DEPARTMENT OF AGRICULTURE, SOUTH AUSTRALIA

Agronomy Branch Report

THE POPULATION OF *Culex annulirostris* ALONG THE RIVER MURRAY IN SOUTH AUSTRALIA IN 1975

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The population of *Culex annulirostris* along the River Murray in South Australia in 1975

by Roger Laughlin *
and Peter Allen **

C. annulirostris may be a principal vector of Australian Arbo-encephalitis (Murray Valley encephalitis). The mosquito can infect chickens with the virus for 40 days after a latent period of about a week (McLean 1953). Virus has been detected in *C. annulirostris* in the field (Doherty 1964).

Miles & Howes (1953) suggest that outbreaks of encephalitis in man in the lower Murray Valley may be caused by a rise in the bird population of the river. Following high rainfall in the enzootic areas of Northern Australia, there is a general spread of birds, down to the river from the northern breeding grounds. These birds carry the virus and start to breed in the river area. The increase in numbers of viraemic nestlings and young birds is followed by an increase in the number of infective mosquitoes. Given the right conditions, the infective mosquitoes may carry the virus to man.

An outbreak may also require an unusually high mosquito population before it can occur.

The 1974 outbreak of encephalitis followed two winters of above average rainfall in the Murray Valley. The winter of 1974 was also wetter than usual, and health authorities throughout the area expected another outbreak in the summer of 1975. So the Public Health Department of South Australia decided to monitor the population of *C. annulirostris* in the river towns to see if any outbreak coincided with a rise in the numbers or activity of mosquitoes. Knowledge of the population level would also help health officers to decide when and if mosquito control programmes should be set in motion.

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Methods

Twenty-seven light/suction traps were located between Renmark and Murray Bridge. Where possible the traps were placed in pairs 100-200 m apart. Three or 4 pairs were allocated to each of the towns of Murray Bridge, Mannum, Loxton and Renmark. One trap was located at Berri beside a pen of sentinel chickens examined regularly for virus. The traps were switched on in the late afternoon and emptied each morning. Male and female C. annulirostris were counted and engorged females noted. The traps ran for 64 days from 30 January to 4 April 1975.

Local government health inspectors and Department of Public Health personnel looked after the traps and also mounted a search for larval populations. Descriptions of waters inhabited by larvae were entered on special forms. Waters with no larvae were not described in detail but each officer returned a statement of the number of hours spent searching for larvae. The search was sporadic and could not be directed or planned because it was only one small item in each officer's multifarious round of duties, a round which had already been expanded considerably by the twice-daily visits to the traps.

A regular (weekly or fortnightly) search for larvae was mounted by Department of Public Health officers from Adelaide. Each week an officer visited 8 sampling areas between Swan Reach and Murray Bridge. Every fortnight the search extended to Morgan to include a further 4 sampling stations.

On one occasion (6 February 1975) an area of 65 ha round one of the more successful traps at Renmark was searched for larvae by the entomologist in charge of the programme. All waters were found, mapped and sampled for larvae. Where larvae were present, an estimate was made of the population of the pool.

In April a similar search was undertaken on the lower Murray by units of the C.M.F.

Results: Trapping adults

Over the whole trapping period, every trap caught at least one Culex annulirostris (Table 1). The baited trap at Berri caught

Table 1. *Culex annulirostris* Light Trap Catches

Place	Trap No.	No. of Nights Operating	Corrected ** Total Catch (59 nights) ♂	♀	Sex Ratio (♂/♂+♀ %)	Catch Corrected for Sex Ratio (♂ + ♀)
Murray Bridge	1	62	2	6	25	8
	2	62	6	23	21	29
	3	59	10	17	37	27
	4	61	7	5	32	22
	5	62	1	6	14	7
	6	60	0	1		1
	7	48	0	1		1
Mannum	1	63	8	9	47	17
	2	62	5	3	63	8
	3	62	4	2	67	6
	4	59	108	23	82*	46
	5	60	63	9	88*	18
	6	58	9	21	30	30
Loxton	E1	51	96	117	45	213
	E2	57	47	16	75*	32
	F1	51	12	82	13*	24
	F2	51	9	50	15*	18
	G1	51	68	57	54	125
	G2	50	60	91	40	151
Berri		45	42	451	9*	84
Renmark	A1	51	54	65	45	119
	A2	51	273	73	79*	146
	B1	49	24	21	53	45
	C1	50	34	40	46	74
	C2	50	5	7	42	12
	P1	50	18	48	27	36
	D2	51	5	14	26	19

