THE ECOLOGY OF *AEDES ALBOANNULATUS* MACQ. AND *ANOPHELES ANNULIPES* WALK. (DIPTERA: CULICIDAE) WITH REFERENCE TO THE TRANSMISSION OF MYXOMATOSIS IN SOUTH AUSTRALIA.

by

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SUMMARY

The ecology of Aedes alboannulatus and Anopheles annulipes (Diptera : culicidae) was studied, with special reference to myxomatosis of rabbits, at Hackham in the Adelaide Hills and at Teal Flat on the River Murray.

At both places, Ae. alboannulatus breeds only in transient pools. At Hackham the larval stage lasted about ten days in summer and about fifty days in winter. The eggs are laid on moist soil just above the water and usually do not hatch until submerged.

An inverse linear relationship was found between oxygen tension and hatching response. A second experiment with eggs from different females showed that all the eggs hatched within twenty minutes when kept at 27°C, but at 5°C to 15°C the hatching response varied from 0 to 100%. At 27°C, embryogenesis lasts about five days. Water is absorbed during the first twenty-four hours, after which resistance to desiccation steadily increases, up to the fifth or sixth day. A Probit analysis of the lethal influence of dryness on mature eggs gave an L.D.33.3 of 1.75 $\pm$ .488 days at 33% R.H. During a hot summer, many eggs laid beneath leaf-litter were exposed to temperatures up to 11°C below air-temperature, and survived for one and a half months.

Although the larvae were restricted to transient pools, experiments in the field showed that eggs were laid around both permanent and transient pools. In the laboratory, first-instar larvae which hatched on moist surfaces, crawled away from light; if eggs hatched around pools without being submerged, this reaction would lead the larvae to water.

The amount of food in natural pondwater was measured in the laboratory. Growth-rates of larvae increased when pondwater was concentrated up to four times normal, indicating that larvae experienced a relative

shortage of food in some pools.

Regular estimates of the population of larvae and pupae at Hackham showed that abundance is related mainly to weather. The numbers of eggs hatching depend on suitable falls of rain in both summer and winter. Two hazards of weather are summer droughts and winter floods.

A new method was developed for estimating the absolute numbers of larvae in a pool, in which the numbers (y) in successive catches (x) are related by $y = Aq^x$; where A is a constant, and $(1-q)$ is the proportion removed in each sample. The estimate is $A/(1-q)$.

A field-experiment on predation by Nectersoma dispar (Coleoptera : dytiscidae) indicated that predators have little influence on abundance.

An. annulipes breeds in permanent pools at Hackham and in vegetated swamps and river-margins along the River Murray. Experiments on larval food showed a relative shortage in treated pondwater and suggested that living food is important for Anopheles.

Regular estimates of the population of larvae and pupae at Hackham showed that Anopheles was abundant during summer and rare during winter. A partial regression of numbers on rainfall and temperature accounted for 37% of the total variability. Numbers were positively correlated with temperature and negatively correlated with rainfall. Other factors influencing numbers were predators and changes in the resources of breeding places. At Teal Flat, abundance was also related to resources of breeding places, which depended on the extent and timing of the annual flood.

Both species were active for a short period at dusk; light intensity determined the onset of biting and other components of weather influenced the numbers biting.

Neither species could be reared in cages. Many experiments in the

laboratory and in large outdoor cages showed that neither species mated, although they fed normally and laid infertile eggs.

The incidence of myxomatosis and rabbits at Hackham was determined by sight-counts over a standard route. The only outbreak of myxomatosis occurred in 1958-59 when An. annulipes was abundant but Ae. alboannulatus rare.

The incidence of myxomatosis at Teal Flat was estimated by shooting rabbits and testing their sera for antibodies. After a severe outbreak in 1958-59, 100% of the rabbits were immune. Susceptible rabbits born in the spring resulted in 29% of immune rabbits in December 1959. No severe outbreak occurred in 1959-60 and by November 1960, the proportion of immune rabbits had decreased to 18%; again no severe outbreak occurred in 1960-61. These results suggest that the high proportion of acquired immunity after an outbreak, prevents further outbreaks for several years. At Teal Flat, an annual rate of increase of 4.8 kittens per female rabbit was estimated from the serological data. In other areas, with a higher rainfall, the rabbits would have a greater rate of increase, which would reduce the effect of acquired immunity.

In September 1958, 50% of the rabbits collected at Coopers Creek in the Far North of South Australia were immune, although no recent outbreak of myxomatosis had been reported. The persistance of myxomatosis in the Far North of South Australia led to the hypothesis that myxomatosis released by Bull and Mules, in 1943, persisted in the area and contributed to the rapid and vast spread of myxomatosis in 1950.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and, to the best of my knowledge, contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

CONTENTS

1. INTRODUCTION.

The European wild rabbit, Oryctolagus cuniculus, was introduced into Australia in 1859; it soon spread over the southern half of the continent and became a major pest. Several attempts were made to use myxomatosis for the biological control of the rabbit, and finally, in the summer of 1950-51, myxomatosis suddenly became widespread in south-eastern Australia. By the end of the summer, myxomatosis was distributed throughout the Murray-Darling River system, an area of 1,000 miles from north to south, and 1,100 miles from east to west (Ratcliffe et al., 1952). The natural spread of myxomatosis was supplemented by innoculation campaigns and by farmers moving sick rabbits from one area to another. By 1954, the distribution of myxomatosis was virtually coincident with the distribution of the rabbit. The economic value of myxomatosis can be judged from the estimate of Reid (1953) that in the sheep industry alone, myxomatosis resulted in an increased production of £30 million in 1952-53.

It soon became apparent that myxomatosis was an insect-borne disease and officers of the Wildlife Survey Section of C.S.I.R.O. studied the ecology of the vectors. Over much of Eastern Australia, including the vast Murray-Darling River system, there was a regular outbreak of myxomatosis every summer, and it was found that the two mosquitoes Anopheles annulipes Walk. and Culex annulirostris Skuse, were the most important vectors (Ratcliffe, 1955).

Work in the laboratory showed that myxomatosis was transmitted mechanically, that is, there is no multiplication of the virus in the vector. This has three important consequences; firstly, any blood-sucking arthropod is capable of being a vector. Secondly, there is no

extrinsic incubation period, as found for example with malaria, and thirdly the mouthparts of vectors became infective when lesions that have a high titre of virus are probed; viraemia is not of direct importance (Fenner, Day and Woodroofe, 1952; Day et al., 1956).

The case-mortality rate was estimated in the field from the observed mortality of rabbits and from serological data. In early outbreaks the case-mortality rate was a high as 99.8%, but within one or two years it had decreased to 90% (Myers, Marshall and Fenner, 1954). Subsequently the case-mortality rate decreased further, due to attenuation of the virus and to an increase in the genetic resistance of the rabbit.

The attenuation of the virus was a result of mechanical transmission. Attenuated strains were favoured by natural selection because they produced lesions which were infective for several weeks, whereas the highly virulent strain produced lesions that were infective for only about five days before the rabbit died (Fenner et al., 1956).

Changes in genetic resistance in wild rabbits were detected by challenging rabbits with a standard virus of grade III virulence (Marshall and Fenner, 1958). Initially the virus killed about 90% of the rabbits, but after the seventh annual epizootic the case-mortality rate was down to 30% (Fenner, 1959a).

Most of the early work was done in Eastern Australia and consequently little was known about myxomatosis and vectors in South Australia. Lines (1952) documented the initial spread of myxomatosis in South Australia and briefly discussed the incidence of mosquitoes. In 1957, an officer of the Department of Agriculture of South Australia told me that outbreaks of myxomatosis occurred in the Adelaide Hills in winter, whereas outbreaks along the Murray River occurred in summer.

I chose two areas in which to study the ecology of vectors with special reference to the incidence of myxomatosis. One area was in the Adelaide Hills, near Hackham, about 25 miles by road from Adelaide. The other area was at Teal Flat, on the Murray River, upstream from Mannum, and about 70 miles by road from Adelaide.

A preliminary survey at Hackham showed that An. annulipes and Aedes alboannulatus Macq. were the most abundant species of blood-sucking arthropods. I selected these two species for study because An. annulipes was known to be an important vector in Eastern Australia; and Ae. alboannulatus was abundant in winter and may have been responsible for the reported outbreaks of myxomatosis in winter.

At Teal Flat, An. annulipes was also the most abundant species, and I hoped that the contrast between Teal Flat and Hackham would be interesting ecologically.

In studying the ecology of An. annulipes and Ae. alboannulatus I have followed the teachings of Andrewartha and Birch (1954). Their approach consists of firstly investigating aspects of physiology and behaviour that are relevant to the natural history of the animal, and secondly of measuring the numbers of individuals in a population as accurately as possible and relating the numbers to the components of the animal's environment.

The original classification of the four components of the environment has since been modified into: (a) weather, (b) resources, (c) other animals and pathogens, and (d) certain characteristics of the place in which the animal lives that cannot be regarded as resources. The idea of resources includes all the material necessities of life such as food and pools in which mosquitoes breed (Andrewartha and Browning, 1961).

The levels of significance of statistical analyses have been denoted by asterisks. One asterisk indicates probabilities between .05 and .01, two indicate probabilities between .01 and .001, and three indicate probabilities greater than .001. I have used both the 5% Fiducial Limits, as defined by Fisher (1954), and the 95% Confidence Limits, as defined by Davies (1957). In each case I have followed the usage of the author who described the particular analysis.

2. AEDES ALBOANNULATUS.

2.1 Description of the study-area at Hackham.

2.11 Physiography. The study-area was confined to the upper reaches of a creek near Hackham, about 14 miles south of Adelaide. A map of the creek is shown in figure 2.01 with the numbers of some of the more important pools. The numbers D1 and D2 refer to two dams made by man, and similarly the numbers W1 to W4 refer to old, disused wells.

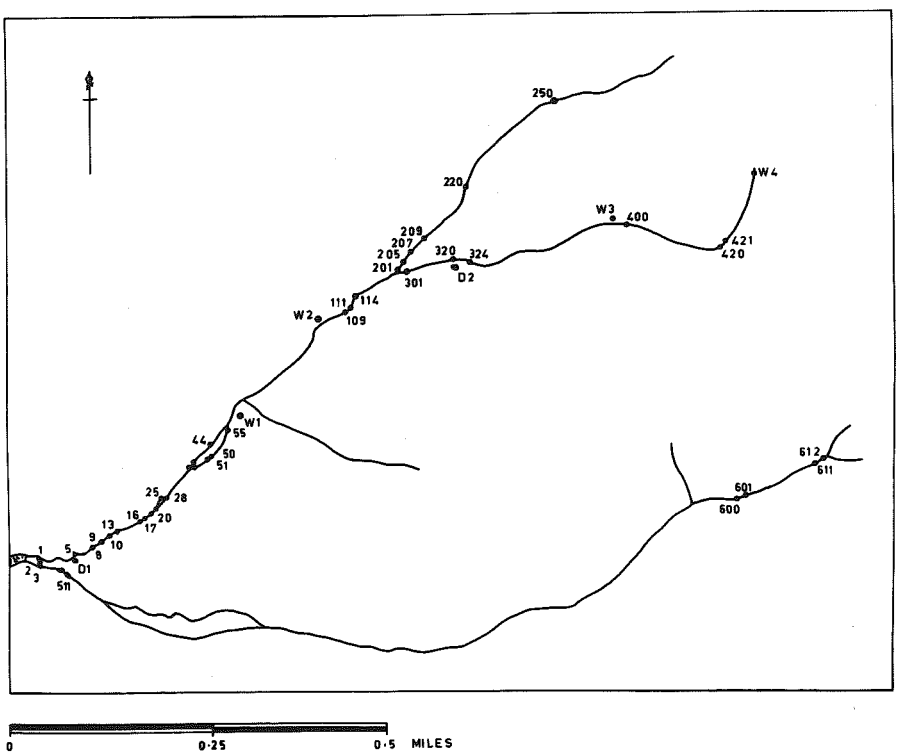


Figure 2.01: A map of the creek at Hackham, showing pool numbers.

The creek rises among rounded hills, the origins of the two main tributaries being 900 feet above sea-level, and about 1 mile from the point where the two tributaries join at pool 1. Pool 1 is about 560 feet above sea-level, which gives the creek a fall of 330 feet in 1 mile. The creek consists of chains of ponds formed in the soil, connected by narrow channels. Rocky outcrops occur at only two places, below pool 20 and between pools 205 and 220.

2.12 Climate. Daily rainfall was measured in a standard rain-gauge at a farm 1 mile from the creek. The records for 1959 were incomplete, so I also used the official records from the post office at Morphett Vale, $2\frac{1}{2}$ miles from the study-area. The mean monthly rainfall at Hackham for the seven years 1953 to 1960 (excluding 1959) is shown in figure 2.02.

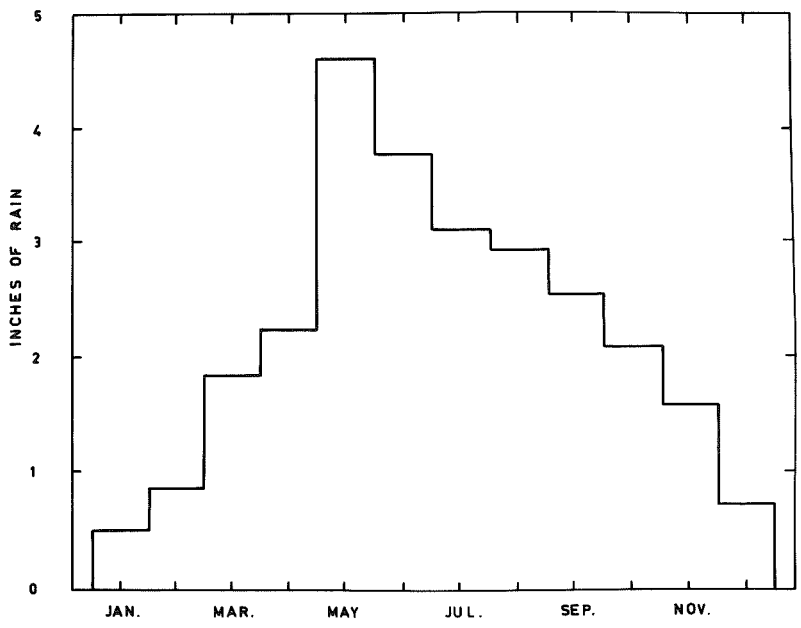


Figure 2.02: The mean monthly rainfall at Hackham for the seven years 1953 to 1960 (excluding 1959).

The nearest records of air temperature were at the Waite Agricultural Research Institute, about 12 miles to the North of Hackham. The maximum and minimum means of the 25 years, 1925 to 1949 are shown in figure 2.03.

The climate of the area is the Mediterranean-type with most of the rain falling in the winter. The summers are dry and hot with the warmest mean monthly maximum temperature above 80°F. The winters are mild with the coldest mean monthly minimum temperature of about 45°F.

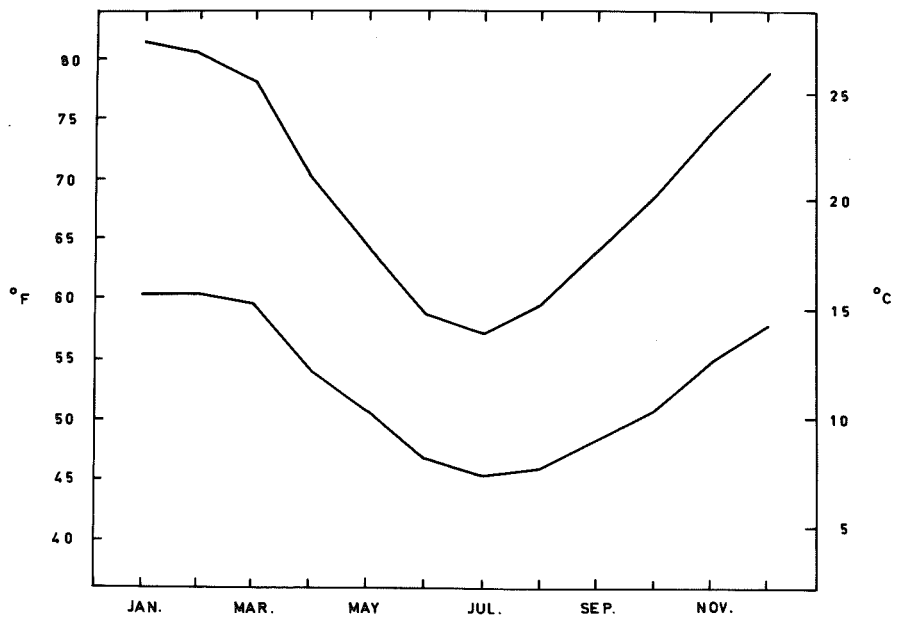


Figure 2.03: Monthly maximum and minimum air-temperatures at the Waite Agricultural Research Institute. Mean temperatures for the 25 years 1925 to 1949.

2.13 Effect of rainfall on the creek. By the end of summer nearly the whole creek is dry, except for two places where pools are kept full from springs. One spring is just above pool 17 and the other is above D2. With the onset of winter rains, the creek begins to fill. The northern tributary fills first, and is followed by the southern tributary later in winter. Then, with the approach of summer, decreasing rainfall is accompanied by increasing temperatures and the process is reversed. The southern tributary dries out first and then most of the northern tributary dries out. This cycle of events will be discussed more fully in section 2.42.

2.2 The natural history of Ae. alboannulatus.

2.21 The life-cycle in relation to temperature. Like many other aedine species, Ae. alboannulatus lays its eggs on the moist soil around the edges of pools. In the laboratory the embryo matures in 4 to 5 days at 27°C, provided the egg remains moist. Once the embryo has matured, the egg can withstand greater dryness. The eggs usually hatch when the water in the pool rises and submerges them. Occasionally, in late summer when some transient pools first filled, large numbers of larvae hatched. Subsequently, the larval instars tended to be synchronized, with all the larvae pupating at about the same time. This result indicates that all the eggs hatched promptly after being submerged.

In the laboratory I tried to measure the influence of temperature on the speed of development of the larvae, but I was unable to get reproducible results. Probably this was due to the difficulty of providing adequate and uniform food in different experiments. However, I was able to measure the duration of the larval stage in the field, both at summer and winter temperatures. The observations are given in table 2.01.

The observations in February and March are quite clear-cut because the pools filled after heavy rains, and no more rain fell before the larvae had pupated. Thus, the pupae counted came from eggs which had all hatched at the same time.

Table 2.01: Duration of the larval stage in the field.

Date	Observation	% pupae	Duration
13 Feb.59	Eggs hatched in pools 8,18,21,301		
	Pool 8, 610 larvae : 257 pupae		
	" 18, 1695 " :1868 "		
	" 21, 181 " :3019 "		
	" 301, 64 " : 160 "		
	2550 " :5303 "	68	9 days
7 Mar.60	Eggs hatched in pool 18		
18 Mar.60	907 larvae : 25 pupae	27	11 days
15 Apr.59	Eggs hatched in pool 301		
7 Jun.59	36 pupae counted	-	54 days
20 May59	Eggs hatched in pool 109		
8 Jul.59	480 pupae counted	-	49 days

During winter the situation was more complicated because the level of the pools fluctuated and more eggs hatched before the first lot of larvae had pupated. However, pupae were observed in pools 301 and 109 on the 54th and 49th days, respectively, after the eggs had hatched. In summary, the larval stage lasts about 10 days in summer and about five times as long in winter.

The influence of temperature on the speed of development of the pupal stage was measured in the laboratory. Fourth-instar larvae were collected in the field and as they pupated were put into distilled water in separate

tubes. The results are given in table 2.02 and in figure 2.04, with a freehand curve drawn through the points.

Table 2.02: Duration of pupal stage at various constant temperatures.

Temp. °C.	No. of obs.	Mean duration in days.	Standard error of mean.
5	12	all died	-
10	16	10.6	± .499
15	16	6.0	± .158
21	19	3.95	± .051
27	19	2.89	± .073
30	13	2.69	± .134
34	17	all died	-

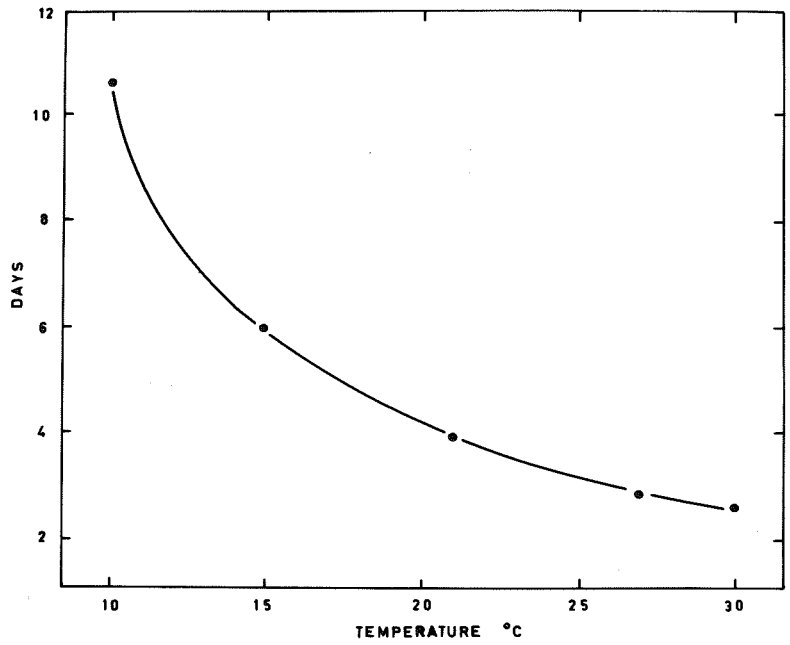


Figure 2.04: Duration of the pupal stage at different constant temperatures.

