged periods.

## Conclusion

While statistical analysis of the results was limited by conditions and larval numbers, it is clear that the larvae of *An. farauti* from freshwater habitats in the Solomon Islands tolerate quite high salinities. Salt tolerance has implications for malaria control strategies involving the breaching of sandbars to raise estuarine salinities as a form of vector control. Further work is needed to exclude the presence of populations of varying salt tolerance.

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