Journal of the American Mosquito Control Association, 15(3):291–294, 1999

OVIPOSITION PREFERENCE FOR FRESHWATER IN THE COASTAL MALARIA VECTOR, *ANOPHELES FARAUTI*

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ABSTRACT. Oviposition preference of the Australasian coastal malaria vector *Anopheles farauti* s.s. for water of varying salinity was determined in the laboratory to help understand the distribution and control of this species in the field. Numbers of eggs laid showed an inverse relationship with salinity; of 5 NaCl concentrations most eggs were laid in distilled water but some were laid in 3.17% NaCl (the salinity of seawater). The association of *An. farauti* with coastal areas occurs in spite of an aversion to salt water by ovipositing females. Factors other than salinity must be the primary determinants of distribution. Increasing the salinity of larval habitats will not totally prevent *An. farauti* from laying eggs. Elimination of this species may not occur unless salinity is kept high enough to prevent complete larval development.

KEY WORDS Oviposition preference, salinity, distribution, malaria, *Anopheles farauti*

INTRODUCTION

Seven species are known to comprise the *Anopheles farauti* Laveran complex (Foley et al. 1994) and members of this complex are important malaria vectors in New Guinea, the Solomon Islands, and Vanuatu (Lee et al. 1987). The factors necessary for the oviposition and larval development of these species are poorly understood but this knowledge may be useful for understanding species distribution and for malaria control through source reduction.

Anopheles farauti s.s. is the most widely distributed of the *An. farauti* complex and this species is mainly confined to coastal areas (Sweeney et al. 1990; Foley et al. 1993, 1994, 1995; Cooper et al. 1995). The remainder of this paper refers to *An. farauti* s.s. The tolerance of *An. farauti* larvae for brackish water (Daggy 1945, Sweeney 1987) has been invoked to explain the coastal distribution of this species (Cooper et al. 1995). However, these authors note that larvae of this species can also tolerate freshwater. Therefore, what prevents *An. farauti* from extending further inland? Oviposition site selection has been identified in other mosquito species as the greatest determinant of larval distribution (Kennedy 1942, Wallis 1954). Assuming that oviposition by *An. farauti* is selective rather than random, is the coastal distribution of this species the result of oviposition preference for brackish water or are other factors more important? We investigated oviposition preference of *An. farauti* s.s. in relation to salinity by measuring the numbers of eggs laid in a laboratory oviposition choice experiment.

MATERIALS AND METHODS

Oviposition choice experiments were conducted at 28°C and 70% relative humidity using colony-bred *An. farauti* housed at the Queensland Institute of Medical Research. This colony was established from specimens collected at Rabaul (Papua New Guinea) in 1965 and supplemented with specimens from Agan (Papua New Guinea) around 1985. Photoperiod was 12:12 light:dark. Female mosquitoes were bloodfed on guinea pigs, then groups of 5 females were allocated to 22 × 27-cm cages. Each cage had 5 oviposition sites, 1 located at each corner and 1 placed at the center of the cage. Salinity treatments were randomly assigned to these positions. Oviposition bowls were ~10 cm wide and 4 cm high and were lined with filter paper. Treatments were 0, 10, 20, 50, and 100% saltwater equivalent (3.17% NaCl = 100% seawater). Oviposition preference was determined by analyzing the number of eggs (n) laid in each treatment (TREATMENT effect), taking note of the cage (REPLICATE effect) and the position within the cage (POSITION effect) and eggs were counted 6 days after bloodfeeding. Ten cages at a time were used and the choice experiment was repeated on 7 days (DATE effect).

Statistical analysis using SPSS 7.0 for Windows (SPSS Inc., Chicago, IL) comprised developing generalized linear models of the main effects of DATE, REPLICATE, POSITION, and TREATMENT on n (transformed to $\log_{10}(n + 1)$). Only cages with more than 30 eggs were considered in the analysis.

RESULTS AND DISCUSSION

Salinity was the only source of variation to significantly affect oviposition choice (Table 1). An inverse relationship between salinity and egg numbers was evident, with 100% salinity the least preferred treatment (Fig. 1).

