CHLORIDE ION TOLERANCE OF FOUR MOSQUITOES OF OKINAWA[1]

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While engaged in mosquito survey work on Okinawa during 1948–1950, an investigation was made to determine whether any appreciable salinity tolerances existed among the mosquito larvae of that area. It was felt that sea water, barring the existence of salt or brackish water mosquitoes, might be successfully employed as a control measure in the fire barrels of those installations located near the ocean. This paper describes the larval distribution of *Anopheles hyrcanus sinensis* Wiedemann, *Culex tritaeniorhynchus* Giles, *Culex quinquefasciatus* Say, and *Culex bitaeniorhynchus* Giles with respect to different salinities and tolerances of these species to sodium chloride solutions.

METHOD. Unless unusually heavy breeding was encountered, a standard of 50 dips in most cases was taken in each collection. Specimens collected were placed in 95 per cent alcohol and later examined microscopically for identification. Keys in Bohart and Ingram (1946) were used for making determinations. Total chloride analyses of the water samples were made by Mohr's method of titration with silver nitrate, the determinations being made within forty-eight hours. To facilitate comparison, soluble chloride values of samples tested are expressed as per cents of the soluble chloride content of sea water. Sea water adjacent to Okinawa was determined to contain 19.72 grams of total chlorides per 1,000 grams of sample.

CHARACTERISTICS OF AREAS SAMPLED. Areas chosen for salinity observations were mainly low, extensive marshes and tidal mud flats located adjacent to the ocean. The majority of them were separated from the sea by walls or dikes. However, faulty flood gates and imperfections in sea walls caused by erosion and faulty construction rendered many of them at least partially defective which permitted the inflow of sea water. Streams and canals with outlets to the sea received minor attention since tidal fluctuations were likely to cause wide variations in chloride content within relatively short periods of time.

One area of particular interest which received major attention was a flat, low-lying peninsula of approximately 2,000 acres surrounded by the sea on three sides. This was located on the eastern side in the southern third of the island. It contained several extensive marshes in addition to tidal streams and canals. Apparently a major portion of this area formerly had been utilized in the production of rice, as remnants of paddy banks were much in evidence. This peninsula was separated from the sea by the ruins of a sea wall on one side and a series of sand banks on the remaining two sides through which had been cut ditches. Water poured through these during periods of high tide and collected in numerous depressions, thereby affording a series of water collections of varied chloride concentrations.

SPECIES DISTRIBUTION IN RELATION TO SALINITY. Sampling was begun in September 1949 and was continued until May 1950. A total of 14,976 dips was taken in the 310 areas investigated. The following larvae were collected in the numbers indicated: 2,970 *Culex tritaeniorhynchus*, 1,067 *Culex quinquefasciatus*, 691 *Anopheles hyrcanus sinensis*, and 10 *Culex bitaeniorhynchus*. A summary of these results is listed in Table I. Salinity values of sam-

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TABLE I.—Larval Densities in the Chloride Ranges Sampled

Percent of soluble chloride content of sea water	Number of dips taken	*Larvae per dip*			
		C. tritaeniorhynchus Giles	*C. quinquefasciatus* Say	*C. bitaeniorhynchus* Giles	*Anopheles hyrcanus sinensis* Wiedemann
100–96	50				
95–91	270				
90–86	100				
85–81	50				
80–76	No samples taken				
75–71	No samples taken				
70–66	140	.04			
65–61	100				
60–56	50				
55–51	150	.01			
50–46	200				
45–41	100				
40–36	160	.04			.008
35–31	250	.04			
30–26	350	.04			.009
25–21	790	.30			.006
20–16	950	.19			.001
15–11	1723	.12	.002		
10.0–9.0	400	.33			
8.9–8.0	750	.13	.001		.001
7.9–7.0	223	.31	.08		.009
6.9–6.0	191	.86			
5.9–5.0	458	.47			.02
4.9–4.0	750	.26	.004		.05
3.9–3.0	1042	.24	.003	.003	.06
2.9–2.0	1915	.34	.004	.002	.11
1.9–1.0	2201	.17	.14	.0005	.10
.99–0	1613	.10	.45	.001	.08

ples are expressed in the percentage of sea water that they contained using total soluble chloride values as a means of comparison. These values are presented in increments of one from 0 to 10 per cent and in increments of five from 11 to 100 per cent. No water in the range of 71 to 80 per cent sea water was sampled. Since no chloride testing was done in the field, the concentration was unknown at the time of larval survey. This also accounts for the small amount of collecting done in water containing more than 35 per cent sea water. Larval occurrence was sporadic in the higher ranges and was not consistently encountered in saline concentrations greater than 40 per cent sea water.

Culex tritaeniorhynchus were found rather consistently in all saline ranges up to those of 40 per cent sea water. One collection of 5 larvae was taken from a tidal stream near its confluence with the ocean in water containing 67 per cent sea water. Two additional larvae were collected in a similar situation, in which the water contained 53 per cent sea water. However, it is felt that the above two collections are of questionable significance as subsequent samples taken from these areas yielded much lower values. This species was not found to approximate its fresh water density of 0.19 larvae per dip in situations greater than 25 per cent sea water. It was taken in the greatest density in areas containing 6.0 to 6.9 per cent sea water, suggesting that possibly this is the optimum range for this species.