Geometric means of trap catch totals

	Murray Bridge	Mannum	Renmark	Loxton
♂	2.2	15.3	25.3	36.0
♀ ***	5.8	7.8	29.4	58.6
♂ + ♀	7.2	24.8	57.4	105.6
♂ + ♀ corrected for sex ratio	7.2	16.4	46.5	62.2

* Only these sex ratios are significantly different from 50% (P = 0.01)

** Corrected catch = Actual catch x 59/No. of operating nights.

*** Means bracketted by a line are not significantly different (P = .05)

452 and the biggest catch for an unbaited trap was 309 for trap A 2 at Renmark. A total of 2500 flies was caught, 52% of them males. Analysis of variance of $\log_e$ (trap catch) between and within pairs of traps for each town in turn (excluding Mannum where only traps 1 and 2 were paired) gives non-significant variance ratios ($P = 0.05$) for a) numbers of females caught, b) total mosquitoes caught, and c) total corrected for sex ratio - see below. Thus there is no evidence that traps placed close together are any less variable than traps placed far apart. Traps placed close together may differ greatly in their success (e.g. Loxton E 1 and E 2, Table 1), supporting the idea (Mattingly 1969, etc.) that activity tends to be concentrated into flight paths and that mosquitoes congregate in particular areas for mating, feeding, resting, and oviposition. In the case of the male catch the analysis for Murray Bridge was just not significant ($P = .06$) and for Loxton was significant at the .02 level. These results may indicate that male activity patterns are less patchy than in the female - that the male mosquito moves more freely in the local environment, not keeping to defined flight paths.

Analysis of the variance between and within towns (excluding the Berri trap and disregarding the pairing of traps) gave significant variance ratios for all analyses. A Tukey's test on the differences between the log means shows that Murray Bridge yielded fewer mosquitoes than Renmark or Loxton and that Mannum stands between, with catches not significantly different from anywhere else except for the female catch at Loxton. This last result is due to the surprisingly high catches from Traps 4 and 5 at Mannum, a further indication of the patchiness of the adult population.

The main conclusion from the 'total trap catches' is that mosquito populations are higher in the Loxton, Renmark area than in the Murray Bridge area. This result supports the suggestion (Waterhouse, personal communication) that the lower Murray's narrow flood plain provides relatively few larval habitats.

The sex ratios ($SR = \frac{100 \times \text{male catch}}{\text{male catch} + \text{female catch}}$) of the trap catches provide some interesting points. Over all traps, all nights, equal numbers of males and females were caught ($SR = 51.9\%$ for 2500 adults, not significantly different from 50%). Yet some traps caught significantly more of one sex than the other (see Table 1).

Four traps (Berri, Loxton F 1 and F 2, and Renmark D 1) caught mostly females. In the case of the Berri trap the reason is obvious: A baited trap attracts large numbers of females in search of a blood meal. Loxton F 1 and F 2 were close to an Old Folks Home which, with its high density of human population at night, probably produced the equivalent of a pair of baited traps. Renmark D 1, on the other hand, was not close to either people or animals, but it was close to one of the few larval habitats found in the course of the survey. The flies caught in this trap may well have been mostly gravid females on their way to oviposit. There was not enough time to study the reproductive state of trapped females in this survey - an omission which would have to be remedied in any future study.

Four traps (Renmark A 2, Mannum 4 and 5 and Loxton E 2) caught mostly males. There were no obvious features to the surroundings of these 4 traps to set them apart from the others. The other traps of the pairs (i.e. Renmark A 1 and Loxton E 1) actually caught fewer than 50% males though not significantly so. There are several possible explanations for a preponderance of males and further information will be needed before any of them might be thought probable. Thus a trap catching mostly males might be set close to, or on the flight path to, a patch of flowers that the males visit to feed; or it might be set in or close to a mosquito shelter or resting place where both males and females may rest during the day but which is inhabited mostly by males at night because female activity is more wide-ranging. Females must search for both blood meals and oviposition sites as well as mates. Males need only find mates and flowers (a static 'prey') and even the former search may, in this species, involve nothing more strenuous than congregating in a likely spot and waiting for the females to arrive.