Observations on the maturation of the ovaries were made on unfertilized females reared from pupae. It would have been preferable to use fertilized females because it has been shown for some species of Anopheles that the ovaries of unfertilized females mature much more slowly that those in fertilized females (Muirhead-Thomson, 1951). However, the only source of fertilized females was in the field, and they may already have engorged blood. In the laboratory, ovaries of females which had not engorged blood developed until the oocytes were about 0.12 mm. long, and no further development took place until the females had engorged blood. The influence of

temperature on the maturation of the ovaries was measured by dissecting females at intervals after they had fed, until mature oocytes were found. The oocyte was assumed to be mature when the pattern of the exochorion could be distinguished. The durations from the meal of blood to maturity are given in table 2.03.

Table 2.03: Development of ovaries at constant temperatures.

Temp. °C.	No. of Obs.	Duration (days)
15°	8	7.0
21°	9	5.5
27°	14	4.0

2.22 Breeding-places. In the creek at Hackham, most of the pools could be classified as permanent or transient. The permanent pools stayed full during summer, being fed with water from springs, whereas the transient pools dried out in summer. The main differences between the two types of pools are summarized in table 2.04. Although most of the pools in the creek could be classified unambiguously, occasionally pools were intermediate between the two types. For example, the summer of 1960-61 was unusually dry and several "permanent" pools dried out and subsequently resembled transient pools. Also, occasionally transient pools remained full long enough for Chara to grow and thus resembled permanent pools.

Table 2.04: Descriptions of permanent and transient pools.

Characteristic	Permanent pool	Transient pool
Water-level	Stable	Fluctuates
Aquatic plants	Mainly *Chara* reeds, sedges and *Typha*	No plants
Predacious insects	Dytiscidae Notonectidae Naucoridae Zygoptera) Nymphs Anisoptera) Nymphs	Dytiscidae
Mosquito larvae	*An. annulipes*	*Ae. alboannulatus*

Observations at Hackham indicated that *Aedes* larvae were restricted to pools with fluctuating water-levels. These pools were either at the head-waters of the creeks or to one side of the main stream. During the winter of 1959 I tested this observation by looking for *Ae. alboannulatus* larvae in other creeks, especially those flowing in the steeper parts of the Adelaide Hills. I was unable to find larvae in a narrow creek near Montacute. The gradient of the creek was about 200 feet in 1 mile, which is less than the creek at Hackham, but the surrounding hills were so steep that the creek flowed continuously during winter and no quiet pools were formed. *Aedes* larvae were found in a small pool to one side of the main current in Fourth Creek at a point where the gradient of the creek was 800 feet in 1 mile. Similarly larvae were found in rock-pools beside the Onkaparinga River, but not in the main stream of the river. At Mannum,

larvae were found in rain-water pools on top of an outcrop of granite.

At Hackham, larvae were also found in old disused wells lined with rock. No water flowed directly into the wells but the water table fluctuated by several feet from summer to winter. Larvae were not found in iron tanks or horse troughs.

The types of places in which larvae were found can be generalized as accumulations of water in which the level fluctuates and which are not disturbed by strong currents.

2.3 Physiology and behaviour.

2.31 Physiology of the hatching of the egg.

2.311 The hatching stimulus. Lees (1955) stated that the eggs of certain aedine mosquitoes would hatch only when stimulated by a low oxygen tension. Turgid eggs of Ae. aegypti even hatched in air when the oxygen was removed with alkaline pyrogallol. Similar experiments were done with the eggs of Ae. alboannulatus to see if they responded in the same way.

Part I of the experiment was done in 1958 with eggs laid by a female caught at Hackham in June. Part II was done with eggs laid by a female caught at Hackham in July 1961. For both parts of the experiment, the eggs had been stored on moist filter-paper at 21°C before being used.

The experiment was done in Conway Units, as used by biochemists to estimate ammonia. The Conway Unit resembles the bottom half of a petri-dish but has a ground edge on which a flat sheet of ground-glass is placed. Vaseline on the join gives a gas-tight seal. Inside the dish is a circular glass wall, half the height of the outer wall, that divides the dish into a central, circular compartment, and an outer annular compartment. The eggs were placed in the central compartment on a sheet of filter-paper which was

either dry, or well moistened with distilled water. Alkaline pyrogallol (61.55 gm. KOH in 50 ml. water plus 3.85 gm. pyrogallol) was placed in the outer compartment. The alkaline pyrogallol rapidly absorbed the oxygen inside the Conway Unit, thus exposing the eggs to a reduced oxygen tension. Two other units were set up as controls, one with water in the outer ring and the other with a solution of KOH at the same strength as in the alkaline pyrogallol.

The arrangement of the treatments and the results are given in table 2.05.

Table 2.05: Hatching of eggs on moist and dry filter-paper.

Date	Outer Compartment	Inner Compartment	No. of eggs	Result
Jun.58	Water	Moist filter-p.	6	None hatched in 2 hours
"	Alk, pyrogallol	" "	6	All hatched in 10 mins.
Jul.61	Water	Moist filter-p.	10	None hatched in 1 hour
"	Alk, pyrogallol	" "	10	All hatched in 11 mins.
"	" "	Dry filter-p.	20	All hatched in over 2 hours.
"	KOH	" "	20	3 hatched in over 2 hours.

The eggs on moist filter-paper with reduced ambient oxygen hatched normally; the larvae pushed the anterior cap out of the way and wriggled out of the shell. In some eggs the cap was pushed right off the shell; in others it remained hinged to the shell.

However, the eggs on dry filter-paper were unable to hatch normally. Each egg had the cap broken away with the characteristic oblique fracture, but the gap was barely wide enough to see the hatching spine. Also, all the eggs had partially collapsed, presumably desiccated by the strong KOH solution. The eggs were flooded with water, but all the larvae were dead.

The strong KOH solution in the alkaline pyrogallol would give a low relative humidity and this may have caused the hatching. This seemed unlikely because when eggs are desiccated they simply collapse, without the cap breaking away from the shell. However, 20 eggs were tested in a Conway Unit with KOH of the same concentration as in the alkaline pyrogallol. Some cobalt thiocyanate paper was placed in the central compartment and the relative humidity estimated with a Lovibond comparator as ten to twenty percent. Seventeen of the eggs remained fully turgid, but three "hatched" and collapsed in the same way as in the other treatment on dry filter-paper. The KOH solution absorbs CO_2 as well as water vapour, and the three eggs that hatched may have responded to the reduced tension of CO_2. The effect is clearly less than that given by a reduced oxygen tension.

All the eggs that did not hatch were subsequently proved viable by hatching them on moist filter-paper in an anaerobic atmosphere.

Apparently the sole stimulus for hatching is low oxygen tension. However, for successful hatching, water is also required.

In nature the eggs usually hatch when covered by water, and hence some experiments were done on the nature of the hatching stimulus in water. Commercial nitrogen bubbled through a buffered solution in a test-tube was a rapid and effective way of producing a low oxygen tension. The solution was buffered to pH 6.5 with 0.001M sodium /potassium phosphate. A pH of 6.5 was chosen because the water in transient pools was usually slightly

acidic. 10 ml. of solution was used per test-tube and the solution was gassed vigorously for 5 minutes with nitrogen. The eggs were introduced in a minimum of distilled water with a capillary pipette while nitrogen was blown in at the top of the tube. The tube was then sealed firmly with a rubber stopper. The experiments were carried out at various constant temperatures either by putting the tubes in a water bath or in an incubator.

At a low oxygen tension, eggs usually started hatching after 5 minutes and frequently all the eggs in the sample had hatched by 20 minutes. Thus, the proportion of the eggs which hatched in 20 minutes was taken as a measure of the effectiveness of various treatments.

When the solution was gassed with oxygen or air, hatching was inhibited, although hatching could be induced by subsequently gassing with nitrogen.

The following experiment was done to see if the stimulus of low oxygen tension was required right up to the time of hatching, or whether eggs which had been triggered by a brief exposure to a low oxygen tension might subsequently complete the hatching process in a normal oxygen tension. The eggs used had been laid by a female caught at Hackham in February 1960. The eggs had been stored on moist filter-paper at 27°C.

Ten eggs were put into each of 3 test-tubes. Two of the tubes were gassed with nitrogen and left for 5 minutes. The tubes were then opened and gassed with air. The third tube was gassed with nitrogen and left closed as a control.

In the control, all the eggs hatched in the interval between 5 and 6 minutes. In one treatment tube, 2 eggs hatched before the oxygen tension was raised, and in the other tube 3 eggs hatched. After the oxygen tension was raised no more eggs hatched. Forty-five minutes later the tubes were

gassed with nitrogen again to lower the oxygen tension, and all the remaining eggs hatched promptly.

The experiment demonstrates that the stimulus must be continuously present until hatching occurs. Horsfall et. al. (1957) postulated that hatching was stimulated by a sudden alteration in the metabolic activity of the embryo. They set out to test the effect on the embryo of various compounds such as sugars, sugar alcohols and the compounds arising in the citric acid cycle. However, the egg-shell proved impermeable to the compounds, so the embryos were carefully dissected out and immersed directly in the various solutions. It turned out that embryos became active in distilled water and Ringer's solution, but that the compounds tested decreased activation. Only 0 to 30% of the embryos became active in glucose, fructose, pyruvate and other compounds in the citric acid cycle.

It is difficult to see any direct relationship between the results of Horsfall et al. and the biochemical nature of the hatching stimulus. My own results showed that the stimulus of reduced oxygen tension must be present for 5 to 10 minutes before the eggs hatch. One hypothesis consisten with this observation is that the accumulation of a product of anaerobic metabolism, e.g. lactate, stimulates hatching. However, the impermeable egg-shell makes it difficult to devise a test of the hypothesis on intact eggs.

2.312 <u>The relationship between oxygen tension and hatching</u>.

Having studied the hatching response at oxygen tensions of nearly zero, I set out to measure the response to oxygen tensions ranging from very low to saturation. One method of achieving a range of oxygen tensions was to bubble known mixtures of N_2 and O_2 through the solutions and

calculate the resulting oxygen tension. Another method was to bubble nitrogen through the solutions for various times and measure the oxygen tensions accurately before introducing the eggs. I chose the second method because it seemed more direct and convenient. Some time after I had finished my experiment I discovered that Horsfall et al. (1958) had done a similar experiment on the eggs of Ae. trivittatus using gas mixtures.

Material.

The eggs were laid by mosquitoes caught at Hackham in October and November, 1958. The eggs had been stored on moist filter-paper at 18°C. Eggs from different females were randomised with respect to treatments.

Apparatus.

Oxygen tension was measured with a manual Polarograph using a dropping mercury electrode. The Polarograph cell held 10 ml. of 0.1M KCl, which was the supporting electrolyte. The dropping mercury electrode was the cathode; the anode was the large pool of mercury at the bottom of the cell. The cell was kept at a constant temperature of 25°C by immersing it in a water-bath.

The method of Petering and Daniels (1938) was used, in which the current is measured at two applied potentials, 0.1 and 1.0 volts. The difference between the observed currents is related linearly to the concentration of the oxygen. The current was measured as the deflection in mm. on the galvanometer scale.

Calibration.

The Micro-Winkler method (Munro-Fox and Wingfield, 1938) was used to calibrate the Polarograph. The KCl solution was gassed with nitrogen or air, or a mixture of the two; the cell was then sealed and the current

measured. The cell was then unsealed and 1.5 ml. of KCl solution quickly collected from near the bottom of the cell and the oxygen tension determined chemically. The calibration curve is shown in figure 2.05. The Winkler determination at the lowest oxygen tension showed a higher oxygen content than would be expected from the upper part of the calibration curve. This was probably due to contamination by atmospheric oxygen as the sample was removed.

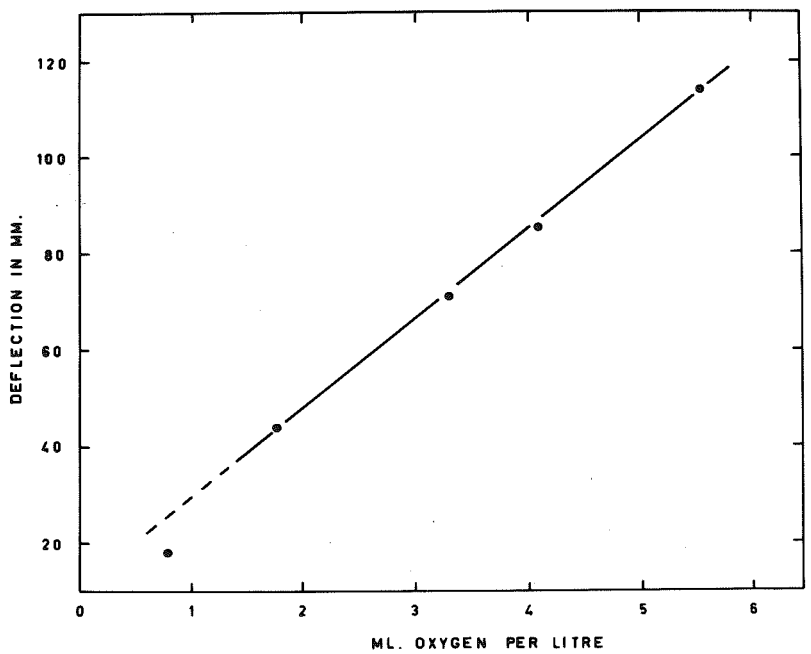


Figure 2.05: Calibration curve for a manual Polarograph to measure oxygen tensions at 25°C.

Methods.

Nitrogen or air was bubbled through the KCl solution until the current passing through the cell indicated that the desired oxygen tension was reached. The eggs were introduced in a minimum volume of distilled water in a capillary pipette. The cell was quickly sealed and an accurate determination of oxygen tension was made. The eggs sank to the bottom of the solution to lie on the surface of the pool of mercury (the anode). There was no evidence of any toxic effect from the mercury, the eggs hatched normally and the larvae swam about in the KCl solution. At the end of 20 minutes the cell was opened, the larvae and eggs removed, and the number that hatched recorded. Any eggs that had not hatched were carefully dissected in insect Ringer with fine needles; viable embryos showed muscular movements of the abdomen. Ten eggs were used at each determination except one with 9 eggs (the 33% hatch at 3.8 ml. oxygen per litre).

Results.

The results are given in table 2.06. The percentages were transformed to angles (Table X, Fisher and Yates, 1948) to enable a linear regression and analysis of variance to be calculated.

Table 2.06: Relationship between oxygen tension and hatching of eggs.

x ml. oxygen per litre	y % hatching	y angles
0.7	60	50.77
0.8	80	63.43
1.0	90	71.57
1.8	30	33.21
2.0	50	45.00
3.5	40	39.23
3.8	33	35.06
4.4	20	26.57
4.5	20	26.57
5.8	0	0
6.2	0	0

A linear regression of y on x was calculated and is shown as an analysis of variance in table 2.07.

Table 2.07: Analysis of variance of data in table 2.06.

Source of variation	Sums of squares	D.F.	Mean S.	Var. Ratio
Due to regression	4312.5535	1	4312.5535	49.05 ***
About regression	791.2643	9	87.9183	
Total	5103.8178	10		

The regression equation is $Y = 68.331 - 10.44285x$

The regression line is shown in figure 2.06.

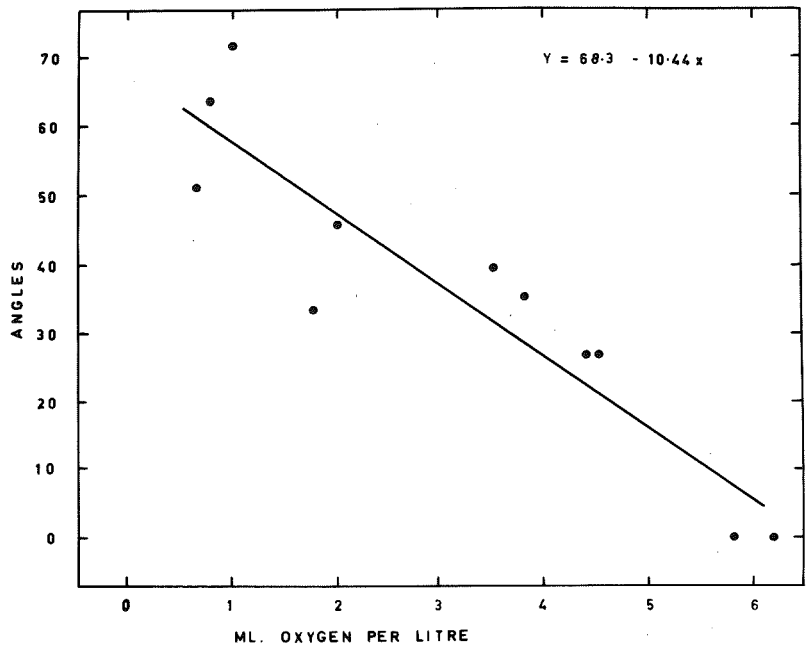


Figure 2.06: Relationship between oxygen tension and the hatching response of Aedes eggs at 25°C. The percentages of eggs hatching have been transformed to angles. Y = 68.3 - 10.44 x.

The standard error of b is 1.49104 and hence the 5% Fiducial Limits are - 10.443 ± 3.373, i.e. -7.070 to -13.816.

2.313. The oxygen tension in natural pools. The experiment in the laboratory on oxygen tension and hatching was followed up by measuring the oxygen tensions in natural pools. Three pools were selected:

(1) Pool 55, a small transient pool which had recently filled. This is the type of pool in which Ae. alboannulatus first-instar larvae were

found most frequently.

(2) Pool 31, a large transient pool which had been half-full for 2 months. First-instar larvae were often found in this type of pool after the water had risen further.

(3) Pool 13, a typical permanent pool with Chara growing in the water. Larvae were rarely found in this type of pool.

The oxygen tensions were measured by the Micro-Winkler Method, (Munro-Fox and Wingfield, 1938). Samples of water were collected just above the soil near the edges of the pools and each determination was made in triplicate. The oxygen tensions, corrected for temperature and pressure, are given in table 2.08 in ml. of oxygen per litre of water. In the third column, the estimated percentages of eggs which would hatch in 20 minutes at the relevant oxygen tensions were calculated from the regression equation given in section 2.312.

Table 2.08: Oxygen tension in natural pools and expected percentage hatching.

Pool	ml. O_2/litre	% Hatch
1. Trans. recently filled	2.72	41.1
2. Trans. half-full	5.02	7.5
3. Perm. with Chara	6.05	0.8

The results show that eggs would hatch promptly in the recently-filled transient pool and somewhat less promptly in the transient pool which had been half-full for 2 months. The oxygen tension in the permanent pool was measured during the day and would be less during the night

when photosynthesis had stopped. Hence, if eggs were submerged in a permanent pool, they would probably hatch. Probably, larvae are absent from permanent pools because the eggs are laid above full-water-level and are never submerged.

2.314 Variation in the hatching response of eggs and the influence of temperature.

In the experiment on oxygen tension and hatching (section 2.312), 90% was the highest percent hatching observed. This was surprising because in earlier tests, 100% of the eggs hatched in 10 or 15 minutes. The eggs used in the earlier tests were laid in winter, whereas the eggs used in the later experiment were laid in summer. I suspected that the reduced hatching response was caused by a weak summer diapause, and the following experiment was done to test this hypothesis.

The eggs were collected in the field during December and January in the course of the experiment on oviposition described in section 2.332. Many of the eggs were laid in discrete batches with the eggs placed side by side in overlapping rows. Each discrete batch was assumed to come from one female. The eggs were left on the absorbent paper and stored moist at 15°C. Samples from 8 batches were tested at low oxygen tensions in phosphate buffer of pH 6.5 at 25°C. The results are given in table 2.09.

The hatching response ranged from 0 to 100%. Although some of the tests are based on small samples, there is considerable variation between batches, and hence presumably between females. It seemed likely that the low hatching response was due to diapause. This seemed plausible because if the eggs hatched after light rain in summer, the larvae would be unlikely to complete their development before the transient pools dried up.

Table 2.09: Percentage of eggs hatching in batches collected in the field.

Date the eggs were laid	Batch	No. tested	No. hatched	% hatched
Jan. 1960	a	10	10	100%
"	b	10	5	50%
"	c	60	12	20%
"	d	20	2	10%
"	e	70	21	30%
March 1960	f	10	1	10%
"	g	10	0	0
"	h	10	0	0

Usually diapause is terminated by exposure to low temperatures (Andrewartha, 1952; Lees 1955). Beckel (1958) studied the eggs of Ae. hexodontus which occurs in Northern Canada. The eggs remain under water during autumn and beneath ice during winter. The eggs hatch in the following spring when the temperature of the water rises a few degrees above 0°C. In the laboratory, eggs laid during summer entered a firm, obligate diapause. Exposure to 1°C and -3°C for 200 days terminated diapause in about 50% of the eggs, whereas less than 2% of the eggs kept at room temperature hatched.

Because the South Australian winter is mild, I expected the diapause in Ae. alboannulatus to be weak, and to respond to relatively high temperatures. Eggs from batches f, g, and h in table 2.09 were used to investigate the termination of diapause.

Four petri-dishes of eggs were put at 12.5°C. After 4, 8, 16 and 32 days a petri-dish was withdrawn and transferred to 27°C for 4 days. The eggs were then tested at a low oxygen tension in phosphate buffer of pH 6.5 at 25°C. Four other petri-dishes were kept at 27°C and one lot of eggs tested at the same time as the lot withdrawn from the cold. Fifteen eggs from batches f and g, and 10 eggs from batch h were put into each petri-dish, making a total of 40 eggs per dish.