The factors influencing oviposition by *An. farauti* are poorly known. In this study, the lack of eggs in some cages indicates that laboratory conditions were not ideal for oviposition. Despite this, a trend towards greater numbers of eggs in less saline wa-

Table 1. Analysis of variance for the effect of salinity on oviposition preference of *Anopheles farauti* s.s.

Source	*SS*	df	*MS*	F	*P*
Replicate	2.497	4	0.624	1.438	0.226
Date	4.518	6	0.753	1.735	0.119
Pos.	1.664	4	0.416	0.958	0.433
Treatment	19.766	4	4.942	11.386	0.000[1]
Error	48.173	111	0.434		
Total	76.155	129			

[1] $P < 0.001$.

ter indicates that NaCl acts as an oviposition deterrent. Petersen (1969) also concluded that salinity is more important as a repelling factor than as an attracting factor in the location of suitable oviposition sites by mosquitoes.

For many species of mosquitoes, salinity tolerance of larvae is not a good indicator of the salinity of typical larval habitats. Grueber and Bradley (1994) found that many species of *Aedes* mosquitoes able to tolerate salt and freshwater occur only in freshwater. Hackett (1937) noted that *Anopheles atroparvus* Van Thiel, typically found in brackish water, preferred to lay eggs in freshwater. Similarly, *Anopheles tenebrosus* Dönitz and *Anopheles mousinhoi* Di Meillon and Pereira tolerate 25% seawater despite being regarded as freshwater-breeding species (Coetzee and le Sueur 1988). Roberts (1996) found that the preferred salinity for oviposition corresponded well with larval survival in *Anopheles stephensi* Liston and *Anopheles culicifacies* Giles, but did not compare well for *Culex quinquefasciatus* Say and *Culex sitiens* Wiedemann. The larvae of *An. farauti* s.l. occur in a wide range of freshwater habitats and have been found in brackish water of 70% salinity of seawater (Daggy 1945, Belkin 1962) or higher (Maffi and Taylor 1974, Cooper et al. 1995). The present study verifies the conclusion of Daggy (1945) that despite the salinity tolerance of larvae, salinity acts as a repellent for adult oviposition. The coastal distribution of *An. farauti* s.s. occurs in spite of both a preference to oviposit and the ability of larvae to survive in freshwater. Factors other than salinity must be responsible for preventing this species from occurring further inland.

In coastal situations, if salinity increases after eggs are laid, larval survival will depend at least on the age of the larva and the salinity (Sweeney 1987). Increases in salinity after eggs are laid are partially counteracted by the increasing ability of larvae to survive highly saline water as they become older. Thus, Daggy (1945) noted that 2nd- and 3rd-stage larvae taken from 68% seawater could complete development in water more strongly saline than seawater. Although the physiologic tolerance of larvae is important, oviposition site selection has been identified as the primary factor in determining mosquito larval distribution (Wallis 1954, Petersen 1969, Bentley and Day 1989).

In analyzing the components of insect oviposition choice, Singer et al. (1992) distinguished acceptance and rejection of an oviposition site, motivation (a nonspecific readiness to oviposit usually influenced by time and egg load), and preference. Preference was divided into rank (the order that sites become acceptable to an insect) and specificity (the strength of an insect's preference). In the present study, all salinities were accepted but the preference rank was 0% > 10% > 20% > 50% > 100% seawater equivalent. Counting eggs 4 days after the 1st bloodmeal would have the effect of standardizing motivation. It is not known what ef-