Even minor, sex-linked variations in flight path or local levels of activity could produce these results. The conclusion to be drawn from these 4 traps at this stage is that the study of male and female behaviour in this mosquito is likely to be fruitful and interesting.

Looking at the nightly total for all traps there are only 2 nights on which significantly more females were caught.

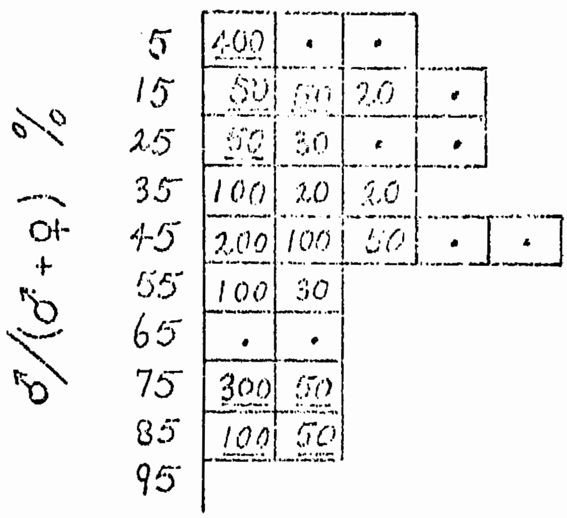
The frequency distributions of sex ratio (Fig. 1) indicate clearly that, while there are 'male-favouring' and 'female-favouring' traps, there are probably not 'male flight nights' and 'female flight nights', i.e. nights in which the flight activity of one sex is markedly greater than the other.

Very few engorged females were caught in the traps. Only 12 were seen in the whole of the study, 8 of these from the Berri trap. Engorged females probably fly very little while maturing the next batch of eggs nor are they likely to be a large class (numerically) in the adult population; hence the low numbers caught may be a true measure of the relative frequencies of engorged and unengorged females in the population and not just a result of a disinclination, on the part of the engorged female, to fly to a light. However, it is impossible to draw definite conclusions from this data; other evidence (obtained by other methods) of the composition of the female population is required.

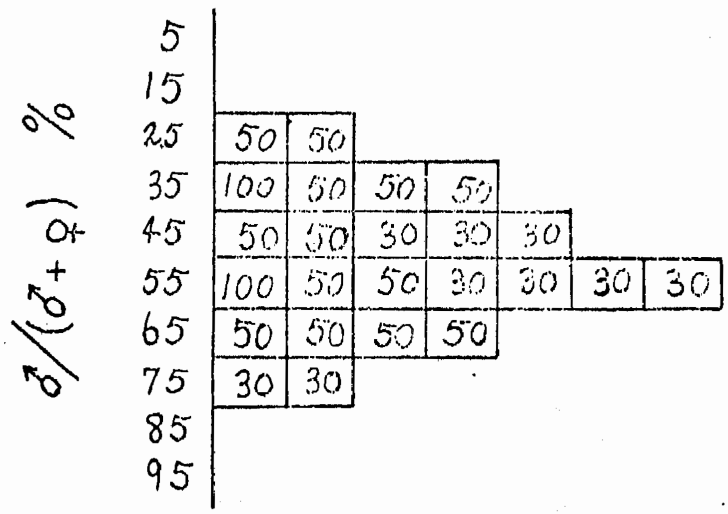
The nightly trap catches for each trap and each district are plotted in Hardy's report. A peak of numbers can be discerned for Loxton, Renmark and Berri about the middle of February (Fig. 2). If the data are plotted as 7-day running means (Fig. 3) the peaks are smoothed and easier to see. The curves also show that there may be an early peak at the beginning of February and a small late peak about the middle of March.

Another statistic plotted on Figure 3 is the 7-day running mean for number of traps which caught any <u>C. annulirostris</u> (successful traps). The close correlation between this curve and the curve for mean mosquito numbers indicates that, in general, the traps were placed within the usual ambit of the adult population, in positions quite likely to catch mosquitoes. Had the traps been placed so that an appreciable number were on the fringes or outside the normal flight ambit, a different picture might have resulted. Thus an increase in the range of activity following a rise in humidity, with mosquitoes spreading out from their usual haunts, could result in a large increase in the number of successful traps with a concomitant rise in total catch. On the other hand, a rise in catch without a rise in numbers of successful traps could indicate an increase in population.