In the first two pairs of tests, after the eggs had been chilled for 4 and 8 days respectively, 100% of the eggs hatched both in the treatments and the controls. Clearly the eggs were not in diapause. Had they been, low percentage hatches would be expected in the controls and perhaps the termination of diapause in a few of the eggs that had been chilled for 4 and 8 days. It seems that exposure to 27°C for 4 days was the important factor; not exposure to cold.

A similar result was reported by Horsfall (1956a) with the eggs of Ae. vexans. Eggs which had been kept at 4°C did not hatch when tested at a low oxygen tension, but if the eggs were exposed to 25°C for 2 days before testing, 96% hatched. With the eggs of Ae. alboannulatus it appears that storing them at 15°C and subsequently testing them at 27°C results in a variable and decreased hatching response.

In a second experiment, 11 female mosquitoes caught at Hackham at the end of March, were put at 21°C. Their eggs were distributed evenly over 4 temperatures and left for 7 and 30 days. At the end of the appropriate period the eggs were tested at a low oxygen tension in phosphate buffer of pH 6.5 at the temperature the eggs had been exposed to. The results are given in table 2.10 as the percentage of eggs hatching out of

the total number of viable eggs.

Table 2.10: Percentage of viable eggs hatching after exposure to 4 temperatures.

Female	5°C		12.5°C		15°C		21°C	
	7 days	30 days	7 days	30 days	7 days	30 days	7 days	30days
a	71	0	71	100	14	100	100	100
b	100	13	100	100	100	100	100	100
c	100	75	100	100	100	100	100	100
d	33	38	100	100	50	100	100	100
e	86	13	88	100	100	75	100	100
f	60	75	100	100	100	75	100	100
g	100	50	100	100	100	100	100	100
h	27	11	100	100	100	100	100	100
i	89	100	100	100	100	100	100	100
j	100	89	100	100	100	100	100	100
k	100	100	100	88	100	100	100	100
Means *	77.5	47.3	96.9	99.0	89.8	96.5	100	100

* means calculated from original numbers.

Different numbers of eggs laid per female resulted in unequal numbers in the 88 subclasses. The mean number of eggs per subclass was 7.5 and a total of 656 eggs was used in the experiment. The percentages were transformed to angles (Table X, Fisher and Yates, 1948) and an analysis of variance computed. The analysis is given in table 2.11 with the non-significant interactions pooled in the sums of squares for errors.

Table 2.11: Analysis of variance of data in table 2.10.

Source of variation	S.S.	D.F.	Mean S.	Var. Ratio
Females	5,819.974	10	581.997	2.40 *
Temperatures	13,765.494	3	4,588.498	18.93 ***
Times	299.296	1	299.296	1.23
Temps. x times	2,670.161	3	890.054	3.67 *
Error	16,971.172	70	242,445	
Total	39,526.097	87		

The analysis shows significant differences between females and between temperatures. From table 2.10 it can be seen that the greater part of the differences is contributed by the data for 5°C. Similarly, the irregular response to 7 and 30 days at 5°C contributes to the significant interaction of temperature x time.

The temperature of water in the pools at Hackham varied from about 10°C in winter to about 20°C in summer. Hence, eggs submerged in winter might not hatch as promptly as eggs submerged in summer, but in general, the effect is probably not important in the field.

In the laboratory, the females and the eggs were treated in the same way, and hence the significant differences between females are probably due to genetical variability. Gillett (1955 a, b) found a similar variation in the hatching response of eggs laid by Ae. aegypti. In a series of experiments he showed that the variation was due to genetical variability in both males and females.

2.32 Relationships between Ae. alboannulatus eggs and water.

2.321 The intake of water during embryogenesis. The eggs were too small to be weighed accurately and hence the intake of water was inferred by measuring the change in length of the eggs. The length was measured with a micrometer in the ocular of a microscope.

Three lots, each of 10 eggs, were put at 27°C, 21°C and 15°C respectively, on wet filter-paper. The eggs were assigned to the treatments at random, but the mean initial lengths seemed markedly different. The mean lengths, in arbitrary units, were 3.14, 3.10 and 3.05, in 15°C, 21°C and 27°C respectively. An analysis of variance was calculated to test whether the differences could be ascribed to chance. The analysis is given in table 2.12.

Table 2.12: Analysis of variance of initial lengths in 3 groups of eggs.

Source of variation	S.S.	D.F.	Mean S.	Var. Ratio
Between classes	4.07	2	2.035	3.25
Within classes	16.90	27	0.6259	
Total	20.97	29		

The variance ratio corresponds to a probability of 0.05 to 0.10 and the differences can therefore be ascribed to chance. The different initial lengths were corrected for by expressing the results as gains in length. The differences were calculated from the logarithms of the original lengths because the eggs probably gained water in proportion to their

initial size. The mean gains in length are given in table 2.13 and plotted in figure 2.07.

Table 2.13: Water intake of eggs measured as mean gains in length at 3 temperatures.

Hours	15°C	21°C	27°C
0	0	0	0
3	.140	.195	.374
18	.339	.329	.453
24	.352	.382	.465
40	.351	.370	.465
48	.364	.370	.465
65	.364	.385	.465
88	.364	.383	.465

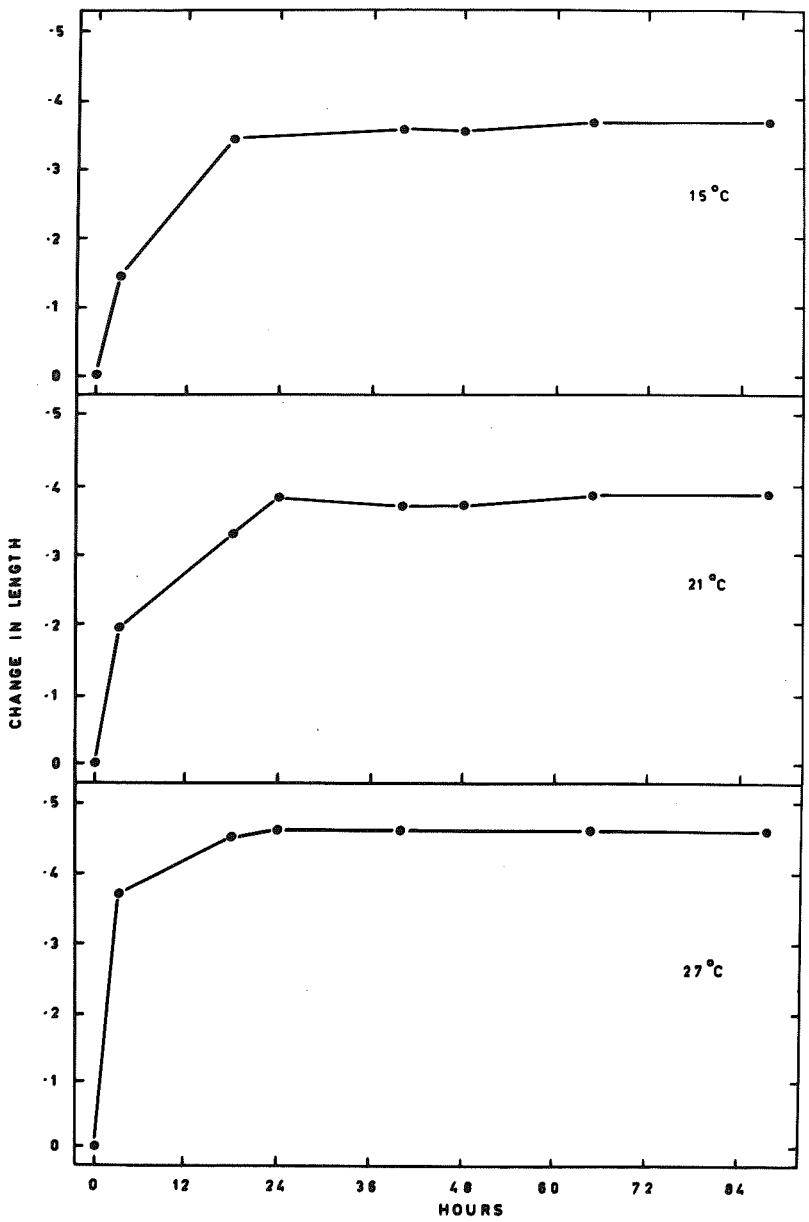


Figure 2.07: The intake of water by Aedes eggs at three constant temperatures. Intake of water was estimated by measuring changes in the lengths of the eggs.

From figure 2.07 it can be seen that all the water is absorbed in the first day, at all 3 temperatures. However, there are trends for more rapid intake and for a greater maximum length with increasing temperature. The significance of the greater rate of intake was tested by calculating an analysis of variance on the lengths after 3 hours exposure. The analysis is given in table 2.14.

Table 2.14: Analysis of variance of data for 3 hours.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Between classes	.002994	2	.001497	24.95 ***
Within classes	.001621	27	.000060	
Total	.004615	29		

Significant comparisons between the three means were made by calculating $s_{\bar{x}}$ = .00245 and hence D = .0086 (Snedecor, table 10.6.1, 1956). Thus, the only significant comparisons are that the mean at 27°C differs from the means at 21°C and 15°C.

Similarly, the significance of the differences between the maximum gains in length was tested by calculating an analysis of variance on the lengths after 24 hours exposure. The analysis is given in table 2.15.

Table 2.15: Analysis of variance of data for 24 hours.

Source of variation	S.S.	D.F.	Mean S.	Var. Ratio
Between classes	.000685	2	.000343	6.35 *
Within classes	.001460	27	.000054	
Total	.002145	29		

Again, $s_{\bar{x}}$ = .00235 and D = .0082, and the only significant comparisons are that the mean for 27°C differs from the means for 21°C and 15°C.

2.322 Permeability of the egg-shell in relation to gross embryology.

While handling eggs in the laboratory, I noticed that newly-laid eggs died rapidly if not kept moist, whereas mature eggs survived drying. In the following experiment the relationship between embryonic development and resistance to desiccation was investigated.

Methods.

Gross changes in the embryo were observed by clearing the black chorion in 83% Aqua Regia (De Coursey and Webster, 1952) and examining intact eggs by transmitted light under a binocular microscope.

Loss of water was estimated by examining each egg under a binocular microscope and assigning it a score, based on the degree of collapse. The method of scoring is given in table 2.16.

Table 2.16: Method of scoring eggs for loss of water.

Description of egg	Score
Turgid	4
¼ collapsed	3
½ collapsed	2
¾ collapsed	1
Wholly "	0

The eggs were scored after 1 day on dry filter-paper at 27°C. The relative humidity, measured with a Lovibond Comparator and cobalt thiocyanate paper, was 30%

In the experiment, parallel samples of eggs were taken; one sample was used to determine the development of the egg, and the other sample its permeability to water. All the eggs were laid in the laboratory by one female. When first laid the eggs were white; they blackened after about 3 hours.

Results.

The results are given in table 2.17.

Table 217: Relationship between development of embryo and resistance to desiccation.

Age of eggs days	State of development		Resistance to desiccation	
	No. of eggs.	Observation	No. of eggs.	Mean score
1	3	Only yolk visible	5	2
2	3	Embryo $\frac{1}{4}$ to $\frac{1}{3}$ length of egg	5	2
3	3	Embryo fills egg, hatching spine	5	2
4	3	More detail visible	5	3.2
4	3	1 out of 3 eggs hatched at a low oxygen tension		
5	5	All eggs hatched	5	3
6	5	All eggs hatched	5	4
7	5	All eggs hatched	5	4

Discussion.

Telford (1957) and Harwood and Horsfall (1959) both found that decreased permeability coincided with the development of the vitelline membrane. Telford worked with Ae. dorsalis and found that permeability decreased rapidly after 20 to 27 hours at 27°C . Harwood and Horsfall worked with Ae. aegypti and found a decreased permeability at 16 to 17 hours after the egg was laid.

With Ae. alboannulatus, observations on eggs from other females showed that the eggs were considerably more permeable when first laid than at one day old. Thus, the decreased permeability found by Telford, and Harwood and Horsfall (loc. cit.) also occured in Ae. alboannulatus, during the first day. The results in table 2.17 show that permeability

steadily decreased up to the sixth day, or about the time when the embryo was ready to hatch.

2.323 The lethal influence of dryness. To get more precise information about the lethal influence of dryness, I planned an experiment which could be analysed by the Probit Method (Finney, 1947). First, two pilot experiments were done to select an appropriate relative humidity and the range of dosages.

Five eggs were put at each of 4 relative humidities, 53%, 74%, 81% and 93%, at 25°C. The relative humidities were obtained by placing a saturated solution of the appropriate salt (Solomon, 1951) in the outer compartment of a Conway Unit, and placing the eggs on filter-paper, in the central compartment. After 48 days the eggs were scored for the degree of collapse and then placed on wet filter-paper at 27°C. After one week, the viable eggs were hatched by lowering the oxygen tension. The results are given in table 2.18.

Table 2.18: Mean score and proportion of eggs dying after 48 days at 4 relative humidities.

Relative humidity %	Mean score	Proportion dead
53	0.8	0.8
74	1.4	0.8
81	2.0	0.4
93	4.0	0

The eggs at 93% R.H. remained fully turgid and viable. At 53% R.H. all the eggs collapsed to some extent, but one egg was still viable after 48 days. Thus, the eggs were fairly resistant to desiccation, and a lower relative humidity (Saturated $MgCl_2$; 33% R.H.) was chosen for the Probit experiment.

The range for the dosage was selected by placing 10 eggs at 33% R.H. and 25°C and observing them at intervals. The mean scores are given in table 2.19. The results indicated that dosages ranging from 0 to 4 days should include the full range of mortalities.

Table 2.19: Relationship between loss of water and days exposure of eggs to 33% R.H.

Dosage (days exposure)	Mean score
0	4.0
0.1	3.28
0.8	2.82
1.3	2.0
2.1	1.0
2.8	1.0
3.7	1.0
4.3	0

For a Probit experiment, at least 5 treatments are required, each with at least 40 eggs. Also, a control is required to estimate natural mortality. I thought that sealing the eggs in Conway Units might increase mortality with time and consequently I used 5 controls, each left for the

same time as a treatment.

Forty eggs were placed at each treatment, and an additional 40 eggs in each of the 5 controls. The eggs of 5 females were used to make up the total of 400 eggs, and distributed amongst the treatments as given in table 2.20.

Table 2.20: Number of eggs per female in each treatment.

Female	No. of eggs per treatment
a	8
b	9
c	9
d	7
e	7
	40

The eggs were arranged in rows on filter-paper and placed in the central compartment of a Conway Unit. At the end of the appropriate period the treatment unit was opened and the $MgCl_2$ replaced with distilled water, to give 100% R.H. After 2 days at 100% R.H., a treatment and a control unit were opened and the distilled water replaced by alkaline pyrogallol. After 30 minutes the number of eggs that hatched normally was counted. All eggs that did not hatch were dissected with fine needles to check that the embryos were dead. The results for the controls are given in table 2.21.

Table 2.21: Number of eggs dying in the five controls.

Dosage (hours)	Number dead
8	4
23	0
47	2
71	3
223	2
	11

There was no trend of natural mortality with time, so the results were combined to give an estimate of natural mortality of 11 in 200, which equals 5.5%. The results were analysed by the method given in Finney (1947). The pertinent figures are given in table 2.22.

Table 2.22: Calculation of Probit regression line of mortality on dosage of 33% R.H.

t	x	n	r	p'	p	Emp. Probits	Y	y	nw
223	2.35	40	21	53	50	5.00	5.04	5.00	22.6
71	1.85	40	15	38	34	4.59	4.74	4.59	21.1
47	1.67	40	17	43	45	4.87	4.64	4.89	20.3
23	1.36	40	15	38	34	4.59	4.45	4.59	19.3
8	0.90	40	8	20	15	3.96	4.18	3.99	15.4
0		200	11	5.5	0				

Where t = dosage in hours.

$x = \log_{10} t$

n = number of eggs per treatment

r = observed deaths

p'= percentage mortality

p = percent mortality corrected for deaths in controls by Abbot's formula (Finney, 1947)

Empirical Probits = Probits read from table I of Finney (1947)

Y = the expected probits read from the first approximate maximum likelihood regression line.

y = working probits from table IV of Finney (1947)

w = weighting co-efficient from table II of Finney (1947)

The probit regression equation is

$$Y = 4.652 + .603289\ (x - 1.6834)$$

i.e. $Y = 3.636 + .6033\ x$

It is plotted in figure 2.08.

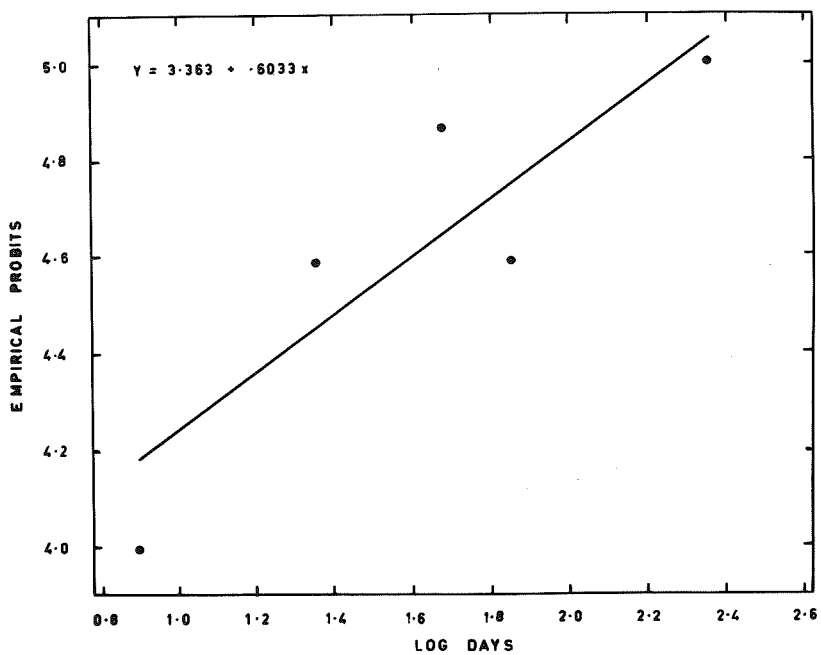


Figure 2.08: Probit analysis of the lethal influence of dryness on *Aedes* eggs. The eggs were exposed for different times to 33% relative humidity at 25°C. Y = 3.636 + .6033 x.

χ^2 for heterogeneity was calculated by the difference:

$$S\ nw\ (y - \bar{y})^2 \ - \ S\ nw\ (x - \bar{x})\ (y - \bar{y}) \ / \ S\ nw\ (x - \bar{x})^2$$

This gave χ^2 for 3 D.F. = 2.75 and $.50 > P > .30$, and thus no correction for heterogeneity is needed. The variance for b is given by the reciprocal of $S\ nw\ (x - \bar{x})^2 = (.2127)^2$,

$$\text{i.e.} \quad b = .6033 \pm .213.$$

Unfortunately, the range of dosages did not include the full range of mortalities expected; the greatest mortality was only 50%. The eggs from individual females were placed in separate rows but the low R.H. buckled the paper and mixed the eggs. However, in the treatment for 223 hours only the last two rows were mixed. The mortalities were 100%, 1%, 33% and 28%, for females a, b, c and (d + e) respectively, indicating great variability between females. Had all the eggs come from female a, the experiment probably would have gone as predicted by the pilot experiment,

The L.D.50 can be calculated as 7.6 days, but because this is at one extreme of the observed values, the Fiducial Limits are too wide to be meaningful. The variance of the L.D.50 (m) is given by

$$V\ (m) \ = \ \frac{1}{b^2} \left(\frac{1}{Snw} \ + \ \frac{(m - \bar{x})^2}{Snw\ (x - \bar{x})^2} \right)$$

Hence, the nearer m is to $\bar{x}$, the closer will be the fiducial limits. For the data in table 2.22, the L.D.33.3, i.e. the dose that is sufficient to kill one third of the eggs in the sample, is nearer to $\bar{x}$ than is the L.D.50.

The L.D.33.3 lacks the advantage of the L.D.50, which represents the mean dose which would be calculated were direct observations possible. However, for this data, the L.D.33.3 and its Fiducial Limits gives an

intelligible and precise statement of the lethal influence of dryness on the eggs of Ae. alboannulatus.

The L.D.33.3 and its Fiducial Limits were calculated by the formula given by Finney (p. 63, 1947), which includes the factor $g = t^2 V(b) / b^2 = 0.477$.

The results were, L.D.33.3 = $1.75 \pm .488$.

Taking antilogs and converting hours into days, the results are:

L.D.33.3 = 2.3 days.

with 5% Fiducial Limits of 0.8 to 7.3 days.

During summer the eggs of Ae. alboannulatus might experience dryness as severe as that used in the Probit experiment. However, the eggs would be exposed to dryness for only a few hours each day, and might take in water from dew overnight. Thus, it is difficult to relate the experimental results to eggs laid in the field. One unexpected result of the experiment was the great variability between eggs from different females, which suggests that some eggs would survive even prolonged dryness. In the next section I set out some observations from the field on the survival of eggs during summer.

2.324 The survival of eggs during summer. From 1 January to 16 January, 1960, Adelaide experienced an unusually severe heatwave; the records of maximum temperature at the Waite Agricultural Research Institute showed that for 5 days the temperature exceeded 100°F and on a further 7 days it was more than 90°F.