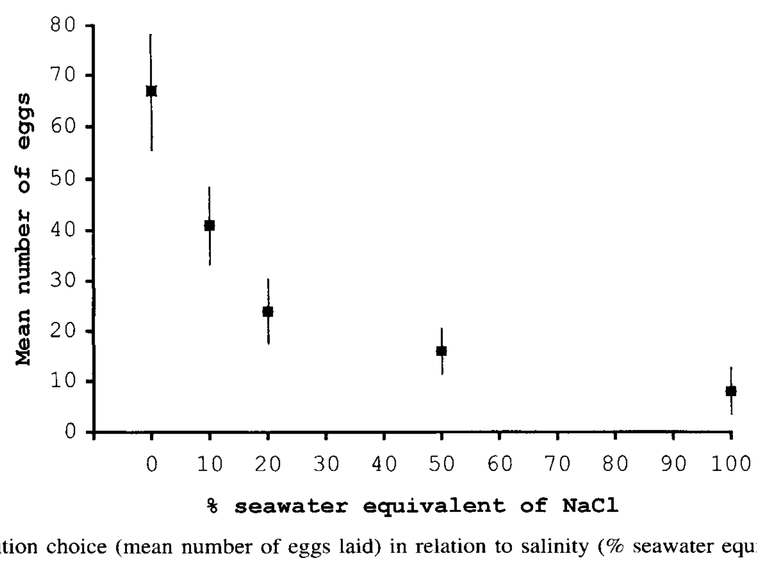

Fig. 1. Oviposition choice (mean number of eggs laid) in relation to salinity (% seawater equivalent of NaCl) for *Anopheles farauti* s.s. Vertical bars are +1 SE.

fect an increase in the motivation to oviposit (e.g., by denying acceptable oviposition sites) would have on preference rank or specificity of *An. farauti.* However, *Anopheles quadrimaculatus,* Say *Anopheles freeborni* Aitken, and *Anopheles aztecus* Hoffmann (Wallis 1954) and *Anopheles annulipes* (Russell 1979) laid eggs in more saline water when lower concentrations were denied them. In addition, Singer et al. (1992) described a situation with 2 populations of the checkerspot butterfly (*Euphydryas editha* Boisduval) where the absence of the preferred host in an area resulted in prolonged oviposition searching and a higher motivation level, which affected oviposition choice.

In a situation where the salinity of a potential oviposition site is increased, such as through evaporation or tidal inundation, 2 results may occur. First, oviposition specificity, which has the potential to be highly heritable (Singer et al. 1992), may come under selection pressure. Second, the motivation of adult females denied preferred oviposition sites would be expected to increase, resulting in egg laying in less preferred habitats, as in the checkerspot butterfly example.

Attempts to control malaria by tidal flushing of *An. farauti* larval sites have been tried in the Solomon Islands and Vanuatu (Daggy 1945). A recent engineering project in Honiara in the Solomon Islands sought to prevent *An. farauti* breeding by increasing the salinity of the Mataniko River. If adult mosquito production is halted because of this intervention, 3 explanations are possible: oviposition continues and larvae cannot survive, larvae cannot survive and oviposition ceases, and larvae can survive but oviposition ceases. In contrast, if adult mosquito production is not halted only 1 possibility exists: larvae can survive and oviposition continues. Thus a consideration of both oviposition and larval survival is necessary to understand the reasons for success or failure.

What can the present study contribute to our understanding of source reduction projects using salinization? Daggy (1945) relates an example of using tidal flushing on Ulilapa Island off the southern coast of Espiritu Santo, Vanuatu, where anopheline breeding (i.e., *An. farauti,* Foley et al. 1994) was eliminated from a small freshwater lake and associated swampy areas wherever tidal flushing was marked. However, heavy wave action temporarily blocked the entrance to the drainage area and "Within a few weeks, anopheline larvae began to reappear in large numbers in the quiet water" that was 68% seawater. Thus, oviposition must have occurred despite the repellency of high salinity. Could it be that in the absence of their preferred habitat highly motivated females were forced to oviposit in saline water? Was selection occurring for females with greater oviposition tolerance to salinity? Or were factors other than salinity such as tidal flushing more important in explaining presence or absence? Daggy (1945) observed that complete development and adult emergence of *An. farauti* took place at salinity levels below 65% seawater although higher salinities could be tolerated in later instars. He also noted that salinization projects "may be a very dangerous practice and may even result in increasing the amount of water surface for anopheline breeding and thus increase the malaria problem." Unless vigilance is maintained to keep salinity above 70% seawater, *An. farauti* adults may continue to oviposit and larvae may complete development, despite a preference for freshwater.

ACKNOWLEDGMENTS

We wish to thank Tony Barnes and Gail Williams for statistical advice and Kay Marshall for maintaining mosquito colonies.

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