Figure 1. Distributions for light trap totals



Distribution of sex ratios among total catches from each trap. Each box represents one catch. The number in the box indicates the approximate number of mosquitoes caught (. = 10 or less). Underlined numbers indicate a catch with sex ratio significantly different from 50% (P = 0.01).



Distribution of sex ratios among nightly totals where over 30 mosquitoes were caught.

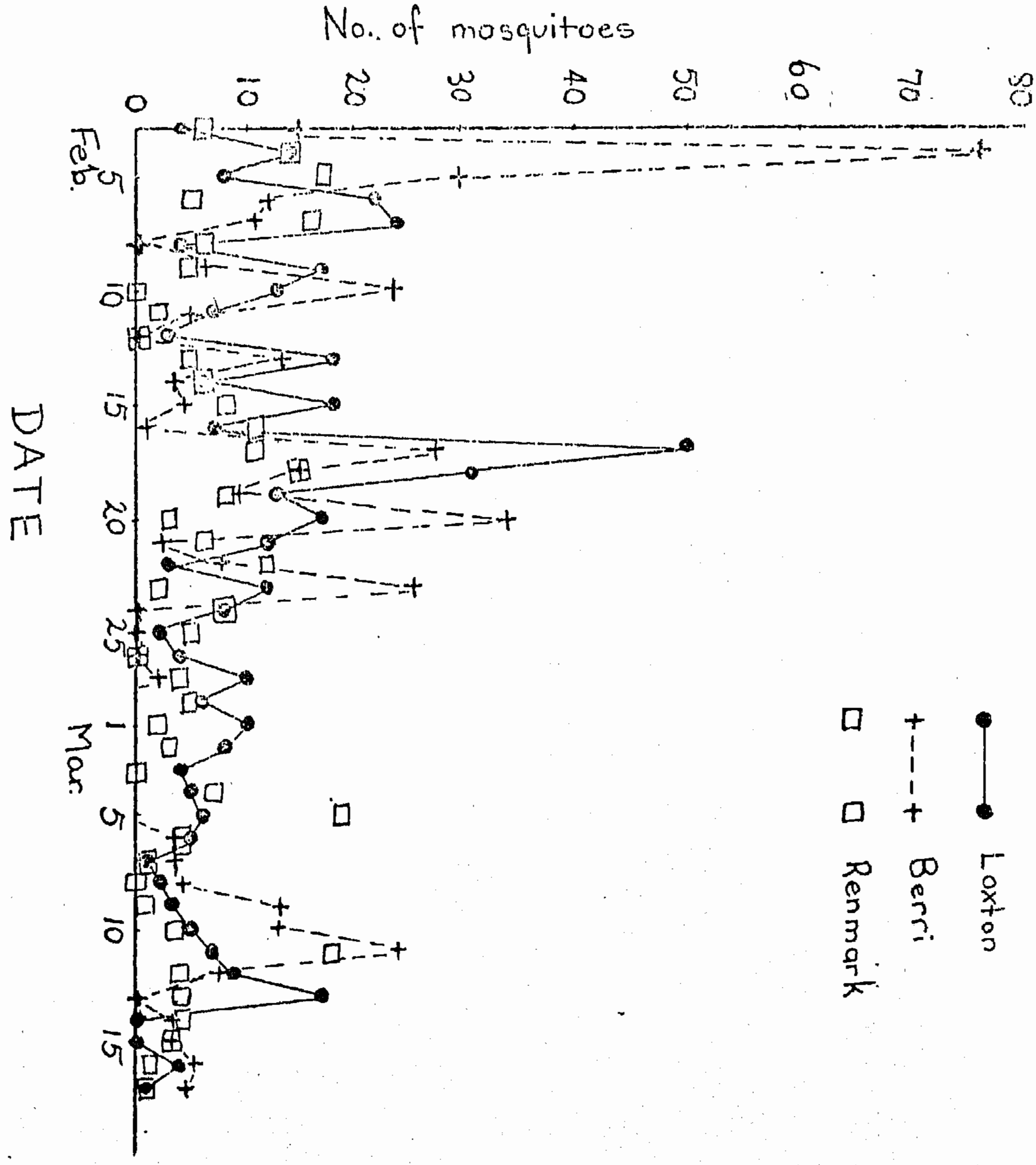


Figure 2. Nightly catch of female mosquitoes caught in light traps.