The observations on the survival of eggs during summer were made on two transient pools numbered 20 and 21. The two pools were full on 29 December, 1959, but only one quarter full on 4 January, 1960. Thus eggs were probably laid during the first week of January. On 18 January the following temperatures

were measured at pool 21 with a standard mercury thermometer (table 2.23).

Table 2.23: Temperatures at different positions in pool 21.

Position of thermometer bulb	Temperature
Air temperature in shade	101°F = 38°C
Dry soil in direct sunlight	123 51
Moist soil in shade	93 39
1" below leaf-litter	87 31
2" below leaf-litter on moist soil	80 27

Stratified samples of soil and litter were then collected and washed through sieves. The leaves were also immersed in water in case eggs still adhered to them. The numbers of eggs found are given in table 2.24.

Table 2.24: Number of eggs in stratified samples from pool 21.

Location of sample		No. of viable eggs
Large leaves - upper 1" of litter	Pool 21	none
Small leaves etc. - 1" to 2" zone	Pool 21	23
Moist soil below 2" of litter	Pool 21	149
Moist soil below litter - Pool 20		6

Both pools filled on 14 February, 1960. There were an estimated 2,470 first to second-instar larvae in pool 21 and 296 first to second-instar larvae in pool 20. Thus, large numbers of eggs were laid beneath the leaf-litter where the temperature was as much as 11°C lower than on exposed dry soil.

Both pools had been empty from about 7 January, 1960 to 14 February, 1960. Thus, several thousand eggs survived for 1½ months, including an unusually severe heatwave.

A second series of observations were made during the summer of 1960-61, on three transient pools (numbered 9,21 and 32). On 6 March 1961 soil samples were collected from the pools, which had been empty for 3 months. The only eggs found were either broken or collapsed, and all the embryos were dead. The numbers found are shown in table 2.25.

Table 2.25: Eggs recovered from three empty pools.

No. of pool	Nature of sample	No. of dead eggs
9	Moist soil	11
21	Dry soil under litter	2
32	Dry soil	11

The samples were about the same size as those in table 2.24. Pools 9 and 21 first filled two months later. No larvae were found in pool 9, but there were 9 third-instar larvae in pool 21. Pool 32 did not fill until late winter. Some larvae were found in pool 32 but I was unable to tell whether they came from eggs laid in summer or eggs laid in winter as the water-level slowly rose.

Thus it appears that very few eggs survived for 5 months in pool 21 and none survived in pool 9. The summer was unusually dry and even some pools that were usually permanent dried out.

2.33 The distribution of eggs and oviposition by Ae. Alboannulatus in the field.

In section 2.2 I described the differences between permanent and transient pools, and stated that Aedes larvae usually were found only in the transient pools. During 1959 and 1960 the numbers of larvae were counted in every pool in the creek at monthly intervals (the results are discussed in section 2.42). During these two years Aedes larvae were only rarely found in permanent pools and then usually after the water-level had fluctuated. For example, the summer of 1959-60 was unusually dry and one large permanent pool, D1, had emptied, although the soil at the bottom remained wet. The pool refilled in late April and on 11 May an estimated, 4,840 third to fourth-instar larvae and 21,830 pupae were in the pool.

The observations made during 1959 and 1960 showed that Aedes were quite efficient at utilising most of the unstable accumulations of water in the area. An interesting example was the breeding of Aedes in wheel-ruts. In the summer of 1958-59 I bogged a utility truck in swampy ground near pool 511, which resulted in two wheel-ruts about 8 feet long and up to 8 inches deep. In July 1959 I discovered large numbers of Aedes larvae and pupae in the two ruts; the total estimated numbers being 1,096 first to second-instar larvae, 1,640 third to fourth-instar larvae and 98 pupae. I inferred that eggs had been laid on the sides of the ruts and subsequently submerged.

The observations described above indicate that Aedes larvae are restricted to pools with fluctuating water-levels, that is, to transient pools. It is well known for some species of Anopheles that the females choose specific types of pools to oviposit, even though the larvae can

survive in a wide range of pools (Muirhead-Thomson, 1951). The experiments described in this section were designed to see if Ae. alboannulatus had a similar adaptation with the females ovipositing only around transient pools. Any such adaptation would be advantageous to the species; few eggs laid around permanent pools would hatch because the water would never rise to submerge the eggs.

2.331 The distribution of eggs in the field.

As a first approach I tried to find where the eggs had been laid in the field. In 1958 soil and leaf-litter were collected from empty transient pools and put into tall glass jars filled with water. First-instar larvae appeared, indicating that eggs had been present in the transient pools. I searched many similar samples under a binocular dissecting microscope without finding eggs. Nor was I able to see the eggs in the field, using various portable magnifiers.

In 1959 I developed a method of sieving eggs out of the soil. The method was a simplified version of one described by Horsfall (1956b). A nest of 5 sieves was used, having 7, 16, 30, 60 and 100 meshes to the inch. The soil was broken up and then forced through the sieves by a fan-shaped jet of water. The residues in the 60 and 100 mesh sieves were transferred to a large funnel filled with a saturated solution of sodium chloride. The residue was stirred vigorously for 1-2 minutes by blowing compressed air through the solution. The mixture was then left to settle. The soil and heavy particles would sink while the eggs and other organic material floated to the surface. The eggs were then collected with a Pasteur pipette, rinsed in distilled water and placed on moist filter-paper. There was no evidence that eggs hatched during the process (the larvae would have floated to the surface of the solution of NaCl); nor did the

eggs collapse during the brief immersion in the saturated solution of sodium chloride.

To test the efficiency of the method, a sample of soil collected from a transient pool (31) was thoroughly mixed. Sub-samples of 100 gm. were taken; two were immersed in water and five passed through the sieves. The results are shown in table 2.26.

Table 2.26: Comparison of methods of sieving and immersing soil in water to detect eggs.

Eggs found by sieving	Larvae hatched from samples in water
35	11
40	32
35	-
40	-
31	-

The mean and its standard error for the sieving method is 36.2 ± 1.72, indicating little variation between trials. Fewer larvae emerged from the two sub-samples immersed in water, probably because some larvae were trapped between layers of soil.

Further samples of soil were collected, each sample covering about 1 square foot and taken to a depth of $\frac{1}{2}$ to 1 inch. The origin and size of each sample is given in table 2.27.

Table 2.27: Number of eggs recovered by sieving soil collected in the field.

Number of pool	Type of pool	Size of sample	No. of viable eggs
30	Transient	1 sq. ft.	0
31	"	2 sq. ft.	110
32	"	1 sq. ft.	1
Wheel-rut a	"	0.75 sq. ft.	211
Wheel-rut b	"	0.5 sq. ft.	30
13	Permanent	2 sq. ft.	0

The samples were collected in July 1959. Pools 30 and 32 had been empty for 7 months; pool 31 had started to fill, the water being 6" deep, about $\frac{1}{4}$ the maximum depth. Both wheel-ruts were full and it was unlikely that the eggs would ever have been submerged. Pool 13 had been full all the time.

The results indicate that eggs are present around transient pools and also that sometimes eggs are laid above full-water-level where they are unlikely to hatch.

Further evidence for the absence of eggs around pool 13 was obtained by raising the water-level above the normal full-water-level. A weir built across the end of the pool increased its depth to 4 inches above the normal full mark. The water stayed 4 inches above normal for 5 days; on the sixth day water was pumped into the pool and the level increased to 9 inches above normal for 1 day. Any eggs around the banks should have hatched, but although I searched carefully, I failed to find any larvae.

The observations on the wheel-ruts prompted me to do the following experiment on the breeding of Ae. alboannulatus in newly-formed pools. On 29 July, 1959 I dug three "artificial wheel-ruts", each 3 feet long, 6 inches wide and 6 inches deep. These were dug in the sloping, swampy ground near the original ruts. One artificial rut was dug near the bottom of the slope where seepage would fill it completely. The second was dug near the upper edge of the swampy ground where seepage would partially fill it, and the third midway between the other two. The sods removed from the ruts were smoothed into position along the sides to imitate the way the soil had been forced up along the sides of the original ruts.

The three ruts filled rapidly but by 2 September, 1959 they had started to dry out. Samples of soil were collected from the ruts and examined for eggs. The results are given in table 2.28.

Table 2.28: Eggs recovered by sieving soil from artificial wheel-ruts.

Position of rut	Eggs counted	Eggs per sq. ft.
Upper (empty)	7	14
Middle ($\frac{1}{2}$ inch deep)	88	176
Lower (3 inches deep)	4	8

The artificial ruts were empty during March and early April in 1960, but filled in late April. On 11 May there were 22 first to second-instar larvae, and 63 third to fourth-instar larvae in the lower rut and 20 third to fourth-instar larvae in the middle rut.

The history of the artificial ruts may be summarized as follows. The ruts were dug in winter and eggs were found one month later. The ruts then dried out during summer. Larvae first appeared in late April, about 9 months after the ruts were dug. After two and a half years the ruts were completely silted up and overgrown.

The sieving method had proved adequate for extracting eggs from small samples of soil, but it involved too much work to be used for an extensive survey of transient and permanent pools. Rather than increase the capacity of the sieving method, I sought another method to find where the eggs were laid.

2.332 Oviposition by Ae. alboannulatus in the field.

During the winter of 1958 absorbent paper was placed around the banks of pools to see if Ae. alboannulatus females would oviposit on it. Rolls of commercial paper towelling 8 inches wide were used. The bottom of the strips dipped into the water, the strip being held in place by nails driven through cardboard squares. Several trials were made during winter in which the paper was dyed various colours, but no eggs were laid.

In February, 1959, many adults emerged after heavy summer rains had filled some of the transient pools. By 1 March the creek was dry except for five permanent pools (D1, 13, 14, 16, 17) and two transient pools (7, 8). It seemed likely that many Ae. alboannulatus females would be ovipositing, consequently transient pool number 10 was selected for an experiment on oviposition. On 1 March the bottom and sides of the empty pool were entirely covered with absorbent paper, an area of ten square feet. In the late afternoon the pool was filled with 80 gallons of rain-water. The water seeped away overnight and by morning the pool was empty. Thus, during dusk and overnight the water-level was falling, exposing the wet paper. The next morning the paper was removed and examined for eggs. As a control, a strip of paper was placed along one bank of a permanent pool (13) about 30 yards upstream. This paper was left in place and examined on the spot. The numbers of eggs laid overnight are shown in table 2.29.

Table 2.29: Oviposition by females in the field.

Date 1959	No. of eggs laid overnight	
	Pool 10	Pool 13
1 March	0	0
7 "	530	0
9 "	204	0
11 "	84	0
18 "	136	0
22 "	0	0

On the 22 April the creek started flowing and the experiment was stopped.

This experiment, in the summer, probably succeeded for two reasons. Firstly, only a few pools held water. When similar experiments were done in winter, several hundred pools were full and the proportion of the total area that could be covered with paper was much less. Secondly, many adults had recently emerged and would be seeking places to oviposit.

In December 1959, the situation was again similar to that in March 1959, and the experiment was repeated, this time more extensively. One inch of rain during November and two inches during December had kept water in several transient pools, and many pupae were present in late November and early December. Strips of paper were placed around the accessible banks of the pools, usually for $\frac{2}{3}$ to $\frac{3}{4}$ of the perimeter, and the lengths of the paper strips recorded. Up to 9 transient pools were used (numbered 8, 8a, 9, 10, 21, 25, 31, 109, 114) and 3 permanent pools (13, 16, 17).

The transient pools began to dry out in January, but the permanent pools remained full throughout the experiment. The results are given in table 2.30.

Table 2.30: Oviposition by females in the field.

Date when eggs were counted 1959-60	Transient			Permanent		
	Total no. of pools used	Length of paper strips in feet	No. of eggs	Total no. of pools used	Length of paper strips in feet	No. of eggs
18 Dec.	7	101	0	none	-	-
22 Dec.	8	112	330	1	49	0
29 Dec.	6	85	0	3	104	43
1 Jan.	7	93	0	3	95	127
4 Jan.	6	92	145	3	95	440
11 Jan.	6	81	0	3	95	1134
Total			475			1744

After the 11th January all the transient pools dried out. However, observations were continued on the 3 permanent pools until the creek flooded on 20th April. These results are given in table 2.31.

The data from tables 2.30 and 2.31 are combined in figure 2.09. The height of the column represents the total number of eggs counted, the shaded portion of the column represents eggs laid around permanent pools, and the cross-hatched portion represents eggs laid around transient pools.

Table 2.31: Oviposition by females around permanent pools.

Date 1960	Permanent pools		
	Total no. of pools used	Length of paper strips in feet	No. of eggs
18 Jan.	3	49	0
12 Feb.	3	28	100
16 Feb.	2	11	0
11 Mar.	2	11	105
6 Apr.	2	11	70
13 Apr.	2	11	0

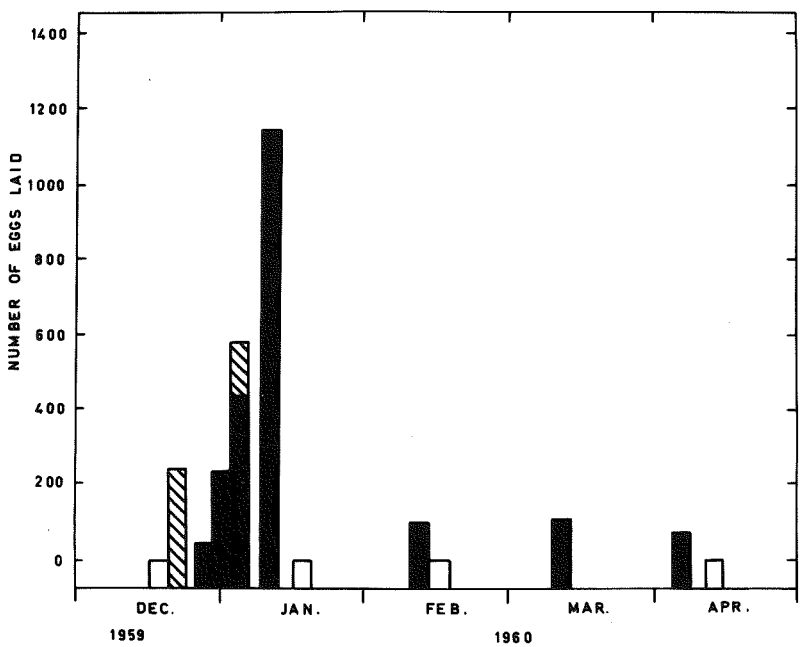


Figure 2.09: The numbers of eggs laid around pools at Hackham. The height of the column represents the total number of eggs laid. The shaded part of the column represents eggs laid around permanent pools; the cross-hatched part refers to eggs laid around transient pools.

The results of the second experiment show clearly that Ae. alboannulatus females oviposit on paper around both types of pools and do not specifically select transient pools.

Some other interesting facts came out of the observations on the permanent pools. Firstly, pool 13 was treated in the same way as in the previous experiment and once more no eggs were laid on the paper. Secondly, eggs were laid around pool 17 on 6 occasions, and on 5 occasions all the eggs were laid in the same corner of the pool, within the same area of 1 square foot. The numbers of eggs ranged from 43 to 440. On the sixth occasion 1,026 eggs were laid, some in the same corner, the rest scattered along the banks. On pool 16, also, eggs were laid on two occasions, each time in the same place on the bank. Thus, although the females oviposit on permanent pools, it seems they frequently choose small specific areas around those pools, but I was unable to recognize the quality of these places that stimulated the females to lay eggs there.

Also, contrary to expectation, some eggs hatched on the moist paper without being submerged. One compact group of 105 eggs, presumably from a single female, had all hatched; in another compact group of 70 eggs, 14 had hatched. Both groups had been laid around pool 17. This may be an example of variability in the hatching response of eggs from different females, as discussed in section 2.31. Doubtless the larvae would have crawled to the water as described in section 2.34. Indeed, on 11 May, one second-instar and two third-instar larvae were found in 14 samples, each covering $\frac{1}{2}$ square feet, taken from the pool.

2.333 The survival of Ae. alboannulatus larvae in permanent pools.

If the results of the experiments on oviposition indicate what happens in nature, a considerable number of eggs are probably laid around permanent pools. The virtual absence of larvae in these pools could have arisen because the eggs were never submerged and hence so few hatched that the larvae were not noticed. However, in the second experiment, 119 out of 2,019, or 6%, of the eggs laid around permanent pools hatched without being submerged. If the value of 6% was not purely fortuitous, it would be expected that occasionally quite large numbers of larvae would hatch in the permanent pools. Therefore, the absence of larvae could indicate that the larvae were unable to survive in permanent pools. In the experiment on oviposition, nearly all the eggs laid on the two permanent pools (16 and 17) were laid above the deepest parts of the pools (3 feet and 2 feet respectively).

Now, Anopheles larvae survived in the permanent pools, but they fed mostly at the surface. In contrast, Ae. alboannulatus larvae fed both at the surface and on the bottom. It seemed possible, then, that the permanent pools were too deep for the first-instar larvae to survive. To test this hypothesis, four holes, 1 foot square, were dug in swampy soil and allowed to fill from seepage. Two holes were $3\frac{1}{2}$ feet deep, and the other two 6 inches deep. The holes were arranged at the corners of a square, with the deep holes at diagonally opposite corners. The holes were dug on 6 November, 1960. I had intended to add 50 first-instar Ae. alboannulatus larvae to each hole, but before I had found enough larvae, the two shallow holes had dried out during the dry summer of 1960-61. The water level of the two deep holes fell by 9 inches, but the water remained clear. Chara plants subsequently grew and Anopheles

larvae were present from December 1960 to April 1961. That is, the deep holes resembled permanent pools. In March the water rose and subsequently second-instar Ae. alboannulatus larvae were found in one of the deep holes. On 6 April, 1961 the holes were full and there were an estimated 140 second-instar and 20 fourth-instar larvae in the deep holes. The two shallow holes also filled; there were four second-instar larvae in one, but the other was stagnant and covered with a ferruginous scum. Although the results did not come from the planned experiment, the survival of 20 fourth-instar larvae in the deep holes is sufficient evidence to reject the original hypothesis.

2.334 Physical differences between transient and permanent pools.

One shortcoming of the experiment on oviposition was that the eggs were laid on paper rather than on the soil. The permanent pools stayed full during summer and it would be expected that constant evaporation of water from the soil of the banks would leave a high concentration of salts on the surface of the soil. Transient pools, however, were full for a shorter period during winter and therefore ought to have a lower concentration of salts in the soil. Wallis (1954) found that female mosquitoes, including 3 species of Aedes, could discriminate differences between solutions of NaCl as small as 0.02 M with contact chemoreceptors located on the tarsi. Similarly, Woodhill (1941) found that Ae. aegypti and Ae. concolor (= Ae. australis) could discriminate between dilutions of seawater differing by 0.5%. Since Ae. alboannulatus females would have to rest on the soil to oviposit, they could be repelled by high concentrations

of salts.

The hypothesis was tested by measuring the amount of salts in the soil around a permanent and a transient pool. Two samples were taken from each pool; in each sample two square feet of the surface soil was removed from just above water-level. The amount of soluble salts was estimated by the method described by Piper (1950); the soil was dried, ground, and 200 gm. shaken in 1 litre of distilled water for 1 hour. The supernatant was then drawn through a filter-candle and 100 ml of the filtrate evaporated to dryness. Hydrogen perixode was added to oxidise soluble organic matter. The results are given in table 2.32, expressed as milligrams of soluble salts per 100 gm. of dry soil.

Table 2.32: Amount of soluble salts in soil around permanent and transient pools.

		Permanent pool (16)	Transient pool (32)
Sample	1	210	415
	2	295	275
Total		505	690
Mean		252.5	345.0

There is considerable variation between samples from the same pools and not a great difference between the two means. Furthermore, the trend is for more salts around the transient pools which is contrary to the hypothesis.

There was one further difference between transient and permanent pools that seemed to be worth testing. The water-level of transient pools

fluctuates and when the water-level was falling an expanse of wet soil would be left which could attract gravid females. For example, in the first experiment on oviposition the water level of pool 10 was falling each time eggs were laid.

The hypothesis was tested by lowering the water-level of two permanent pools (numbers 13 and 16) on four successive nights. The two pools were pumped out to 9 inches below full-water-level on the first three nights. On the fourth day the two pools were emptied and left to fill by seepage. Emptying the pools ensured that any eggs laid could develop completely before the water rose and submerged them. Eight days later both pools were full again. The two pools were searched carefully on several occasions, but no larvae were found.

Thus the experiment provides no evidence for eggs being laid on permanent pools when the water-level fluctuated. However, the absence of oviposition may have been because few gravid females were about. The experiment was done in April, 1959, one month after the experiment shown in table 2.29. The data in table 2.29 indicate a period of intensified oviposition following the emergence of many adults, and it is apparent that oviposition was declining by the end of March. A similar wave of oviposition is shown in figure 2.09.

Conclusions.

Since there is no difference in concentrations of salts between transient and permanent pools, the results of the experiments on oviposition (section 2.332) are probably valid. Hence, it appears that eggs are laid indiscriminately on all types of pools in the creek, and in general, the only eggs which hatch are those subsequently submerged. Often, many of the eggs must die because they are never submerged. This would happen

to eggs laid around permanent pools and also to eggs laid above the full-water-level of transient pools.

2.34 The reaction of first-instar larvae to light.

During the course of an experiment on the hatching stimulus, some eggs hatched in a Conway vessel on filter-paper that was wetter than usual. I noticed that as soon as the larvae emerged they started to crawl away from their egg-shells and that they all crawled away from the light of a window. When the filter-paper was rotated through 180°, the larvae promptly stopped crawling, paused for a few seconds, then turned back on themselves and crawled away from the light again. This definite reaction to light rather surprised me. Omardeen (1957) studied the reaction of larvae and pupae of Ae. aegypti in a gradient of light, and found that second and third-instar larvae did not respond to the gradient, but that fourth-instar larvae moved to the dark end. The photonegative reaction of the pupae was even more pronounced and Omardeen concluded that the increasing response was correlated with the increasing development of the imaginal compound eye.

