THE EFFECT OF SALINITY ON OVIPOSITION AND EGG HATCHING IN *CULICOIDES VARIIPENNIS SONORENSIS* (DIPTERA: CERATOPOGONIDAE)[1]

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ABSTRACT. The preferences of *Culicoides variipennis sonorensis* females for oviposition on different salinities were tested in experiments using small wells in a 4 × 4 latin square array. The mean numbers of eggs deposited on wet surfaces on salinites of 0.0, 9.9, 19.0 and 34.0‰, were 1212, 659, 287, and 0, respectively. Within a higher range, salinites of 19.0, 23.0, 28.0 and 34.0‰ yielded mean numbers laid of 110, 3, 6 and 0, respectively. The overall relationship could be reasonably well described by linear regression, implying, under experimental conditions, a decline of 458 eggs laid for every 10‰ increase in salinity. Eggs laid directly onto salinities up to 19.0‰ survived and hatched equally well; at 34‰, however, no eggs became tanned and none hatched. In contrast, when eggs were laid onto fresh water, then transferred to different salinities 24 hr later, they survived and hatched even at 34‰.

INTRODUCTION

Because of its incrimination as the major vector of bluetongue disease of sheep and cattle in the United States (Foster et al. 1963, 1968; Jochim and Jones 1966), *Culicoides variipennis* (Coq.) has assumed a status of considerable economic importance. The species is distributed throughout the United States as a complex of 5 subspecies (Wirth and Morris 1985). Within this extensive distribution, the larval habitats are unusually diverse and are broadly associated with particular subspecies (Wirth and Morris 1985). Larvae are often found in moist, non-saline soil polluted with animal feces, especially in cattle pastures or adjacent to dairy farms (Wirth and Jones 1957, Hair et al. 1966, O'Rourke et al. 1983). However, extensive breeding may also occur in alkaline or substantially saline environments (Jones 1961, Zimmerman and Turner 1984).

Since the behavior of the ovipositing gravid female is in one sense a primary determinant of the larval habitat, it is of interest to know how physical and chemical factors affect the selection of oviposition sites. Some information on oviposition by *C. variipennis* in the environment of the laboratory colony has been provided by Jones (1957, 1967), but specific experimental work on factors that may stimulate or inhibit oviposition is lacking. This paper reports results from experiments to determine the effects of salinity on oviposition and egg survival of *C. variipennis sonorensis* (Coq.).

MATERIALS AND METHODS

Insects used in the experiments were from a laboratory colony of *Culicoides variipennis sonorensis* maintained according to Jones et al. 1969. Males and females were kept in 470 ml (1 pint) ice cream cartons and given a blood meal daily from a rabbit. When used in the experiments, the females were between 1 and 2 weeks old.

To test for the effects of salinity on selection of an oviposition site, about 200 gravid females were placed in a 3.8 liter (1 gal) ice cream carton, with a lid consisting of a disc of Plexiglas® fitted tightly to the inside of the carton. In the bottom of the carton was placed a 15.5 cm diameter plastic dish to which 16 small wells, 2.2 cm wide and 1 cm deep, had been cemented in a 4 × 4 array (Fig. 1). This was used as a latin square, in which 4 salinities were tested simultaneously (i.e., 1 well of each salinity in each row, in a position randomly selected at the start of each series of experiments). The latin square design was used as a precaution against possible bias due to positional differences within the carton. To prepare the array for an experiment, each well was half-filled with clean cotton, which was then wetted with a predetermined quantity of artificial sea water (Instant

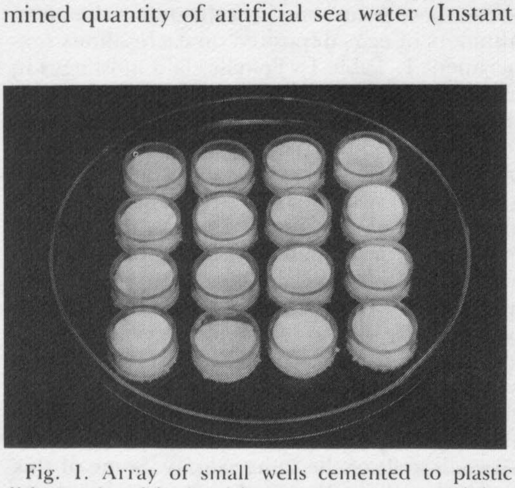

Fig. 1. Array of small wells cemented to plastic dish, as placed in 1 gal ice cream carton containing gravid females.