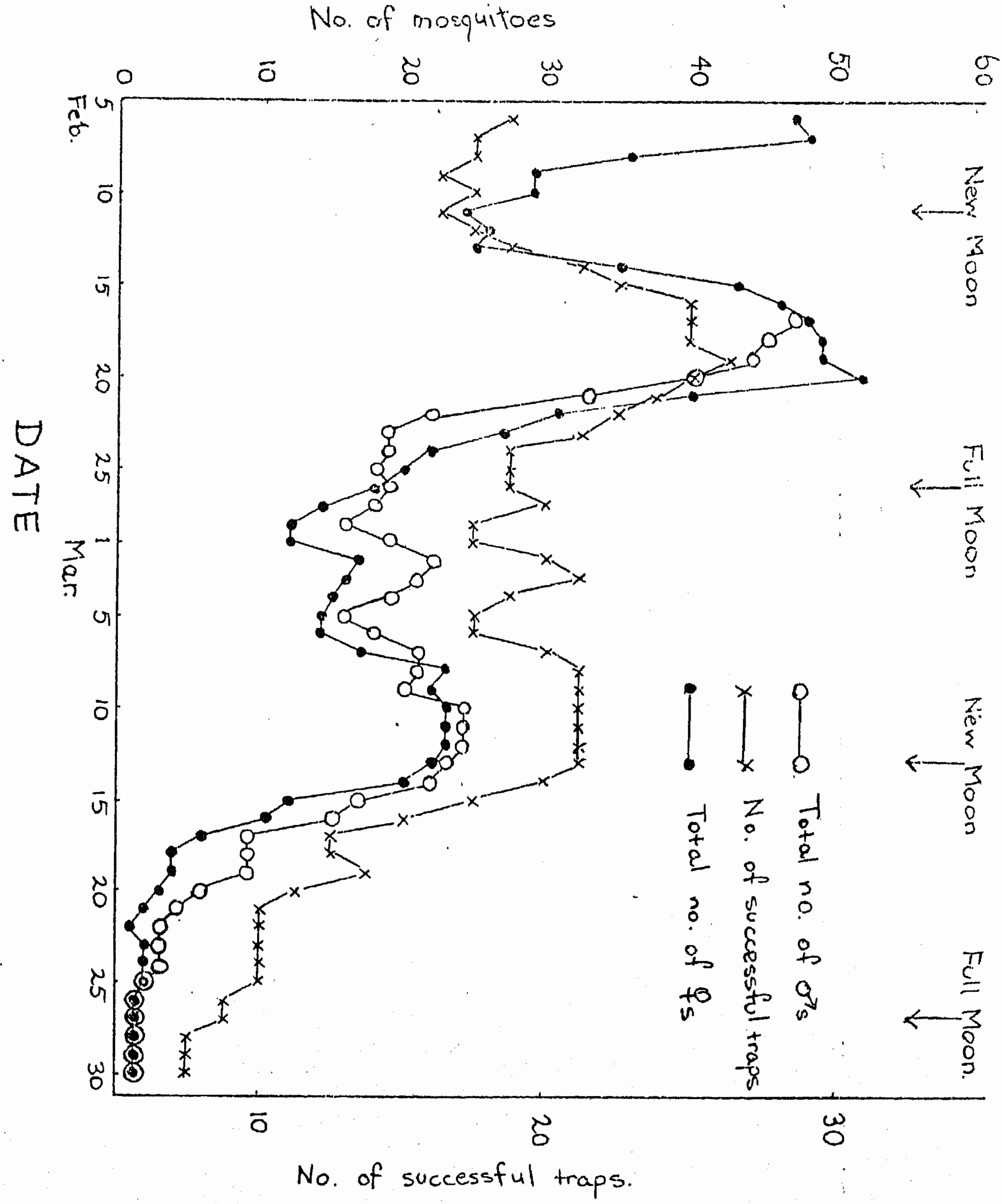


Figure 3. Seven day running means for catch of mosquitoes from all 27 light traps and for number of 'successful traps' (see text).