My own observations in the field indicated a similar response to light by Ae. alboannulatus. First and second-instar larvae were usually distributed fairly uniformly throughout a pool irrespective of light and shade, whereas fourth-instar larvae and pupae tended to aggregate under shadows.

The observations discussed in section 2.33 indicate that some eggs laid in the field hatched without being submerged. If these larvae are to survive, they have to find their way rapidly to water. In most places, crawling away from the light would lead the larvae straight to the water and the photonegative response seems to be an adaptation which would have some

survival value.

The photo-negative reaction was studied in greater detail by using an experiment with two light-beams crossing at right angles in a darkened room. Two microscope-lamps were focussed to give parallel rays of light in beams about 2 inches wide. A sheet of wet filter-paper resting on glass was used for the larvae to crawl on and the two light-beams were arranged to cross at right-angles in the centre of the filter paper. Larvae were placed at the mid-point where the beams intersected, each larva facing in a different direction, and after they had crawled 2 cm. the path they took was recorded. The newly-hatched larvae were so small that it was difficult to plot their paths accurately; accordingly the circle of 2 cm. radius was divided into sectors, each of 15°, arranged as shown in figure 2.10. Three combinations of lighting were used: (a) both lights of equal intensity (1:1); (b) one light at one quarter the intensity of the other (1:$\frac{1}{4}$); and (c) one light switched off altogether (1:0). The light intensities were checked with a light-meter.

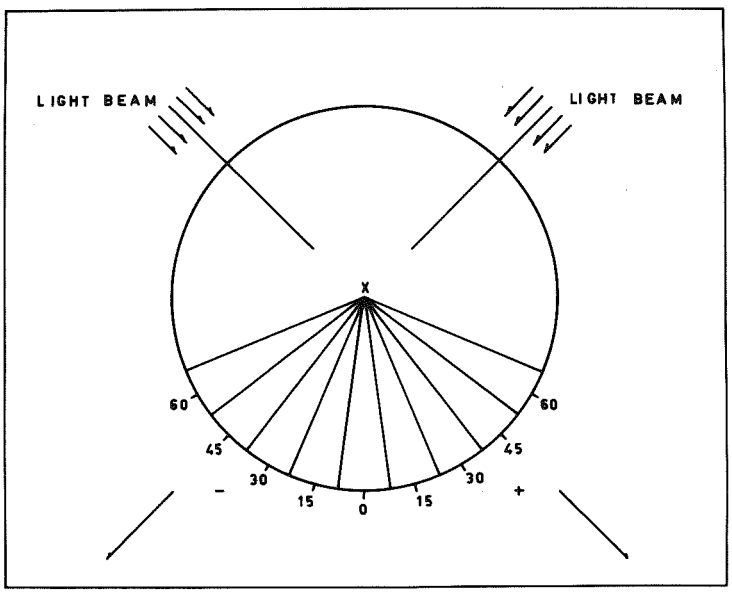


Figure 2.10: Arrangement for an experiment on the reaction of first-instar larvae to light. The larvae were released at x.

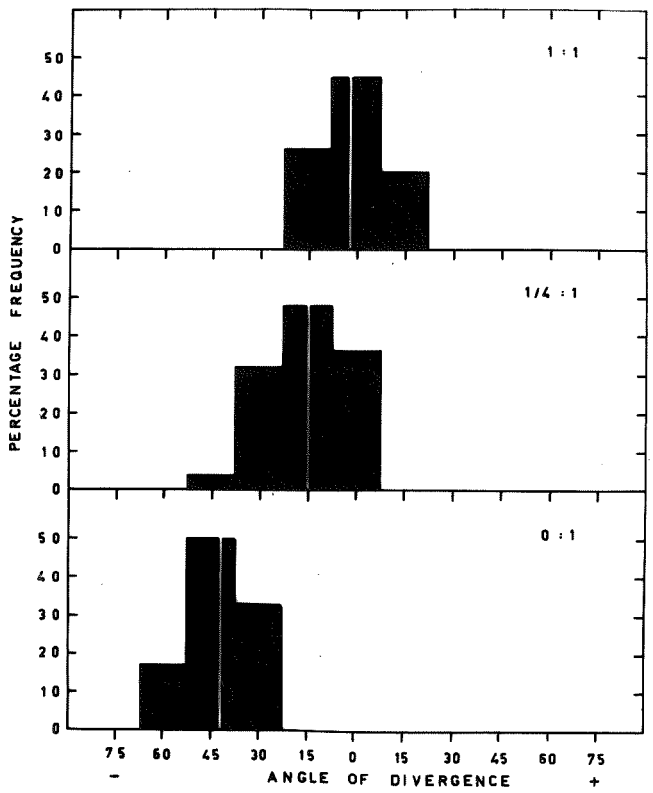


Figure 2.11: The paths taken by first-instar larvae crawling away from two beams of light crossed at right angles.

The results are shown in figure 2.11 and also given in table 2.33.

Table 2.33: Orientation of first-instar larvae to two beams of light, expressed as percentage frequencies.

Angle of divergence Class intervals	Relative light intensities		
	1:1	1:¼	1:0
+ 22.5 - + 7.5	20		
+ 7.5 - -7.5	44	16	
- 7.5 - -22.5	36	48	
- 22.5 - -37.5		32	33
- 37.5 - -52.5		4	50
- 52.5 - -67.5			17
Mean divergence	-2.4	-15.4	-42.6
Total no. of larvae	25	25	6

When only one light was switched on, the larvae crawled directly away from the light. When two lights of unequal intensity were used, the larvae crawled away from both lights along a path nearer the brighter beam. When both lights were of equal intensity, the larvae crawled directly away from both lights. One other point is that the light rays were parallel which indicates that the larvae were crawling away from the source of the light and not responding to a gradient in intensity.

These reactions are typical of the behaviour called Tropo-taxis by Fraenkel and Gunn (1940), although the experiment does not exclude Klino-taxis. However, the larvae have two well developed eyes placed on either side of the head and it seems most likely that the behaviour is a tropotaxis.

Some further observations were made on the behaviour of the larvae. The larvae also crawled away from a diffuse source of light, consisting

of a 40 watt fluorescent tube in a darkened room. Larvae were also tested on wet filter-paper held vertically. When illuminated with an incandescent bulb from above, the larvae crawled downwards. But when the bulb was placed below the larvae, they all crawled upwards, away from the light and against the force of gravity.

To test the influence of gravity in the absence of light, larvae were put on moist filter-paper held vertically in a darkened room. After 5 minutes the direction taken by each larva was recorded. The directions taken were grouped into 4 equal zones, such that zone 1 faced upwards, zone 2 to the right, zone 3 downwards and zone 4 to the left. The numbers of larvae in the zones on 6 trials are given in table 2.34. Each lot of larvae was used for two trials.

If the larvae did not respond to gravity, equal numbers would be expected in each zone. That is, a total of 12 larvae per zone. The observed totals were tested against the expected totals by χ^2.

$\chi^2 = 3.66$ with 3 D.F.

and $.50 > P > .30$.

Hence the results are consistent with the hypothesis that the larvae do not respond to gravity.

Table 2.34: Orientation of larvae with respect to gravity.

Trial	Zone				Total
	1	2	3	4	
a	3	2	2	1	8
b	2	2	1	3	8
c	2	1	1	3	7
d	3	1	2	1	7
e	3	0	3	3	9
f	2	1	2	4	9
Observed totals	15	7	11	15	48
Expected totals	12	12	12	12	48

2.35 The relationship between larvae and food in natural pools.

An animal's chance to survive and reproduce may be influenced by a shortage of a material necessity such as food or living-space. Andrewartha and Browning (1961) proposed the name "resources" for the material necessities of life. They analysed the idea of resources, giving particular attention to the concept of absolute and relative shortages of resources. An absolute shortage occurs when an animal consumes all its stocks of a resource. A relative shortage occurs when some of the resource is inaccesible to the animal; for example several rabbits may have enough blood to feed a large population of adult mosquitoes, yet if the rabbits are dispersed over a large area, many mosquitoes may go short of a meal of blood because they never find a rabbit. Similar reasoning applies to mosquito larvae. Their food consists mainly of small particles which the larvae filter out of the water with their mouth brushes. If the concentration of particles is low, a larva may not get enough food to complete its development, even though the total amount of food, were it concentrated, would be sufficient for many larvae. The experiments in this section were designed to see if larvae experienced a relative shortage of food in natural pools.

It would be expected that as the concentration of food was increased, the growth rate would also increase, until the larva was growing at its maximum rate. This type of relationship was found by Trager (1937) when he reared the larvae of Ae. aegypti in increasing concentrations of Sterile Lilly Liver Extract. He expressed growth-rate as N/T where N is the percentage of larvae reaching the fourth-instar in 9 days at 28°C and T is the mean time to reach the fourth-instar. I wanted to measure growth

rate in various concentrations of pondwater which contained some living food. Because the amount of living food in a sample of pondwater would not be stable over several days, I sought a method for measuring the growth-rate over a short interval of time. Preliminary trials showed that gain in weight over 15 to 20 hours could be measured with satisfactory precision. Because the amount of weight gained was proportional to the initial weight, growth-rate was expressed as:

$$\text{Growth-rate} = \frac{\log.w_2 - \log.w_1}{t}$$

t = duration of experiment

Where W_1 = initial weight in mg.

W_2 = weight after time t.

For simplicity logarithms to the base ten were used. The log. transformation also gave a constant error variance.

2.351 Methods.

(a) The measurement of growth-rate.

Larvae were weighed separately and kept in separate tubes so that variability among individuals could be estimated. Before weighing, the larvae were put briefly into distilled water to rinse off any particles adhering to them. The larvae were then transferred with a pipette to a fine, nylon mesh and the mesh pressed on three layers of filter-paper to remove free water. The larvae were transferred with a fine sable brush to the pan of an "A.S.E." torsion balance and weighed. The torsion balance was graduated in units of 0.05 mg. with a range of 0 to 25 mg. The scale was read to 0.01 mg. with the aid of a magnifying glass.

Fifteen ml. aliquots of the various concentrations were put into flat-bottomed glass tubes stoppered with cotton-wool to reduce evaporation. Larvae were put into the tubes at random with respect to treatments and the larvae were also weighed in a random order so that any systematic error, such as a drift of the zero position on the balance, would be randomised over treatments. Because it took up to three hours to weigh 60 larvae, the larvae were weighed in the same order for the initial and final weighings. At the final weighing each tube was examined for exuviae and any larvae that had moulted were discarded from the experiment.

(b) Concentration of the pondwater.

Water was collected in the field and brought to the laboratory as quickly as possible. For the experiment described in section 2.352(c), the water was thoroughly mixed and 250 ml. aliquots put into polythene bags and frozen. For the other experiments, the pondwater was collected in 5 gallon polythene containers and stored at 4°C.

The pondwater was concentrated in a "Quickfit Rotary Film Evaporator". At 30°C and under reduced pressure from a water pump, just over 100 ml. of water could be evaporated per hour. Concentrations were expressed as multiples of normal pondwater; e.g. 4x means that the sample of pondwater was reduced to one quarter of its original volume. Thus, if there were x gms. of food per 100 ml. in the normal pondwater (1x), there would be 4x gms./100 ml. in the concentrated water (4x).

(c) Osmotic pressure.

The water was concentrated by evaporation because there may have been some food in solution, and this may have been important in the nutrition

of the larvae. Because the concentrating process would also increase the osmotic pressure of the water, the osmotic pressure was measured and controls included in the experiments to detect effects due to osmotic pressure. The osmotic pressure was measured by the Beckmann Cryoscopic Method, after filtering the pondwater through Whatman's No. 1 filter-paper to remove the larger particles.

2.352 Experimental Results.

(a) The influence of concentration and temperature on growth-rates.

The experiment was designed for a factorial analysis, with the following treatments:

Class A: 1x, 2x, 3x, 4x, 5x, 6x, 3x + NaCl, 3x No. 21.

Class B : 15°C, 21°C, 27°C.

Class C : Experiments 1 and 2.

Replication : 6 larvae per sub-class.

The treatment 3x + NaCl was included as a control to detect effects of osmotic pressure. The osmotic pressures, determined by the Beckman Cryoscopic Method, of three concentrations are given in table 2.35.

Table 2.35: Osmotic pressures of concentrated pondwater.

Concn. of water	% NaCl equivalent to O.P.
Distilled water	0
1x	.15
3x	.45
4x	.60

The osmotic pressure is related linearly to concentration. Accordingly NaCl was added to a sample of 3x water to give an osmotic pressure equivalent to 0.9% NaCl, which is the expected osmotic pressure of 6x water.

The treatment 3x No. 21 was included as a comparison for when the experiment was repeated using water from pool 21. Class C was included because the experiment was done in two halves.

I expected considerable variability between individuals and also that some larvae would moult. Accordingly I replicated each treatment 6 times, making a total of 144 larvae. I could handle only about 70 larvae at the one time, so I divided the experiment in half, each half being the full number of treatments with 3 replicates per treatment. Larvae were collected from pool 207 at Hackham and the first half used immediately; the second half were kept at 25°C in pondwater and fed daily on powdered fish-food ("Aquadine") plus powdered milk. The second half of the experiment was done 4 days later.

The results of the two experiments are shown as the means of each sub-class and the number of larvae per sub-class in table 2.36.

Table 2.36: Mean growth-rates of larvae and number of observations.

Treatment	Experiment 1			Experiment 2		
	15°	21°	27°	15°	21°	27°
1x	3 -.0053	3 .0050	2 .0025	3 .0123	3 .0193	2 .0160
2x	2 .0255	3 .0357	2 .0440	3 .0273	2 .0240	2 .0250
3x	2 .0285	3 .0500	2 .0435	2 .0155	3 .0233	1 .0140
4x	2 .0065	1 .0110	2 .0590	2 .0210	3 .0230	3 .0243
5x	3 .0237	3 .0340	1 .0340	3 .0220	3 .0337	2 .0325
6x	2 .0305	3 .0323	2 .0160	3 .0173	3 .0307	2 .0400
3x + NaCl	3 .0250	3 .0383	2 .0355	3 .0370	3 .0297	3 .0423
3x:No.21	3 .0300	2 .0400	2 .0440	3 .0203	3 .0193	3 .0353

As a preliminary, an analysis of variance was done for each experiment. The unequal numbers in the sub-classes precluded the usual analysis of variance; the approximate method of unweighted means (Snedecor 1956, p.385) was used. However an unbiased estimate of error variance was calculated from the original data treated as a single classification analysis of variance. For comparison with the analysis of the means, the error

variance was divided by the harmonic mean number of replicates. The two analyses of variance are given in tables 2.37 and 2.38.

Table 2.37: Analysis of variance for experiment 1.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Concentrations	.00326977	7	.00046711	4.40 **
Temperatures	.00086513	2	.00043257	4.07 *
Interaction	.00173377	14	.00012384	1.17
Error		32	.00010623	

Table 2.38: Analysis of variance for experiment 2.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Concentrations	.00092873	7	.00013268	1.92
Temperatures	.00020125	2	.00010063	1.45
Interaction	.00046841	14	.00003346	0.48
Error		39	.00006923	

The ratio of $\frac{.00010623}{.00006923}$ = F with 32 and 39 D.F. Hence F = 1.53 indicating that the two error variances are not significantly different at the 5% level of probability.

Both experiments showed similar trends in the table of means and similar results in the analysis of variance. Accordingly the data were tested to see if the distinction between experiments could be discarded.

The data were grouped into a double classification of 24 treatments x 2 experiments. Once more the error variance was calculated from the original data. Unbiased estimates of the sums of squares for both interaction and experiments were found by a weighted means analysis of the 2x24 table of means (Snedecor, 1956, p.382). The analysis is given in table 2.39.

Table 2.39: Combined analysis of variance for experiments 1 and 2.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Treatments	.023620	23	.0010270	5.29 ***
Experiments	.000313349	1	.000313349	1.61
Interaction	.0067563	23	.00029033	1.49
Error	.013789	71	.00019421	

There is no evidence of interaction, nor any reason to expect interaction; also there is no significant difference between the main effects for experiments. Consequently the data were combined into an 8 x 3 factorial design and analysed by the method of unweighted means. The sums of squares for interaction and error in table 2.39 were pooled to give an estimate of error variance based on 94 D.F. The final analysis is given in table 2.40.

Table 2.40: Analysis of variance for pooled data from table 2.36.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Concentrations	.002765518	7	.00039507	4.62 ***
Temperatures	.000941571	2	.00047079	5.50 **
Interaction	.000843016	14	.00006022	0.70
Error		94	.00008556	

The means for the two main effects are plotted in figure 2.12.

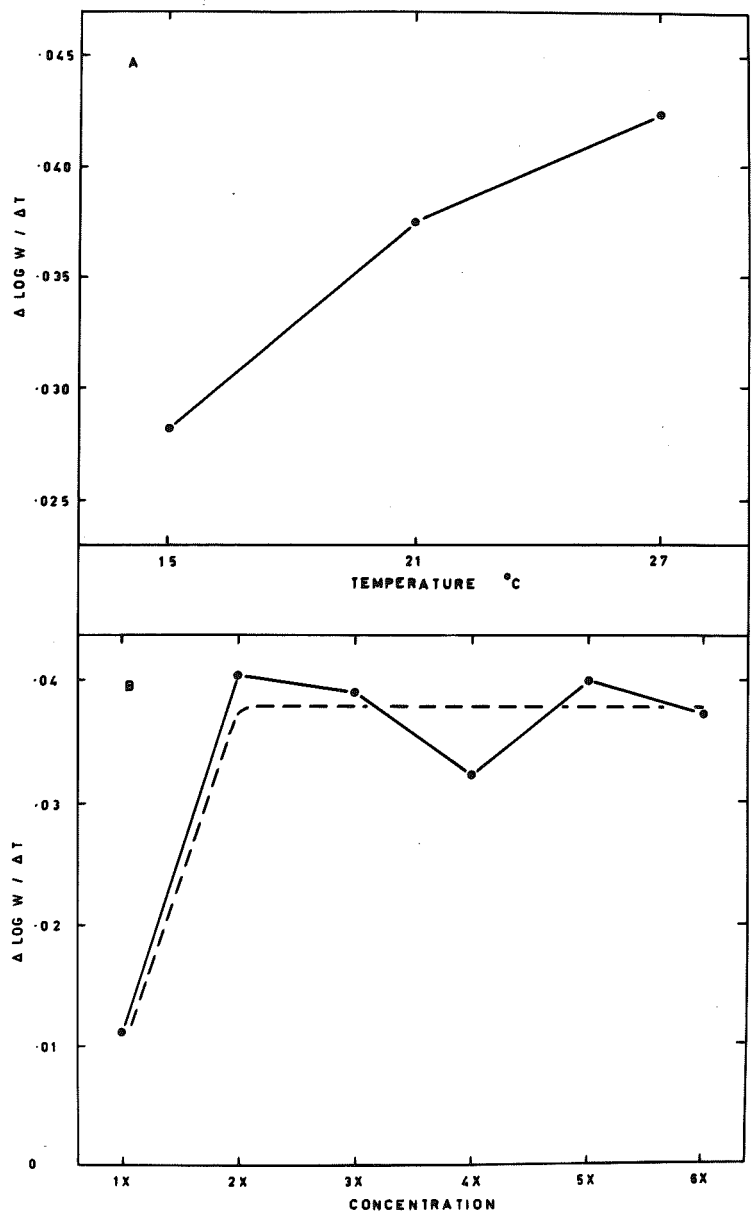


Figure 2.12: A: the relationship between growth-rate and temperature. B: relationship between growth-rate and concentration of pondwater. The broken line represents the hypothetical curve.

Although rather variable, the results for growth at different concentrations are consistent with the model proposed at the beginning of this section, and indicated by the dotted curve in figure 2.12B. At concentrations of 2x and greater, the larvae were growing at their maximum rate. In normal pondwater they were experiencing a relative shortage of food.

The curve for growth-rates at different temperatures shows the expected increased growth-rates with increasing temperatures. The slope seems to be decreasing above 21°C, which is similar to the results for the duration of the pupal stage (section 2.21).

An experiment with a similar design was repeated with water from another pool, No. 21. One treatment (2x) was left out to reduce the number of larvae used. The mean growth-rates are given in table 2.41.

Table 2.41: Mean growth-rates of larvae in concentrated pondwater at 3 temperatures.