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Ocean®) at the appropriate dilution (salinity checked with a refractometer) and covered with a circle of filter paper (Fig. 1). The filter paper became glistening wet, but without sufficient surface water to entangle the females. Cartons were placed about 60 cm (2 ft) below a 200 watt lamp, which provided light/dark 12:12, with simulated sunset and sunrise (about 45 min duration each). Full illumination in the bottom of the carton was about 400 ft-candles. Females were left in the cartons for 24 hr, then were removed and the number of eggs in each well counted under a stereomicroscope. Three replicates were done for each of two ranges (see results).

For experiments on salinity and egg survival, eggs were obtained by decapitation of fully gravid females (Linley 1965). Two experiments were done, (1) in which eggs were deposited directly onto filter paper wetted to one of four salinities (see results) and, (2) in which eggs were laid onto distilled water, kept 24 hr, then transferred to the different salinities (only eggs that darkened normally and completely were transferred). Salinities in the results are expressed as parts per thousand (⁰/oo).

RESULTS

OVIPOSITION AND SALINITY. With both ranges of salinity, analysis of variance for the latin square showed no significant differences between well rows or columns, indicating no positional bias within the carton. Thus, only the salinity effects are considered further. The first experiment tested for oviposition preference between salinities of 0.0, 9.9, 19.0 and 34.0 ⁰/oo, approximately equivalent to 0, 29, 56 and 100% sea water. The analysis of variance indicated highly significant differences ($P < 0.001$) between these treatments, as reflected in the mean numbers of eggs deposited on each salinity (experiment 1, Table 1). Females laid most eggs in wells wetted with distilled water and progressively fewer as salinity increased. No eggs were found at 34 ⁰/oo. To resolve further the response of females at salinities between 19.0 and 34.0 ⁰/oo, a second array of wells (experiment 2, Table 1) allowed females access to 19.0, 23.0, 28.0 and 34.0⁰/oo (approximately 56, 68, 82 and 100% sea water). At 19.0 ⁰/oo, the mean number of eggs was lower but not significantly so, than in experiment 1 (Table 1), while very few were found at 23.0 and 28.0 ⁰/oo and, again, none at 34 ⁰/oo.

The ranges and standard errors in Table 1 indicate considerable variation in egg count between wells of the same salinity. Some of this variation was probably due to the relatively large number of eggs laid by *C. variipennis* females. A few more females ovipositing on a particular well, as opposed to another, could result in quite large differences in egg count. In experiment 1, for example, a total of 25,881 eggs was laid in the 3 replicates. Assuming 125 eggs per female (Jones 1967), a total of about 200 females oviposited overall, or about 70 per replicate. In the case of the wells at 9.9 ⁰/oo, which accrued a total of 30.6% of all eggs laid, this would imply that only about 21 females laid on one or other of the 4 wells, hence the possibility for variation.

Table 1. Summary of experiments testing effect of salinity on oviposition of *Culicoides variipennis* females.

Experiment	Salinity (⁰/oo)	No. eggs laid/well Mean ± SE	Range
1	0.0	1,212.3 ± 748.4	419–2668
	9.9	658.8 ± 415.6	145–1492
	19.0	285.6 ± 264.8	4–804
	34.0	0	—
2	19.0	110.3 ± 100.5	0–261
	23.0	2.7 ± 5.7	0–18
	28.0	5.7 ± 15.7	0–57
	34.0	0	—

The mean number of eggs at 19.0 ⁰/oo did not differ significantly between experiments (Table 1), despite the presence of approximately equal numbers of gravid females in each. Only 1,423 eggs, in total, were deposited in experiment 2, comparing with 25,881 in experiment 1. Approximately the same number of females, however, laid in the 19 ⁰/oo wells in both experiments. At higher salinity, evidently, females did not lay preponderantly at 19.0 ⁰/oo because it was the most acceptable site, nor, in the lower salinity range, did females not lay at 19.0 ⁰/oo because more acceptable sites were available. Such results would arise if fixed proportions of females in the population become inhibited from laying only when salinity reaches a certain level, but find all lower salinities equally acceptable. Very few eggs were laid at 23.0 or 28.0 ⁰/oo. One well at 28.0 ⁰/oo had 57 eggs, which probably were laid by a single female, and only 2 other wells (out of 12) had any eggs (total 11).

The relationship between mean number of eggs laid and salinity can be described quite accurately ($r^2 = 0.963$) by linear regression (Fig. 2), which, as would be expected, was highly significant ($F = 78.247$, d.f. = 1,3). The regression coefficient implies that an increase in salinity of 10 ⁰/oo caused a reduction in eggs laid (under experimental conditions) of 458 eggs. By extrapolation, the salinity at which oviposition ceases is about 25 ⁰/oo, somewhat less than 75% of the concentration of sea water.