With the present data it is difficult to say whether the observed peaks are due to increased numbers, increased activity or both. The major peak in the middle of February is probably indicative of a high population since it persists for a number of days, is shown by both male and female numbers and is common to all of the regions studied, though very slight at Murray Bridge and Mannum.

Full moon and new moon dates are indicated in Figure 3. Moonlight is known to reduce light trap catches and could have caused the low catches between 24 February and 6 March. Perhaps, then, there is but one population peak lasting from mid-February to mid-March. The trough of February 9 - 13 occurs at the new moon and would not be removed by any correction for the effect of moonlight.

The correction for sex ratio mentioned on p. 4 was made only on those traps with a sex ratio significantly different from 50% (P = 0.01). There were 8 such traps (marked * in Table 1). Two assumptions were made: 1) That the more frequent sex was present in high numbers for one or other of the reasons given on p. 5; 2) that the numbers of the less frequent sex were likely to be representative of the general catching efficiency of the trap. So the number caught of the less frequent sex was doubled to give the corrected catch. The correction does not affect the outcome of the analysis of variance and Tuhey's test of differences between means. It does group the means a little closer, moving Mannum closer to Murray Bridge and Loxton closer to Renmark while slightly widening the gap between Renmark and Mannum.

Correlations of trap catch at Loxton and Renmark with maximum temperature and relative humidity are given in Table 3 of Hardy's report. There was a significant correlation between catch and daily maximum at both places and a significant negative correlation between catch and humidity (measured at 9 a.m.) at Loxton only. The correlation persisted if humidity readings at sunset (only available for Loxton) were used.

To take this analysis a little further (Table 2) the trap catches can be subtracted from each day's value of the 7-day running mean. These departures from the running mean are likely to be caused more by fluctuations in mosquito activity than by changes in population. If the departures are correlated with the weather data for Murray Bridge, Loxton and Renmark (using means of the daily figures from all

Table 2. Correlation Coefficients for Weather Data and Trap Catches.

	Departures from 7-day running mean ♂	♀
Maximum temperature	.390	.221
Minimum temperature	.502*	.288
Relative humidity (9 a.m.)	- .571*	- .366*

	Maximum	Minimum	Humidity
Murray Bridge } Loxton	.887	.648	.550
Loxton } Renmark	.959	.913	.816
Murray Bridge } Loxton } Renmark	.797	.709	.628

3 places

1) the correlation with maximum temperature is no longer significant for males and females. The original correlation probably arose through the coincidence of peak trap catches with the hottest part of the trapping period. As temperatures declined through February and March, so did the population of mosquitoes.

   On the other hand, the negative correlation between catch and humidity (Hardy's report Table 3) is present and significant for both male and female departures from the running mean (Table 2). One possible source of this phenomenon is in the relation between activity and temperature.

2) activity in insects shows a positive correlation with temperature over at least part of the temperature range of the environment; relative humidity shows a negative correlation with temperature; perhaps then the meaningful correlation is with night temperature – the best available measure of which, in this case, is the nightly minimum. However, partial correlation of 'catch departure', humidity and minimum temperature give inconclusive and conflicting results which may be clarified by further data but which cannot profitably be discussed further here.

Correlations between the weather data values for each place are included in Table 2 to show that the use of a general mean for the whole of the Murray Valley in South Australia is a reasonable procedure. The coefficients are high enough to allow the region to be considered as a single unit if a large and widespread change of activity is being studied. Just such a change was postulated by Waterhouse (personal communication): That with a rise in humidity C. annulirostris adults quit their normal ambit of activity and range far and wide away from the river. However, as mentioned above, relative humidity did not rise very high in the Riverland during the study period. The summer of 1974-75 was remarkably dry. Normally the river towns experience at least one period of 3 or 4 days of cool, misty weather with drizzly rain. These periods are known as the 'Currant Rains' since they frequently occur when the currants are drying, hindering the drying process with their days and nights of high humidity.

Possibly, then, there were no nights damp enough for really intense activity. Below a particular humidity, there may well be a negative correlation with activity, revealed here in the absence of high RH values.