Anopheles hyrcanus sinensis was found only in water of saline ranges from 0–35 per cent of sea water, but was only collected in significant numbers in the 0–5.9 per cent sea water range, and in densities

TABLE II.—Larval Mortality Rates in Various Concentrations of Sodium Chloride Solutions

Culex tritaeniorhynchus Giles

Per cent of NaCl	Number Started	Cumulative Mortality at 24 hour Periods in Per cent														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
distilled water	50	20	26	34	48	52	56									
0.25	40	10	15	25	32	40	42	42	45	45	45	45	47			
0.50	40	15	20	27	35	45	47									
0.75	10	10	30	30	30	40	50	50								
1.00	50	36	50	60	66	68	72	74								
1.25	20	25	80	85	90	95	95									
1.50	20	20	80	100												
1.75	20	70	95	95												
2.00	20	80	100													

Anopheles hyrcanus sinensis Wiedemann

Per cent of NaCl	Number Started	Cumulative Mortality at 24 hour Periods in Per cent														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
distilled water	30	30	43	37	60	60	67	70	80	87	93	97	97	100		
0.25	20	30	40	50	50	55	60	60	75	75	75	75	75	80	80	80
0.50	20	15	50	50	55	60	75	75	80	80	80	85				
1.00	30	67	90	90	90	90	90	90	100							

Culex bitaeniorhynchus Giles

Per cent of NaCl	Number Started	Cumulative Mortality at 24 hour Periods in Per cent														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
distilled water	15	48	54	74												
0.25	15	27	54	54	80											
0.50	15	19	60	60	67	67										
1.00	15	40	100													

Culex quinquefasciatus Say

Per cent of NaCl	Number Started	Cumulative Mortality at 24 hour Periods in Per cent														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
distilled water	10	20	20	20	20	*										
0.25	10	0	10	10	10	*										
0.50	10	10	10	10	20	*										
0.75	10	10	10	40	40	*										
1.00	10	60	90	90												
1.25	10	100														
1.50	10	100														
1.75	10	100														

* Discontinued on the fifth day.

exceeding its two year average of 0.04 specimens per dip in the 0 to 4.9 per cent sea water range. It was taken in the greatest density in the range of 2.0 to 2.9 per cent sea water, suggesting an optimum for this species.

Culex quinquefasciatus was found in numbers that approached its fresh water density of 0.49 only in the 0–0.99 per cent range. Extensive collecting was done on the peninsula previously described where the drainage from a large native village flowed into the area. Normally this material which contained effluent from animal pens and cesspools would have afforded ideal breeding areas for this species. However, none was found in any range higher than the above figure.

Culex bitaeniorhyncus was not taken in densities that approached its two-year

average of 0.04 larvae per dip in any saline range. The scarcity of this species in the situations investigated might be attributed at least partially to its preference for clean water in shaded situations, since the vast majority of the areas sampled were relatively unshaded.

LABORATORY OBSERVATIONS. Limited laboratory tests were conducted at room temperature to determine which of those species had the most tolerance for sodium chloride solutions. Third and fourth stage larvae were placed individually in 10 cc capacity bottles containing various concentrations of sodium chloride as shown in Table II. Checks of distilled water were run in each test and pablum was used as food. Observations were made at twenty-four hour intervals.

High mortality rates were encountered in checks of the three species on which observations were completed. It is felt that had checks of water from rice paddies or other natural habitats of these species been used, mortality rates in checks would possibly have been lower. The average chloride content of water tested from 16 rice paddies was found to be 0.35 per cent of the chloride value of sea water.

In all cases *Culex tritaeniorhynchus* exhibited the lowest mortality rates. Adult emergence in the case of this species occurred in all sodium chloride solutions up to and including 1.25 per cent. Adult emergence did not occur in the cases of *Anopheles hyrcanus sinensis* and *Culex bitaeniorhynchus* in sodium chloride solutions in excess of 0.50 per cent.

SUMMARY AND CONCLUSION. Tests were made at Okinawa during 1949 and 1950 to determine the feasibility of using sea water in fire barrels as an anti-mosquito measure. Chloride ion determinations were made of water taken from 310 areas. *Culex tritaeniorhynchus* larvae were found in water with a chloride content as high as 67 per cent of the chloride value of sea water, but were not collected consistently in water with a chloride value greater than 40 per cent. *Anopheles hyrcanus sinensis* larvae were encountered in chloride ranges as high as 33 per cent, but were taken in significant numbers only in ranges up to 6 per cent of sea water. *Culex quinquefasciatus* larvae were taken in quantity only in water containing up to 4 per cent of sea water chloride content.

Tests were conducted to determine what tolerance these species possessed for sodium chloride solutions. *Culex tritaeniorhynchus* larvae exhibited the highest tolerance. Tests on *Culex quinquefasciatus* larvae were incomplete. Larvae of *Culex bitaeniorhynchus* and *Anopheles hyrcanus sinensis* exhibited approximately equal tolerances.

Culex tritaeniorhynchus larvae exhibited the highest tolerance to soluble chlorides. However, *Anopheles hyrcanus sinensis* larvae were also collected in water of fairly high chloride content. It is felt that because of the dilution factor, due to heavy rainfall, use of sea water to prevent or inhibit the breeding of these two species in fire barrels would be inadvisable. *Culex bitaeniorhynchus* and *Culex quinquefasciatus* would probably be prevented from breeding for prolonged periods of time.

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