	15°	21°	27°
1x	+ .009	+ .007	+ .038
3x	+ .014	+ .008	+ .016
4x	+ .016	+ .021	+ .031
5x	+ .008	+ .006	- .015
6x	- .061	- .052	all dead
3x + NaCl to 6x	- .090	- .090	all dead
3x : No. 207	+ .035	+ .024	+ .022

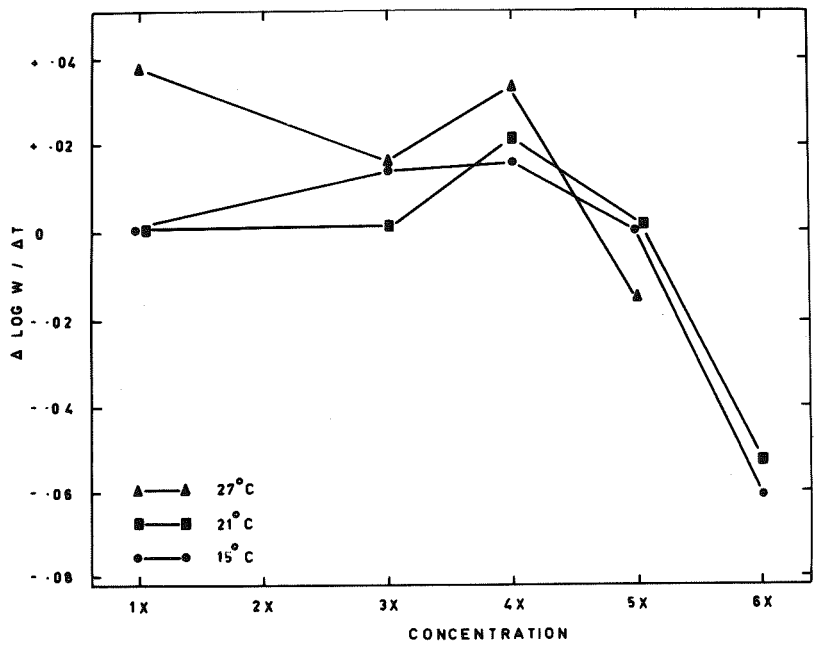


Figure 2.13: The relationship between growth-rate and concentration at 3 temperatures.

The curves for growth-rate at 5 concentrations and 3 temperatures are shown in figure 2.13. The shape of the curves indicate drastic effects of osmotic pressure. This inference is confirmed by the control 3x + (NaCl ≡ 6x) which gave a greater loss of weight than 6x pondwater.

The osmotic pressure of 3x pondwater was measured and found equivalent to 1.07% NaCl. Thus, the osmotic pressure of pool 21 was 1.07/0.450 = 2.4 times greater than pool 207. The experiment was not analysed

further because it is not relevant to a discussion on food. However, it does show the deleterious effect of increases in osmotic pressure. The influence of osmotic pressure is discussed more fully in section 3.22.

(b) Quality of the food.

In the field, larvae were observed foraging on the bottom of pools. The bottoms of transient pools are usually rich in decaying organic matter, such as leaves and grasses, that accumulates over the summer. I attempted to estimate the influence of solid matter at the bottom of the pool by collecting two samples of water from pool 44. The first was taken from the surface; the pool was then vigorously stirred with a spade, bringing up mud and organic matter, which was included in the second sample. Each sample was used at concentrations of 1x, 3x and 5x. The results are given in table 2.42 as mean Δ log. w/Δ t.

Table 2.42: Mean growth-rates in 2 types of pondwater.

Concentration	Surface water	Water + solid matter
1x	.008	.033
3x	.046	.036
5x	.039	.046

Again, numbers in the sub-classes were unequal because 7 larvae moulted. The data were analysed as a 2 x 3 table using weighted differences between means, which gave unbiased estimates of the sum of squares for interaction and sum of squares for pondwaters. The analysis of variance is given in table 2.43.

Table 2.43: Analysis of variance of data given in table 2.42.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Pondwaters	.000441	1	.000441	1.46
Concentrations	.003353	2	.001677	5.50 **
Interaction	.001628	2	.000814	2.67
Error		52	.000305	

Thus, there are significant differences between concentrations but not between pondwaters. The means in table 2.42 indicate that the larvae are growing at about their maximum rate in 3x pondwater. Therefore, the interesting comparison is between the 2 pondwaters at 1x concentrations. A t test was computed from the original data giving t = 3.37 ** with 18 degrees of freedom. Thus, including the solid matter significantly increased growth and the ratio of means indicates about a fourfold increase. Further comparisons between means were made by calculating the least difference between 2 means (D) which is significant at the 5% level of probability. The test given by Snedecor (1956, p.251) was used in which $D = Q\, s_{\bar{x}}$.

Where Q is the upper 5% point in the Studentized range

$s_{\bar{x}}$ is the standard error estimated from the analysis of variance table.

$D = 4.19 \times .00181$

i.e. $D = .008$.

Among the 3 means for water without solids, the means for 3x and 5x differ from 1x but not from each other. Thus, maximum growth was reached

at 3x. Among the 3 means for water plus solids, the mean for 5x differs from the other 2 and the mean for 3x differs from 5x. Thus, there is evidence of increased growth when the concentration is increased from 1x to 5x, but the increase is not as great as that shown with water alone.

(c) Growth-rates in other pools.

(i) Water was collected from pool 209 and 10 larvae put at each of the following concentrations: $\frac{1}{4}$x, $\frac{1}{2}$x, 1x, 2x, 4x. The dilutions were made with distilled water, and the experiment was done at 27°C. The results are given in table 2.44.

Table 2.44: Mean growth-rates of larvae in diluted and concentrated pondwater.

Concn.	$\frac{\Delta \log w}{\Delta t}$	No. of replicates
$\frac{1}{4}$x	.006	10
$\frac{1}{2}$x	.008	9
1x	.008	10
2x	.013	10
4x	.018	8

A linear regression was fitted to the means of the five treatments. The regression equation was $Y = .0056 + .00325\ x$.

The analysis of variance for the regression is given in table 2.45.

Table 2.45: Analysis of variance of regression for data in table 2.44.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Due to regression	.0000338575	1	.000033857	70.9 **
About regression	.0000014325	3	.000004775	
Total	.0000352900	4		

The means are plotted in figure 2.14. It can be seen that the mean increase of growth-rate with concentration is approximately linear and that b is significantly different from zero. There is no sign of maximum growth-rate being reached up to 4x concentration and this indicates that the larvae experienced a moderate relative shortage of food in the normal, 1x, concentration.

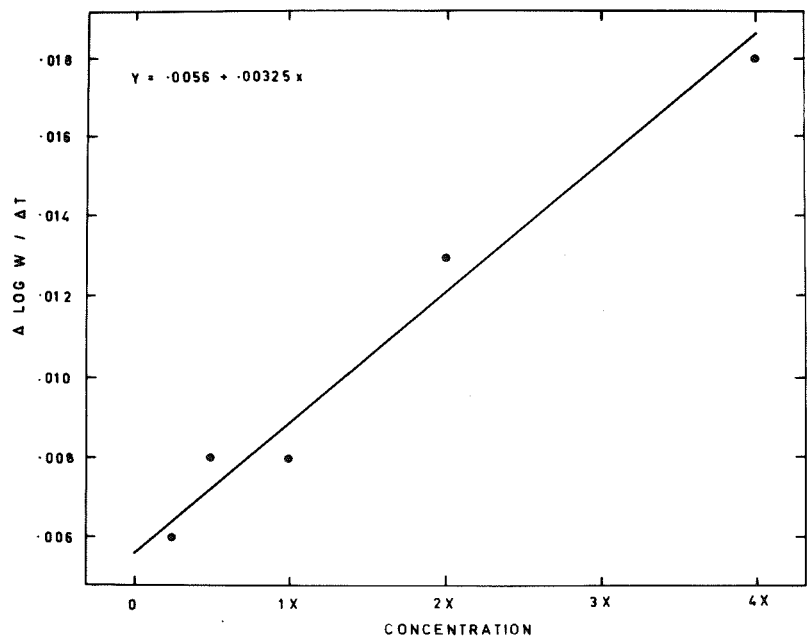


Figure 2.14: The regression of growth-rate of larvae on concentration of pondwater from pool 209. Y = .0056 + .00325 x.

(ii) A second example illustrates a severe relative shortage of food. In October 1960 I set out to estimate larval mortality in pool 400, using the removal method described in section 2.43. Samples were collected on 2 occasions, 7 days apart. The data from the second occasion did not conform to the model on which the removal method is based and consequently larval mortality could not be estimated. However, the larvae had been classified into their instars and the data indicated only slight development over the seven days. A summary of the data is given in table 2.46.

Table 2.46: Proportion of different instars in samples collected from pool 400.

Date	L_1	L_2	L_3	L_4	P	Actual total caught
19 Oct. 60	5%	12%	77%	5%	1%	231
26 Oct. 60	0	11%	78%	8%	3%	823

The weather during the week was warm and dry, and from previous experience I expected most of the individuals to be in the fourth-instar or pupae. It was unlikely that any more eggs hatched after the first sample was taken because the level of the pool had been falling. Certainly more larvae were caught in the second sample, but that was probably because the smaller volume of the pool made the larvae more accessible. On the second occasion some water was collected in order to estimate the amount of food in it. First, the osmotic pressure was measured and found equivalent to 0.067% NaCl, far too low to cause osmotic stress.

The mean larval growth-rate was measured in 1x pondwater from pool 400 and compared with the mean larval growth-rate in 1x pondwater from pool 209. The pondwater from pool 209 was from the same sample that was used in the previous experiment, having been stored frozen. Twenty-four larvae were put into each treatment but 6 moulted during the experiment and were discarded. The results are given in table 2.47.

Table 2.47: Mean growth-rates of larvae in normal pondwater from pools 400 and 209.

Pondwater from pool	No. of larvae	Mean growth rate $\frac{\Delta \log_e W}{\Delta T}$	Pooled estimate of variance	t
400	22	.0148	.0905	3.94***
209	20	.0038		

Thus there was a significantly greater amount of growth in pondwater 209, the ratio of the means indicating approximately 4 times as much food in pondwater 209. Now, growth-rates from different experiments cannot be compared accurately because the larvae were caught in the field and I would expect different collections of larvae to respond differently to identical food. However, in the first experiment on pondwater 209, growth-rate increased linearly with increasing concentrations up to 4x, whereby indicating a moderate relative shortage at 1x. The second experiment showed even less food in 1x pondwater 400 than in 1x pondwater 209. This evidence together with the 2 counts of larvae, indicates that the larvae in pool 400 were experiencing a severe relative shortage of food.

2.353 Discussion.

The results of the first experiment are consistent with the model proposed in the introduction to this section. But the important question is whether the results apply to larvae living in natural ponds. There are probably two major differences between the experiments and nature. Firstly the pondwater has passed through various processes that kill the larger organisms, though probably not all the micro-organisms, and secondly, the larvae were confined in small tubes whereas in a pool they are free to browse on various substrates as well as filtering particles from the water.

Probably the experiment on pool 44, in which sediment was included, was the nearest to nature. It is noteworthy that the treatments including sediment showed virtually no relative shortage, in contrast to a moderate relative shortage in pondwater alone. Possibly, larvae living in pool 207, which was tested in the first experiment, did not experience a relative shortage when they also had access to sediment on the bottom of the pool.

In the last experiment, however, the severe relative shortage was judged both from observations in the field and by the experiment in the laboratory. Thus it is reasonable to conclude that the experiments in the laboratory do indicate what happens in nature, although they probably exaggerate the severity of the relative shortage.

2.36 Mosquitoes biting at dusk.

The counts of mosquitoes biting at dusk were made primarily to estimate the incidence of adult mosquitoes, and this aspect of the results is discussed in section 4.12. For most of the counts, records were kept of temperature, relative humidity, light intensity and wind velocity to see if these factors were associated with the activity of the mosquitoes.

2.361 Methods.

(a) Mosquitoes were caught as they settled on my bare legs. They were identified as they were caught, put into small cages, and the identifications checked on the following day. The catches were grouped into intervals of 15 minutes. Usually counting was started before the mosquitoes became active, and was continued until an interval of 15 minutes was completed in which no mosquitoes were caught.

(b) Temperature and relative humidity were measured with a whirling hygrometer every 15 minutes.

(c) Light intensity was measured with a "Weston" photographic meter every 15 minutes. The meter was moved around until the maximum light intensity of the western sky was noted. The meter was calibrated against a "Weston Photronic Foot-candle Meter, Model 514" lent to me by the Electricity Trust of South Australia. The photographic meter was calibrated by reading both meters at dusk, over the range of 0 to 30 foot-candles. Over this range the photographic meter was reading directly in foot-candles, although the scale could not be read as accurately as the foot-candle meter.

(d) Wind velocity was estimated roughly by noting the movement of leaves and branches of trees and referring to the table of wind-velocities and noticeable effects given by Kimble (1951).

2.362 Results.

Counts were made on 25 occasions and on 16 of these less than 5 mosquitoes were caught. On the remaining 9 occasions the numbers ranged from 5 to 18 per night. Only 5 out of the 9 counts were sufficiently complete for comparing the effect temperature, humidity, light and wind velocity.

(a) Wind velocity. Most of the counts were deliberately made on calm nights and hence the data are not extensive enough for any detailed analysis. However, warm nights with no wind seemed favourable to activity whereas cold nights with wind-velocities above about 10 miles per hour seemed unfavourable.

(b) Temperature, relative humidity and light. On most nights, as light intensity and temperature decreased, the relative humidity increased. These associated changes are shown in figure 2.15a, in which the temperature fell by 17 degrees while the relative humidity rose from 60% to 98%. However, on some nights the changes were slight and on 10 November, 1958 the trend was almost reversed as shown in figure 2.15b. This evening's count followed the passage of a thunderstorm in the afternoon; the wind was force 0 after the storm, but just before the count was started, the wind blew at about force 3. The changes shown in figure 2.15b probably resulted from mixing air-masses at different temperatures. The results are given in table 2.48.

Table 2.48: Meteorological measurements and mosquitoes biting at dusk.

Interval	A				B			
	Temp °F.	Rel. Hum.%	Light f.c.	No. of Mosquitoes	Temp. °F.	Rel. Hum.%	Light f.c.	No.of Mosquitoes
1	77	59	375	0	72	74	400	0
2	74	59	200	0	72	65	175	0
3	70	65	75	0	70	69	37.5	1
4	67	69	11	5	71	75	3.2	11
5	66	68	0.4	5	67	78	0	4
6	60	96	0	1	66	78	0	1
7	60	96	0	0	71	57	0	0

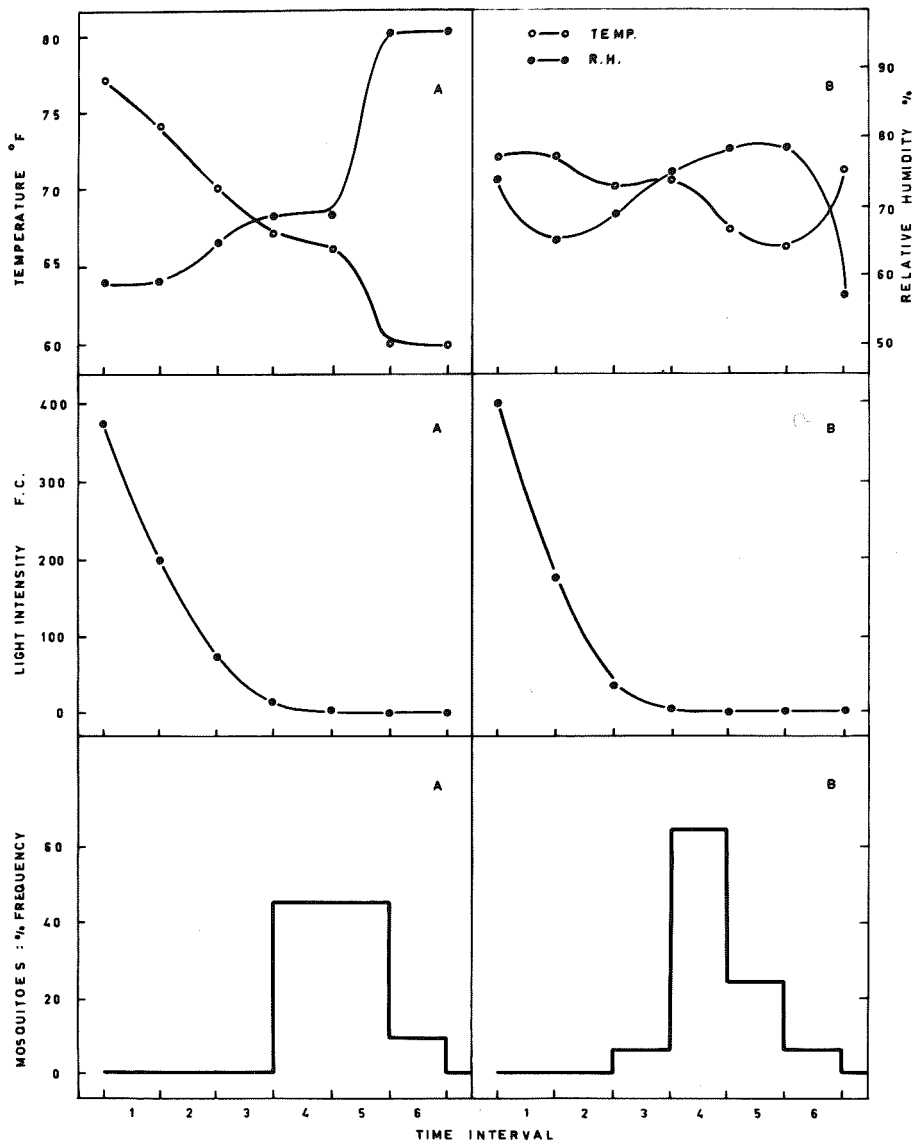


Figure 2.15: Measurements of temperature, relative humidity, light intensity and mosquitoes biting at dusk. The measurements were made at Hackham; A : 3 April,58; B: 10 Nov.58.

It is apparent that on these two nights the mosquitoes were active for a short period and that the activity was correlated with light intensity rather than with temperature and relative humidity. Similar results were obtained on other nights, but the changes in relative humidity and temperature shown in figure 2.15 were the most extreme encountered.

(c) The effect of light intensity alone.

The meter used to measure light intensity was not sensitive to low levels of illumination, and registered zero when about half the mosquitoes had been caught for each night. However, I could estimate light intensity by graphical interpolation at the lower quartile, i.e. when 25% of the mosquitoes had been caught. The results are given in table 2.49.

Table 2.49: Light intensity at which 25% of the mosquitoes had been caught.

Night	Light intensity f.c.
3 April, 1958	3
10 Nov. 1958	1.5
18 Nov. 1958	3
11 March, 1959	2.5
1 Nov. 1959	3.5

The mean of the light intensities is 2.7. Because it is a small sample, Bessel's correction (Moroney, 1953) was applied and the standard error of the mean calculated at $\pm$ 0.379. Thus the 95% Confidence Limits are 1.7 to 3.8 foot-candles. In view of the rapid change in light intensity at dusk, the 95% Confidence Interval is remarkably small.

(d) The period of activity at dusk.

To give an idea of the period of intensified activity after sunset, the results for the 5 nights were treated as follows. A particular light intensity was selected and the time (t_i) it occured was found by graphical interpolation. For each t_i, the number of mosquitoes (p_i) that had bitten up to that time was found by interpolation in the grouped data, using the formula given by Moroney (1953, p.77). The p_i were then summed and divided by the total number of mosquitoes caught for the five nights, thus giving the proportion of mosquitoes caught during the 5 nights up to the particular light intensity. The value for the light intensity was chosen to give the proportion caught equal to 0.25, i.e. the lower quartile. This value for the light intensity was found by iteration to be 2.4 foot-candles. By exactly the same method of iteration, the period between catching 25% and 75% of the mosquitoes was found to be 17.6 minutes. Thus the data can be summarized by saying that during the 5 nights, 25% of the mosquitoes had bitten by the time that the light intensity had fallen to 2.4 foot-candles, and 75% had bitten 17.6 minutes later. The period of 17.6 minutes is the inter-quartile value and is a measure of the spread of the data. The inter-quartile value indicates a limited period of activity because 50% of the mosquitoes were caught within 18 minutes.

2.363 Discussion.

Lee et al. (1957) found a similar, brief period of activity at dusk at Colo Vale, in the Great Dividing Range, N.S.W. They were unable to correlate activity with temperature and humidity, although the period of activity was longer when cloudy weather was combined with a high relative

humidity. With reference to light they stated "The sudden increase in attack occurred before darkness set in but may well have been triggered-off by a reduction in light values to a very low threshold." The situation at Colo Vale was more complex than at Hackham; Lee et al. recorded 11 species of mosquitoes (including Ae. alboannulatus) attracted to man at dusk, whereas at Hackham there were seldom more than 2 species.

It seems reasonable to conclude that the onset of the period of activity is related to light intensity. The numbers of mosquitoes caught would be related to other meteorological conditions and to the density of the population of adult mosquitoes at that time.

2.37 Experiments on rearing Ae. alboannulatus.

The main reason for trying to rear Ae. alboannulatus in the laboratory was to provide a supply of all stages of the life-cycle, which could be used for experiments.

During a project for the Honours Degree of B.Sc., I found that Ae. alboannulatus would not mate in cages 2 feet square by 3 feet high. Bates, (1949) stated that species that will not mate in small cages often do so in large cages. Consequently, the first experiments were done in large cages, both indoors and out of doors.

2.371 An experiment in a large indoor cage.

In small cages, Ae. alboannulatus adults lived for 2-3 weeks provided that the relative humidity was kept at about 80% and that drinking-water was supplied.

For the first experiment, a cage 11.5 feet high and 6 feet x 4 feet was made by building a partition across a room that was maintained at a constant temperature of 25°C. The relative humidity was kept constant at about 80% by means of a bundle of hair that switched a heating element in a water-bath. A small fan was connected in parallel with the heating element to circulate the steam. A "daylight" 20w. fluorescent tube was switched on for 15 hours each day and a 60w. opalescent globe connected to a variable transformer was used to simulate dusk at the end of the 15-hour light period. Small tubes of water stoppered with cotton-wool were placed around the room for the adults to drink from.

About 100 adults emerged from larvae collected at Hackham in June 1958, and were subsequently released in the cage. On the second and third days I stayed in the cage during the simulated dusk. While the main light

was on no mosquitoes were flying, but after it was switched off adults began flying around the cage, and 8 females engorged from my arms.