Another possibility is that the mosquitoes did in fact range further afield as the humidity rose. The effect of this would be to lower the density of flies in the vicinity of the traps (all placed well within the normal flight range) and hence to produce the negative correlation. This speculation is included to illustrate the difficulty of interpreting trap data where no supporting data is available.

Results: Sampling for larvae

An area of 65.2 ha round traps A1 and A2 (Grimshaw's Caravan Park), on both sides of the approach road to the Paringa bridge was surveyed. With the aid of a 1:10,000 scale aerial photograph, all pools were mapped and their areas measured. There were 15 pools ranging in size from 30 x 60 cm to 350 x 150 m and 3 groups of empty bottles.

Larvae were found in one place only, a weed-covered pool beside the road, 2000 m^2 in area. Nine samples of water from this pool yielded 3 larvae and 1 pupa of C. annulirostris (3 positive samples and 1 negative) and gave an estimate of about 1000 for the total number in the pool.

One working day was required for this piece of information and it is the only area estimate of larval numbers made during the research programme, apart from 2 similar samples, one near Murray Bridge and one near Mannum, taken by units of the C.M.F. under the direction of Mr. Smith. These samples covered a total of 4000 ha and yielded only 4 larvae of C. annulirostris. No estimate of larval population was made.

These 3 samples illustrate one of the difficulties attached to studies of mosquito ecology. A 2-stage sample is required for an estimate of larval population. First, areas must be searched for pools of water, then the pools must be searched for larvae. The areas must constitute a properly chosen (in the statistical sense) sample of the region under study. Then each pool within the area must be properly sampled for an estimate of numbers of larvae. A third sampling procedure may be required if there are so many pools in an area that it is impossible to sample them all in the time available.

Thus each area requires at least a day's work to yield a reasonable estimate of larval numbers. At least 10 areas are desirable for a regional sample, and if the population is being monitored through a period of time, then samples must be repeated every week to 10 days. Succeeding samples take less time, of course, since, if the same areas are studied, the mapping is already done - unless there has been rain, flooding, effluent discharge, irrigation, etc. going on in the interval between samples.

The results of the larval search by DPH officials and Local Board personnel have been ably summarized in Mr. Smith's report. Only 5 pools containing C. annulirostris larvae were found, all in the Upper Murray region. The survey was of only minor help in defining the habitat of C. annulirostris but did yield the valuable information that mosquitoes were scarce on the Murray in 1975. This fact could not be deduced from the light trap catches since there was no way of relating trap catch to absolute population size.

Future larval searches must be better planned and a standard procedure must be used. It has proved impossible to collate the data on negative samples provided by the different people involved.

Future Work

If the traps are to be used again to monitor adult population and activity levels then fewer traps, operated less frequently, would suffice. Thus, 3 traps per town, unpaired, sited in the same places as before and operated on week nights only, would give sufficient information to assess major increases in numbers from week to week and from year to year.

If the object is to gather information on flight paths and behaviour then all the traps should be operated in one area as part of a detailed study programme requiring trained personnel over a long period. Since the Victorian DPH appears to be attempting this sort of project, this course is not recommended unless it is undertaken in close collaboration with Victoria.

If a search for larvae is to be conducted, its objectives must be clearly defined. If the object is to seek out populations and apply control measures, the diffuse and wide-ranging system used in 1975 would be appropriate (if improved). If, on the other hand, the object is to estimate the number of mosquitoes being bred in a region, a set of area samples similar to that described above would be required.

The work planned for 1975 attempted to do many things: The light traps monitored the rise and fall of population, investigated the effect of propinquity, might have shown the effect of control measures if they had been applied; the larval search was designed to demonstrate periods of egg-laying, to measure the density of the larval population, to correlate habitat with larval occurrence. Future work should be less ambitious and should plump for one or two objectives only. Scientific objectives in mosquito ecology require sustained effort and a great deal of time and money. Practical objectives may be more readily attainable and are certainly more easily funded.

All of this report has been concerned with Culex annulirostris. This species is almost certainly a vector of AAE but is not necessarily the only or even the most important species involved. Research into the ecology of other species could be more profitable, especially if the project was widened to include the transmission of the virus responsible for polyarthritis.

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Mr. Curtin, a resident of Caloote Landing, operated one of the traps.

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