EGG SURVIVAL. Data summarizing the experiments on egg survival are given in Table 2.

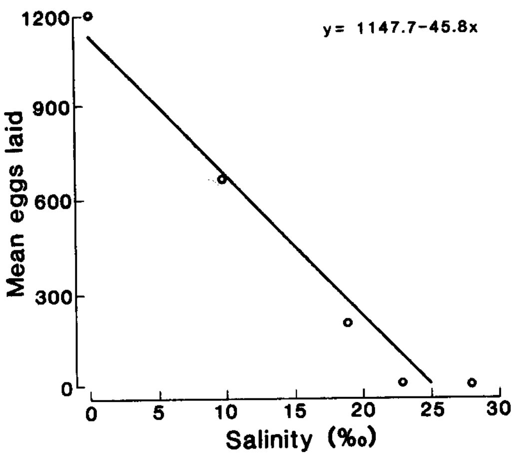

Fig. 2. Regression of mean number of eggs laid per well on salinity (‰). Value at 19.0 ‰ is mean of experiments 1 and 2.

In experiment 1, when the decapitated females deposited eggs directly on filter paper wetted to different salinities, only the results at 34 ‰ showed a sharp difference from the other groups. No eggs darkened (tanned) normally within about an hour, as healthy eggs do, and none hatched. From 0 to 19 ‰, comparable if not entirely consistent proportions tanned, failed to hatch even though embryonated (detectable by larval eyespots through the chorion), and hatched (Table 2). Results in the second experiment were similar, with one notable difference, namely that once eggs had become tanned (on fresh water), they were capable of surviving and hatching at 34 ‰ equally as well as those at lower salinities (Table 2). Survival was poor at 19 ‰, but this was almost certainly a chance event connected with low viability among the particular eggs in this experiment. The numbers of eggs in experiment 2 were rather low due to difficulties with obtaining enough females from the laboratory colony.

DISCUSSION

Consistent with its very wide distribution throughout the United States and into Mexico and Canada, *Culicoides variipennis* is a species of great biological diversity. Currently, it is considered to include 5 subspecies, the biologies of which have been discussed by Wirth and Morris (1985). The subspecies are associated with larval habitats that lie within broad ranges of salinity or alkalinity. Thus, *Culicoides variipennis variipennis* is found in freshwater habitats in the northern and eastern parts of the country, while subspecies *occidentalis* Wirth and Jones, *australis* Wirth and Jones, and *albertensis* Wirth and Jones are found in highly saline or alkaline environments (Wirth and Morris 1985). *Culicoides variipennis sonorensis*, of present concern, does not breed under saline conditions. Perhaps to a lesser degree than anticipated, the oviposition behavior is in accord with natural habitat selection, since about 56% of the total eggs (expt. 1, Table 1) were deposited on a non-saline substrate. The readiness of a considerable proportion of females to lay on salinities up to 19 ‰, and even somewhat higher, may be ascribed to the broad genetic variability known to be intrinsic in the subspecies *sonorensis* (Wirth and Morris 1985). Presumably, females of the more halophilic subspecies would be expected to oviposit predominantly on more saline sub-

Table 2. Summary of experiments testing effect of salinity on egg survival.

Experiment	Salinity (‰)	Total eggs	No. tanned (%)	No. embryonated but not hatched (%)	No. hatched (%)
	0.0	1291	916 (70.9)	22 (1.7)	436 (33.8)
	9.9	1060	950 (89.6)	14 (1.3)	400 (37.8)
1[1]	19.0	1560	1363 (87.4)	44 (2.8)	759 (48.7)
	34.0	816	0 (0.0)	0 (0.0)	0 (0.0)
	9.9	459	—[3]	6 (1.3)	111 (24.2)
2[2]	19.0	407	—	3 (0.7)	29 (7.1)
	34.0	610	—	15 (2.5)	250 (40.9)

[1] Eggs laid directly onto salinities.
[2] Eggs laid onto distilled water, transferred to salinities after 24 hr.
[3] Only tanned eggs were transferred after 24 hr.

strates, perhaps within a more limited range. It would be interesting to know if their eggs are capable of survival when laid directly on wet surfaces of 34 o/oo salinity and higher. Eggs of *C. variipennis sonorensis* cannot survive under such conditions (Table 2), but the behavior of the females ensures that the eggs would not normally encounter such environments. An increased understanding of the oviposition preferences of other subspecies is of potential practical importance as a means of identifying breeding sites. Among factors that might affect oviposition, only salinity was tested here. The experimental method worked well and should be applicable to tests involving other physical and chemical factors. With a larger array in an appropriate container, simple factorial designs might be attempted.

ACKNOWLEDGMENTS

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