I did not see any mating behaviour, and by the fifth day, all the mosquitoes were dead. Apparently, longevity was less in the large cage than in the small one, even though the relative humidity and temperature were the same.

2.372 Experiments in large outdoor cages.

Rather than modify the indoor cage to increase longevity, further experiments were made in large cages built over the creek at Hackham. Also I hoped that mosquitoes in the cages would experience a natural environment which included stimuli to mate.

Three cages were built one week after heavy rain in February 1959 had filled several transient pools. The cages were 6 feet square by 8 feet high and were made of green mosquito netting with a strip of headcloth around the bottom where they were fastened to the ground. One cage was built over pool 8, with all 4 sides dipping into the water. The other 2 cages were built side by side over pool 9 and were fastened to the tops of the banks. Thus, these two cages included overhanging banks about 2 feet high.

Before the cages were erected, the numbers of larvae and pupae were estimated by the method of removal sampling (described in section 2.43). The estimated absolute numbers are given in table 2.50. The cages over pool 9 divided it in half so that approximately half the larvae and pupae were in each cage.

Table 2.50: Absolute numbers of larvae and pupae in pools 8 and 9.

Date	Pool	Larvae	Pupae
21 Feb.59	8	2,812	0
23 Feb.59	9	8,953	351

One of the cages on pool 9 was left as a control, in which the mosquitoes were not given a chance to engorge blood. In the other two cages, rabbits were provided for the adults to feed on; they were left in for 5 hours, including the period of dusk, on the 8th, 10th, 12th and 16th days, and overnight on the 19th day. Soon after the cages were erected, the pools began to dry up, so I refilled them with rainwater. Pool 8 was refilled on the 29th and 30th days and pool 9 on the 3rd, 12th, 15th, 24th and 27th days.

I sat inside the cage over pool 9 on several nights, and as the sun set, I saw mosquitoes flying out from under the overhanging banks. The females fed from me, especially at dusk, although they also fed during the day. On most nights, 10 to 15 mosquitoes were observed hovering around the rabbit at dusk, and some were seen to engorge blood.

Every night that I watched them, the mosquitoes in all three cages, gathered in the upper, western corner, facing the setting sun. At sunset 50 to 100 adults, both males and females, were flying about in the corner. When I returned before dawn, the mosquitoes were still aggregated in the western corner, sitting motionless on the cage. They did not move when the sun first rose, but by mid-morning the adults had all disappeared,

presumably into resting-places near the bottom of the cage.

Three criteria were used for detecting mating.

(1) On several occasions a few females were dissected and their spermathecae examined for sperms. A total of 12 females from the cage on pool 8, and 8 females from the cage on pool 9 were dissected, but all were unfertilized. Also 2 males were dissected; both had motile sperm in their vesiculae seminales.

(2) The edges of the pools were lined with absorbent paper towelling which made it easy to see any eggs that were laid. No eggs were observed.

(3) If some eggs were laid on the soil between small gaps in the paper, they should have hatched when the pools were refilled, but no first-instar larvae were found. Also no mating or swarming was observed.

When the cage on pool 9 was dismantled on the 62nd day, no adults were found. The cage on pool 8 was left up until the 104th day. When it was dismantled 30 male and female mosquitoes were caught and transferred to a similar large outdoor cage at Adelaide, described below.

The fourth large cage was built in an orchard at Adelaide. It was provided with an aquarium, a small conifer to give shelter to the adults, and some Agonis flowers to provide nectar. Ae. alboannulatus larvae and pupae were collected from the creek at Hackham and put in the aquarium. A rabbit was put in the cage periodically for the females to feed on.

The adults behaved in the same way as the ones at Hackham. At dusk they flew out of the conifer, and some females engorged blood. The males and females also aggregated in the western corner each night. The aquarium was lined with absorbent paper and on the 34th day 37 eggs were

laid. However, these proved to be infertile .

Discussion.

While the experiments with large cages were being done, another experiment on oviposition was set up in pool 10, about 5 yards upstream from pool 9. The results, given in section 2.332 showed that large numbers of eggs were being laid by 7 March, 1959, which corresponds to the 16th day after the cages were erected. The eggs were probably laid by females which emerged about 26 February, 1959. Thus, 9 days after emergence, the females were laying fertile eggs around a pool similar to the 2 inside the cages.

It appears that even in a cage of nearly 300 cubic feet, set in natural surroundings, the mosquitoes do not mate.

2.373 Experiments on stimuli to mating.

One interesting observation in the outdoor cages was the aggregation of mosquitoes in the western corners at dusk. Some species are known to disperse a considerably distance from their breeding-places on the first day after emergence, and then not move much on later days, e.g. Ae. taeniorhynchus (Provost, 1957). It seemed that the cages may have prevented a similar dispersal flight in Ae. alboannulatus, which may be necessary before mating occurs. The following experiments were devised to make the mosquitoes fly as much as possible.

Kennedy (1939) found that both moving patterns and slow-moving air induced mosquitoes to fly. These results suggested that changes in light intensity or slow currents of air might stimulate mosquitoes in a cage to fly. For preliminary trials 10 Ae. alboannulatus adults were put in a cage 2 feet x 1.5 feet and 3 feet high. Switching from darkness

to light stimulated some mosquitoes to fly, but the effect did not persist over repeated trials. Similarly, slow currents of air from a 12 inch fan had little effect. When the fan faced directly at the cage all the mosquitoes clung to the mesh sides, but when the fan was switched off the mosquitoes flew for several minutes.

The best results were obtained by combining both stimuli. The fan and light were wired to a time-switch so that the light went off when the fan came on and vice versa. After the fan was on for 1 minute, 3 of the mosquitoes flew for about half a minute. Similarly after the fan was on for 10 minutes, 3 mosquitoes flew for about half a minute. However, after the fan was on for 5 minutes, 7 to 8 of the 10 mosquitoes flew for about 6 minutes.

For the experiment the time-switch was set to switch the fan on, and the light off, for 5 minutes every 20 minutes. In the second experiment, this cycle was used 390 times and the mosquitoes were flying as actively on the last time as at the start. The 2 experiments were done in a constant temperature room at 25°C at a constant relative humidity of 80%.

In the first experiment about 70 adults, both males and females, were put in the cage. An immobilised rabbit was put in the cage regularly and most of the females fed from it. The fan and light cycle was switched 164 times, during the day only. The cycle was stopped after the eighth day because many mosquitoes were dying. To estimate the amount of time a mosquito flew, I assumed that half of the mosquitoes flew for 4 minutes after each cycle. This gives an estimate of 5.5 hours flying.

Some mosquitoes lived until the 30th day, and on the 26th and 27th days 231 eggs were laid. The eggs subsequently proved infertile.

A second experiment was done with 130 females and 160 males, in which the light and fan cycle was continued for 24 hours a day. The cycle was switched 390 times in the first 6 days, after which the cycle was stopped because mortality increased. On the same assumptions as above, the estimated time a mosquito spent flying was 13 hours. Each day the dead females were dissected and their spermathecae examined for sperm. On the 18th day one female had sperm in 2 of the 3 spermathecae. On the 21st day the 18 surviving females were removed from the cage and placed individually in small vials provided with moist paper. Six of the 18 females laid a total of 313 eggs that all proved to be infertile. The remaining 12 females were dissected as they died and again all were unfertilized.

Thus, out of 130 females, only 1 mated, but died before laying eggs. If this female had laid eggs I might have been able to select for a strain of mosquitoes that mated in small cages. However, larval, pupal and early adult mortality would have made it difficult to establish a colony.

2.374 Experiments with males.

Roth (1948) studied the behaviour of Ae. aegypti, which mates readily in small cages. He found that the male perceived the sound made by the wings of the female by means of his antennae. When the antennae were removed, the male no longer perceived the female and made no attempt to copulate, even when brought close to a female. Downes (1958) studied the swarming of Ae. hexodontus, and found that males

caught in swarms had the antennal setae erected, whereas males caught in resting places had the setae folded along the shaft of the antenna. He stated that the antennal setae of Ae. aegypti were erected about 1 day after emergence and then stayed erect during the lifetime of the male. He suggested that this might be why Ae. aegypti mates in small cages.

In all the experiments with Ae. alboannulatus, I never saw the antennal setae of the males erected. Thus their inability to mate in cages may be due to the lack of a stimulus to erect their setae.

Observations in the field showed that Ae. alboannulatus adults were more active at dusk than at any other time. Hence, if some environmental factor stimulates erection of the antennal setae, it probably occurs at dusk. Obvious factors at dusk which could act as stimuli are changes in light intensity, relative humidity and temperature. But these changes were experienced by the adults in outdoor cages with no result.

The adults that aggregated in the corner of the outdoor cage flew spasmodically, frequently resting on the mesh. A final experiment was done out of doors at dusk in which several males, mounted side by side, were made to fly continuously. A piece of fine wire was fixed to the thorax with wax and the other end attached to a light, metal strip. A jewelled bearing near one end of the strip was placed on the point of a steel needle, which was mounted vertically, so that the strip turned freely in a horizontal plane. Thus, when the mosquitoes flew, they rotated the strip and went round the circumference of a circle of about 20 cm. diameter.

Two revolving strips were used, 1 with 4 males and the other with 3. As controls, 20 males were crowded in a small cage 4 x 4 x 6 inches and 5

males put in individual tubes. The males on the revolving strips flew for most of the two-hour period of dusk, but did not erect the antennal setae. Nor did the males crowded in the cage or in the tubes.

2.375 Induced copulation with decapitated males.

McDaniel and Horsfall (1957) found that after a male's head was cut off, it could be induced to mate with an immobilized female. Apparently a nerve-centre which normally inhibited mating was removed with the head. Ae. stimulans and Ae. vexans copulated within seconds when the tips of their abdomens were brought together, ventral sides uppermost.

I tried the technique with Ae. alboannulatus and found that decapitated males attempted to copulate, but the females pushed the males away with their legs. Females were then glued to strips of cardboard which fitted snugly into glass tubes, and gently pushed backwards in the tubes so that their legs were bent back over their heads, while their abdomens projected from the tubes. Even then, males did not properly contact the females when held in the simple position described by McDaniel and Horsfall (1957). I managed to induce some males to copulate by first holding the mosquitoes with their ventral sides together, and the tips of the abdomens touching. Then as the male attempted to insert the genitalia, it was swung back until the bodies were in line, with the heads pointing in opposite directions.

Twenty pairs of mosquitoes, which had been reared from larvae, were tested. Two females laid batches of eggs in which about half the eggs were fertile. The other 18 either laid eggs which were all infertile or did not have sperm in their spermathecae when dissected.

This technique could be useful for genetical experiments, but it was too unreliable to be used to supply eggs for physiological experiments.

2.4 The numbers and distribution of Ae. alboannulatus larvae in the creek at Hackham.

During 1958 and 1960 the trends in the population of Ae. alboannulatus were followed by estimating the total numbers of larvae and pupae in the creek at monthly intervals. The total or absolute number of larvae in the creek was found by summing the estimated absolute numbers of larvae in each individual pool. The results for each pool were kept separately so that the history of individual pools could be followed.

2.41 Estimating the absolute numbers of larvae and pupae in a pool by random sampling.

Altogether there are about 350 pools in the creek. Ae. alboannulatus was not found in all these pools, but in winter a large number of pools had to be examined each month, and consequently not much time could be spent on any one pool. Thus, limitations of time restricted the accuracy of the sampling program. Samples were not taken strictly at random, but rather in a random manner, without reference to the distribution of larvae and pupae. Most of the pools had dark backgrounds, which made it difficult to see the larvae and pupae and thus reduced the risk of unconscious bias.

A sample of larvae and pupae was taken from the pool by sweeping a rectangular net, 6 inches wide, up the longest axis of the pool. The larvae and pupae were counted, grouping the instars approximately into 3 classes; first to second-instar larvae; third to fourth-instar larvae; and pupae. The larvae and pupae were then returned to the pool. Only 1 sample was taken from each pool because once the net had been swept through the water, all the remaining larvae swam to the bottom and remained there for 5 to 10 minutes.

The area traversed by the net was measured and the numbers of larvae and pupae per square foot were calculated. The area of the pool was estimated by various geometrical approximations. Most of the pools were elliptical, others rectangular, etc. In each case the appropriate measurements were taken and the surface-area calculated. The numbers of larvae and pupae per square foot were then multiplied by the surface-area to give the total numbers of larvae and pupae in the pool.

The accuracy of the method depends on the assumptions that firstly, all the larvae are at the surface of the pool when the sample is taken, i.e. sampling is on the basis of area rather than volume, and secondly, that the sample is a representative sample. The first assumption is a reasonable approximation; provided that the larvae have not been disturbed by water movement or shadows, the majority seem to be at the surface. However, in clear water a few can always be seen feeding on the bottom and there seems to be a few larvae continually moving between the surface and the bottom of the pool. The second assumption is less reasonable. The net was swept from one side of the pool to the other and thus sampled larvae from the edges as well as the centre. However, on sunny days, third to fourth-instar larvae and particularly pupae tended to aggregate under shadows, and this patchiness of the distribution made it hard to get a representative sample.

To test the accuracy of the sampling, another method was sought which was independent of estimates of area or volume of the pool. The marking, release and recapture method seemed promising, provided that the larvae could be marked. In the laboratory various vital stains such as Methylene Blue and Giemsa were suitable for marking larvae, but when the technique was tried in the field the stains did not persist. The

"removal method" for estimating absolute numbers was then tried and is described in section 2.43 below.

2.42 Results and analysis of the regular counts of larvae and pupae in the creek.

The results from the monthly counts are given in table 2.51 and figure 2.16.

Table 2.51: Estimated absolute numbers of larvae and pupae in the creek at Hackham.

Date	L_{1-2}	L_{3-4}	P	Total	Rainfall
1959					
Feb. 12	0	0	0	0	8
Feb. 22	500	3,600	5,300	9,400	99
Apr. 5	40	10,500	0	10,540	159
May 6	500	800	100	1,400	13
Jun. 7	3,400	14,800	100	18,300	42
Jul. 8	4,600	7,700	2,200	14,500	29
Jul. 29	5,700	3,000	300	9,000	152
Sep. 2	1,900	15,000	3,200	20,100	180
Oct. 16	1,300	2,800	2,900	7,000	190
Nov. 1	24,000	1,800	1,700	27,500	86
Nov. 18	1,100	4,400	3,800	9,300	9
Dec. 10	5,200	2,900	6,000	14,100	151
1960					
Jan. 15	300	1,100	100	1,500	158
Feb. 12	100	1,000	12,900	14,000	148
Mar. 11	17,100	100	100	17,300	193
Apr. 6	200	100	10	310	15
May 11	1,600	11,100	21,900	34,600	600
Jun. 2	200	400	10	610	372
Jul. 11	500	800	10	1,310	272
Aug. 15	700	1,000	20	1,720	239
Sep. 15	700	1,200	700	2,600	328
Oct. 19	1,400	900	400	2,700	194
Nov. 13	300	900	500	1,700	97
1961					
Jan. 5	80	600	300	980	50
Feb. 7	70	0	0	70	9

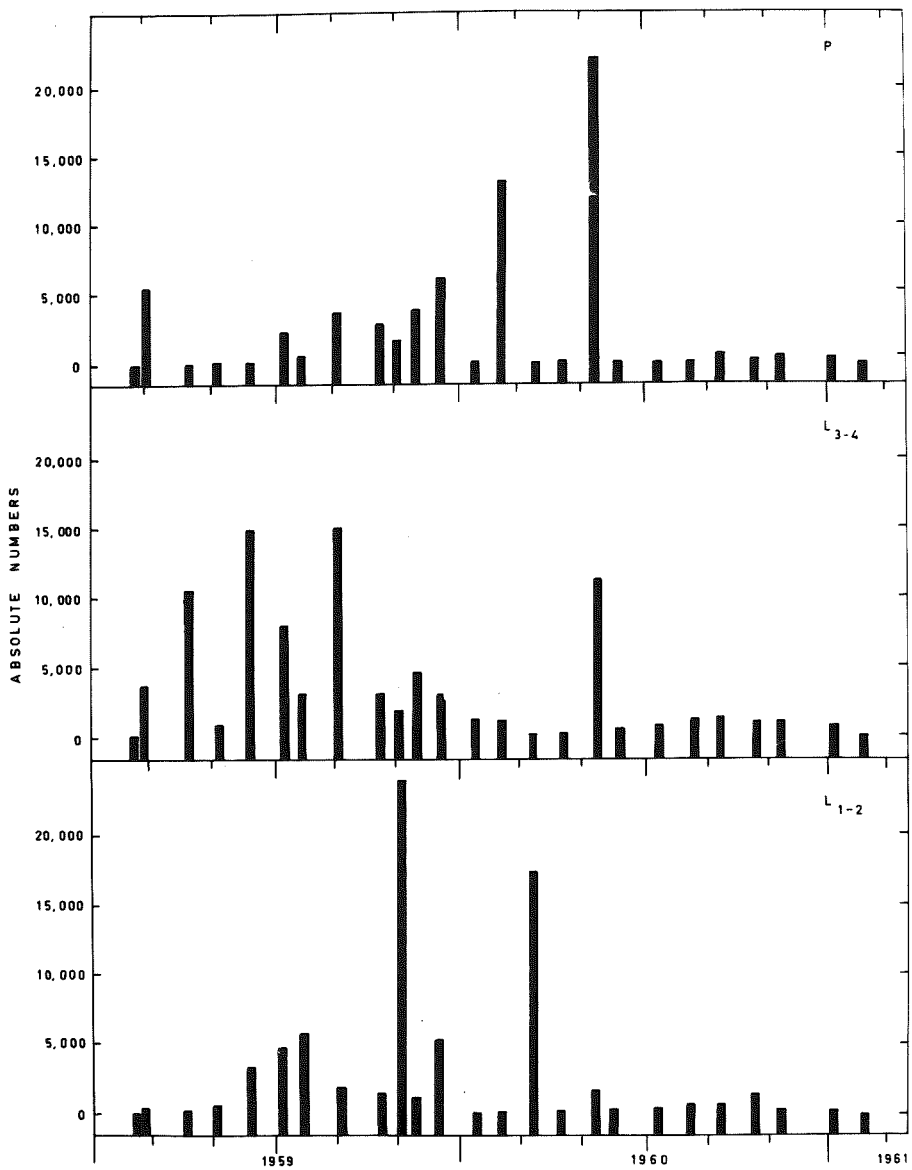


Figure 2.16: The estimated absolute numbers of first to second-instar larvae (L_{1-2}); third to fourth-instar larvae (L_{3-4}); and pupae (P) in the creek at Hackham.

Discussion.

In section 2.2 it was said that the eggs of Ae. alboannulatus hatch after water submerges them. This being so, a high positive correlation between rainfall and the numbers of larvae would be expected. The ideal factors to use in the correlation would be rainfall between 2 counts and the number of larvae hatched during the same interval. However it was difficult to estimate the number of larvae hatched in each interval. With the life-cycle lasting only 2 weeks in summer it was possible to miss completely a whole generation of larvae. In winter, the life-cycle was extended to 2 months and larvae present in 1 count could still be present in the following count. The history of each pool could be followed but even so, an objective decision could not always be made.

As a first approach, I calculated the correlation co-efficient between the total numbers of larvae and pupae at each count with rainfall during the interval up to that count (5th and 6th columns in table 2.51). This grouping had the advantage of including most of the larvae that hatched between counts in summer, but the counts in winter would be biased by including some larvae that had hatched in the previous interval. This bias would enhance the correlation by exaggerating the counts in winter. However, the correlation co-efficient of $r = 0.332$ with 23 D.F. corresponded to a probability of about 10%. Thus, the data are consistent with the null hypothesis that there is no relationship between the 2 variables. I tried various other groupings of rainfall, such as the number of rainy days, or the number of days with more than 10 points, more than 25 points, and so on, but none gave a significant correlation.

Since there is no adequate statistical summary of the influence of weather on the numbers of larvae, a summary of the data is presented in

the form of a diary in table 2.52. In the second column the type of rainfall is described, because 1 inch of rain in 1 day has a much different effect to 1 inch of rain spread evenly over the interval between 2 counts. In the third column is given the ratio of the number of new pools, which did not contain larvae in the previous count, to the total number of pools containing larvae. In the fourth column, pertinent changes in the numbers of larvae and pupae are given.

Table 2.52: Changes in the numbers of larvae and pupae in the creek at Hackham in relation to rainfall.

Date	Rainfall	new pools/ total	Changes in nos. of larvae and pupae.
1959			
Feb. 12	15 pts. since Jan.1	0	No larvae.
Feb. 22	1 in. in 1 week	11/11	9,400 larvae and pupae.
Apr. 5	1 in. on Mar. 31	7/17	10,500 larvae hatched Mar.31
May 6	13 pts. since Apr. 5	1/12	500 L_1 hatched, other larvae from Apr. 5.
Jun. 7	½ in. since May 18	14/26	15,000 out of the 18,000 larvae were in the 14 new pools.
Jul. 8	¼ in. evenly over interval.	8/28	4,000 out of the 4,600 L_{1-2} in the 8 new pools.
Jul. 29	1½ in. evenly over interval.	10/33	Further 5,700 larvae hatched.
Sep. 2	70 pts. on Aug. 2. Another 1 in. evenly over interval.	3/21	About 17,000 larvae in all 21 pools.
Oct. 16	1½ in. evenly over interval.	3/22	Further 1,300 larvae hatched. Most of L_{3-4} from Sep. 2.
Nov. 1	¾ in. on Oct. 28	6/31	24,000 larvae hatched in all 31 pools on Oct. 28.

Date	Rainfall	new pools/ total	Changes in nos. of larvae and pupae.
1959			
Nov. 18	¼ in. evenly over interval.	1/8	24 pools had dried out with mortality of larvae. 9,300 larvae and pupae in remaining pools.
Dec. 10	1½ in. evenly over interval.	14/20	Further 5,200 larvae hatched.
1960			
Jan. 15	1½ in. in Dec. and early Jan.	2/4	Most pools drying out. 1,500 larvae and pupae in remaining 4 pools.
Feb. 12	¾ in. on Feb. 1	4/7	14,000, mostly pupae from Feb. 1.
Mar. 11	1 in. on Feb. 14 and 1 in. on Mar.6	7/14	17,100 larvae hatched on Mar. 6.
Apr. 6	10 pts. on Apr. 1	1/3	200 larvae hatched on Apr.1.
May 11	3 in. over Apr.21-28	44/44	34,600 larvae and pupae mostly from Apr. 21-28.
Jun. 2	A total of 972 pts. since Apr. 6	4/6	Only 610 individuals left, many larvae swept downstream.
Jul. 11	¾ in. evenly over interval.	25/26	1,310 larvae and pupae. Creek still flowing strongly.
Aug. 15	2½ in. evenly over interval.	1/21	1,020 larvae from Jul. 11. Further 700 larvae hatched in old pools.
Sep. 15	3¼ in. evenly over interval.	9/17	Most of the 1,900 larvae in the 9 new pools.
Oct. 19	2 in. evenly over interval.	21/34	2,700 all instars in all pools.
Nov. 13	¾ in. on Nov. 12	6/20	1,700 first-instar larvae in all 20 pools.

Date	Rainfall	new pools/ total	Changes in nos. of larvae and pupae.
1961			
Jan. 5	½ in. evenly over interval.	2/2	980 all instars.
Feb. 7	8 pts. on 29 Jan.	1/1	70 larvae in 1 permanent pool. All transient pools dry.

The following conclusions can be drawn from the diary of events in table 2.52.

(1) All the larvae that hatched can be explained by rises in water-level.

(2) In summer a relatively heavy fall of rain is needed to fill transient pools, but in winter, smaller falls result in large numbers of larvae hatching. For example, early in February, 1959, 15 points did not fill any pools, but later in the month 1 inch of rain filled 11 pools, resulting in 9,400 larvae and pupae. Between June 7 and July 8, 1959, only one quarter of an inch fell, but the level of 28 pools rose and 4,600 larvae hatched. This differential effect of rainfall at different times of the year is one factor precluding a direct relationship between rainfall and the numbers of larvae.

(3) Ae. alboannulatus breeds at any time during the year, depending on suitable falls of rain.

(4) The larvae and pupae are exposed to 2 hazards from weather, drought in summer and flooding in winter. On 18 November, 1959, 24 pools dried out; soil from the bottom of 2 of the pools was stirred in a

saturated solution of salt and dead fourth-instar larvae were found. Most of the 24,000 larvae which hatched on 28 October, 59 probably metamorphosed to adults, but most of the larvae that hatched later in November probably died. On several other occasions pools dried out before the larvae had pupated, resulting in high mortality in those particular pools.

The effect of flooding was seen on 2 June, 1960, after nearly 10 inches of rain fell during 2 months. A rough estimate of the rate of flow near pool 50 gave 1,000 gallons per minute. On 11 May, 1960, an estimated 1,600 first to second-instar larvae, 11,100 third to fourth-instar larvae and 21,900 pupae were in the creek. All the pupae probably metamorphosed to adults before the flooding, and probably some of the fourth-instar larvae did so too. A conservative estimate of the numbers of larvae present in the creek when severe flooding started would be 10,000. The 10,000 larvae were reduced to about 600, a reduction of 94%. Many of the larvae were found further downstream, on flooded pasture-land outside the study area. The 94% reduction therefore includes unknown proportions of mortality and "emigration". Of the 600 larvae counted within the study-area, 550 were found in 1 large pool formed behind a barrier of debris. The pool was so large that all the water flowed through the centre, leaving undisturbed areas at the edges. The remaining 50 larvae were found in 5 small pools out of the main current.

(5) One interesting aspect of the numbers given in table 2.52 is that the 5 monthly counts following the severe flooding on 2 June, 1960 were consistently low, even though 25 new pools filled on July 11 and 21 new pools on October 19. The reduction in numbers could be a result of the severe flooding; but this hypothesis could only be tested if counts were continued for several years, and other severe floods observed.

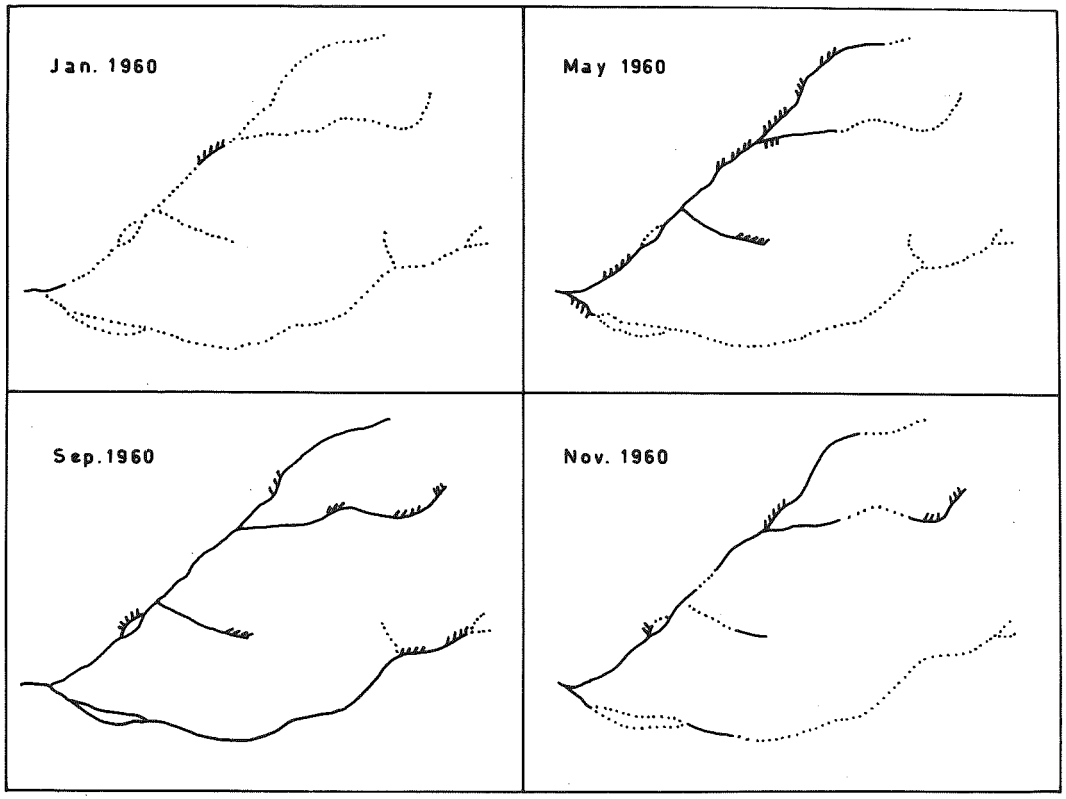


Figure 2.17: The seasonal distribution of Ae. alboannulatus larvae and pupae in the creek at Hackham. The empty portions of the creek system are indicated by dotted lines, the presence of water by solid lines and the presence of larvae and pupae by cross-hatching.

(6) At each count larvae were found in at least 1 new pool, and on most counts about half the pools were new. The figures for new and old pools in table 2.52 reflect striking changes in the spatial distribution of the larvae. The changes in distribution are shown in figure 2.17.

It can be seen that the distribution of the larvae spreads out along the creek as the tributaries fill. Most of the extremities of the tributaries are dry for 6 months of the year. The data given in section 2.324 showed that eggs did not survive in dry pools for 5 months, so it is unlikely that the larvae found in September at the extremities of the tributaries hatched from eggs laid at the end of the previous winter. The distribution indicates that gravid females are dispersing along the creek as it fills.

Conclusions.

The numbers of larvae hatching and surviving in a particular pool depend on a number of factors such as its position in the creek system, the differential effects of rainfall at different times of the year, and the influence of temperature on the speed of development of the larvae. The distribution and abundance of the larvae over the whole creek system result from a complex interaction of these factors together with the powers of dispersal of the females relative to suitable transient pools.

2.43 A new method for estimating the absolute number of mosquito larvae in a pool.

A new method was developed for estimating the absolute number of mosquito larvae in a pool. It is based on the Method of Removal Sampling (Zippin, 1956) in which successive samples of animals are removed from the popula'.ion. Provided the population remains stable, decreasing numbers are caught in successive samples. The number of animals per sample, and the rate of decrease in successive samples, leads to an estimate of the absolute number (N) originally in the population.

It is assumed that each sample removes a constant proportion of the animals remaining in the population. For mosquito larvae, I found that the assumption was realised when each sample consisted of sweeping a net, of 6 x 4 inches aperture, 3 times through the pool. The net was swept vigorously through the pool, stirring up the water and larvae. Samples were taken 20 minutes apart to give the larvae time to resume their usual spatial distribution at the surface of the water. It was desirable to remove obstructions, such as rocks and sticks, from the pool as otherwise pockets of larvae formed which were out of reach of the net. After all the samples had been counted, the larvae could be returned unharmed to the pool.

2.431 The estimation of N.

Zippin (1956) discussed the 2 analytical techniques that had been proposed for the Method of Removal Sampling . One method was developed by De Lury (1947) for estimating the size of populations of fish from records of catch and effort involved. In the special case of equal effort per catch, De Lury calculated the linear regression of the number caught in the ith sample (y_i) on the cumulative total caught (x_i)

prior to the ith sample. The value of x when y = 0 was taken as the estimate of N. Zippin (1956) suggested weighting the regression with weights inversely proportioned to the binomial variance. This method violates the theory of regression analysis because the 2 variates are not independently ascertained. Thus, it leads to only approximate estimates of N and V(N).

The second method was proposed by Moran (1951). Moran derived a maximum likelihood estimate of N which was based on the conditional binomial probability that an animal would be caught in the ith sample. The algebra is complicated and hence N is estimated by iteration (Moran, 1951) or graphically (Zippin, 1956). The variance of N is found by assuming a large sample approximation.

The above techniques are unsatisfactory because inefficient statistics or approximations are used, and hence only an approximate estimate of N is found. Also, the central Confidence Limits for N appear to be unrealistic. I am grateful to Mr. N.S. Stenhouse of the Division of Mathematical Statistics, C.S.I.R.O., for deriving a third technique using efficient statistics that gives an exact estimate of N and a good approximation to the Fiducial Limits of N.

The technique of Stenhouse.

Let each sample remove a proportion p of the larvae remaining in the pool. The expected numbers of larvae in successive samples are given in table 2.53.

Table 2.53: Expected number of larvae in successive samples that remove the proportion p of the population remaining in the pool.

Sample	Larvae removed	
first		pN
second	$p(N-pN) = pN(1-p) =$	pNq
third	$p(N-pN-pqN) = pN(1-p-pq) =$	pNq^2
nth		pNq^{n-1}

Thus, the numbers in successive samples form a geometric progression. Putting $A = pN$, the geometric progression can be expressed as $Y = Aq^x$.

Where y_i = number of larvae in ith sample

x_i = number of sample with i = 0, 1, 2 ... for the first, second, third, etc. samples.

A and q can be estimated by calculating the linear regression:

$$\log Y = \log A + x \log q$$

$$\text{Hence } N = \frac{A}{p} = \frac{A}{1-q}$$

which is the limiting sum of the geometric series as x becomes indefinitely great.

Removal sampling gives data in which the standard deviation is positively correlated with the mean. Besides giving a linear relationship between x and y, the log. transformation has the advantage of giving approximately equal standard deviations which are uncorrelated with the means.

The Fiducial Limits for N are difficult to derive because N takes

the form:

$$N = \frac{e^{A}}{1-e^{q}}$$ where e = base of natural logarithms.

Thus the Fiducial Limits involve the compound fiducial probability of A and q. Mr. Stenhouse hopes to derive the exact Fiducial Limits for N; for the present, approximate Confidence Limits are calculated which seem to be good approximations to the exact Fiducial Limits.

The approximate Confidence Limits are found by calculating N for the extreme limits of A and q. The Fiducial Limits for A and q are $A \pm t\, s_A$ and $q \pm t\, s_q$. The value of t is calculated for P = .158 and (n-2) degrees of freedom, to give a compound probability of $2(.158)^2 = .05$. The limits for N are found by dividing the greater value of A by the smaller value of (1-q) and the smaller value of A by the greater value of (1-q).

The technique is illustrated with the following example, using counts of third to fourth-instar Ae. alboannulatus larvae collected from pool 9 on 23 February, 1959 (table 2.54). This set of data gave the best fit to the mathematical model.

Table 2.54: Third to fourth-instar larvae counted in successive samples collected from pool 9 on 23 February, 1959.

Sample no. (x)	Larvae caught (y)	Log_{10} y
0	2,483	3.3950
1	2,115	3.3253
2	1,398	3.1455
3	814	2.9106
4	673	2.8280
5	398	2.5999
6	358	2.5539

The regression of log Y on x is

$$Y' = 3.425270 - 0.153271x$$

The analysis of variance is given in table 2.55.

Table 2.55: Analysis of variance of regression calculated from data in table 2.54.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Due to regression	.657780	1	.657780	242.81 ***
About regression	.013544	5	.002709	
Total	.671324	6		

The regression line is shown in figure 2.18.

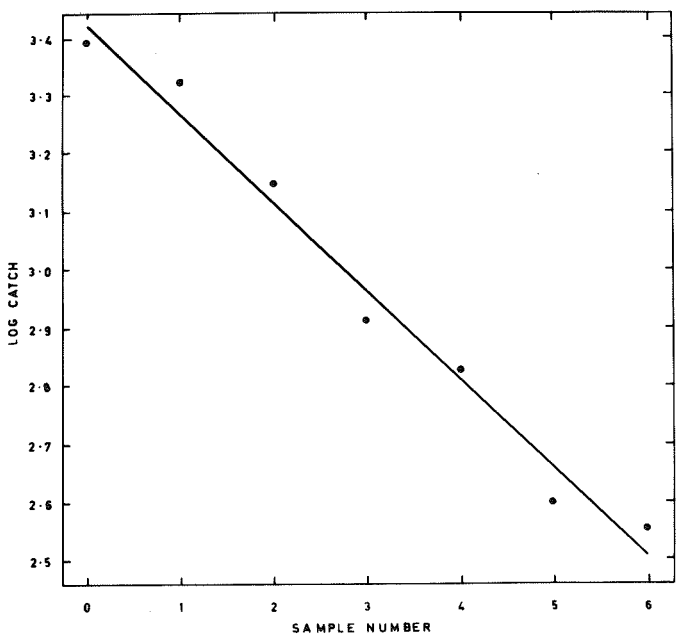


Figure 2.18: The regression of the logarithm of the numbers of larvae caught in successive samples on sample number.

N is found by taking antilogs of the regression equation to give:

$$Y = 2662.38\ (.702634)^X$$

Whence $N = 2662.38/(1-.702634)$

i.e. $N = 8953$.

The relationship between the actual numbers of larvae caught and the fitted regression curve is shown in figure 2.19.

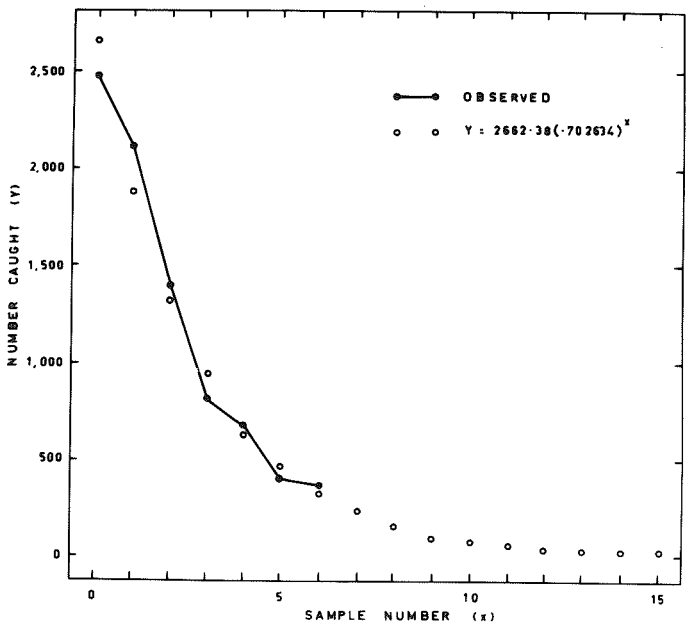


Figure 2.19: The curvilinear regression of the actual number of larvae in successive samples on sample number. Derived from the regression shown in figure 2.18. $Y = 2662.38\ (.702634)^X$.

95% Confidence Limits of N.

$$V(A) = s^2 \left\{ \frac{1}{n} + \frac{\bar{x}^2}{S(x - \bar{x})^2} \right\}$$ whence the S.D. (A) = .035465.

S.D.(q) = .0098362. Exact interpolations in the t table were supplied by the Division of Mathematical Statistics of C.S.I.R.O.; t for P = .158 and 5 degrees of freedom = 1.1133. The 15.8% Fiducial Limits for A and q are given in table 2.56.

Table 2.56: The 15.8% Fiducial Limits for A and q.

		Logarithms	Antilogarithms
F.L. (A)	(	3.4647532	2915.77
	(	3.3857868	2431.01
F.L. (q)	(	$\bar{1}$.8357786	.685139
	(	$\bar{1}$.8576794	.720575

Hence the 95% Confidence Limits for N = 8,953 are:

2915.77/(1- .720575) = 10,435

and 2431.01/(1- .685139) = 7,721.

The limits are non-central because the sampling distribution of N is skewed.

The accuracy of the method was checked by selecting a small pool, with a surface-area of 21 square feet, and taking successive samples until all the larvae had been caught. The number of larvae in successive samples are given in table 2.57.

Table 2.57: Number of Aedes larvae in successive samples from pool 18 on 18 March, 1960.

Sample number (x)			
0 - 9	10 - 19	20 - 29	30 - 37
746	179	63	21
283	140	45	15
440	94	44	21
309	35	41	23
332	17	12	23
156	29	28	8
124	46	44	8
115	36	34	0
168	44	33	
142	40	15	

The total number caught = 3,953. The regression of log. Y on x is $Y' = 2.474718 - 0.041039x$ and the analysis of variance is given in table 2.58.

Table 2.58: Analysis of variance of log Y on x for the data given in table 2.57.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Due to regression	7.104077	1	7.104077	140.42 ***
About regression	1.770746	35	0.050593	
Total	8.874823	36		

Taking antilogs gives $Y = 298.344\ (.909831)^x$

Whence N = 3,309. The 95% Confidence Limits were 2,582 to 4,272.

Thus, the estimated N is close to the true value of 3,953 and the true value is included in the 95% Confidence Limits.

2.432 Results of the Method of Removal Sampling from other pools.

The results and a summary of the analysis for 6 other estimates are given in tables 2.59 and 2.60.

Table 2.59: Counts of Ae. alboannulatus in successive samples. Bottom row of table gives estimates of N.

Pool 9 Pupae 23 Feb.59 y_1	Pool 18 Larvae 28 Feb.59 y_2	Pool 10 Larvae 18 Feb.59 y_3	Pool 18 Larvae 11 Mar.60 y_4	Pool 8 Larvae 21 Feb.59 y_5	Pool 9 Larvae 17 Feb.59 y_6
97	129	119	1094	720	539
116	95	109	386	538	200
61	55	97	405	586	246
25	33	71	330	311	99
14	14	72	163	190	219
11	28	93	103	128	153
2		80			152
		17			153
		45			185
		40			94
		54			173
		57			113
		58			192
		42			91
		42			101
		33			88
		26			64
		26			75
		22			99
		23			82
351	373	1,355	2,608	2,812	3,957

Table 2.61: Summary of analysis of data given in table 2.59.

y	n	Regression M.S.	Residual M.S.	Var.Ratio	t(P=.158)	N	95% C.L. (N)	
1	7	2.141109	.038652	55.39***	1.1133	351	226	533
2	6	.520078	.029924	17.38*	1.1446	373	221	668
3	20	.772052	.022887	33.73***	1.0310	1,355	992	1,910
4	6	.574893	.013809	41.63***	1.1446	2,608	1,832	3,831
5	6	.413737	.008263	50.07***	1.1446	2,812	2,091	3,884
6	20	.552949	.020882	26.48***	1.0310	3,957	2,862	5,699

2.433 Accuracy of the random sampling method.

Routine estimates of the absolute number of larvae in a pool were made by taking random samples and calculating the number of larvae per square foot. This figure was then multiplied by the surface-area of the pool to give N.

The accuracy of the random sampling method was tested by taking random samples in the usual way, returning the larvae to the pool, and then using the Removal Method. The estimated values of N from the 2 methods are given in table 2.61 and the discrepency is expressed as the percentage error of the more precise Removal estimate.

The errors are reasonably small; 4 of the 6 estimates from random sampling lie within the 95% Confidence Limits from the Removal method. Discrepencies occur in both positive and negative directions, indicating that on the average, random sampling will probably give reliable estimates of the absolute numbers of larvae in the pools.

Table 2.61: Comparison of N estimated by the Removal Method and by random sampling.

y	Removal estimate of N.	Random sampling estimate of N.	% error
1	351	582	+ 66
2	373	516	+ 38
3	1,355	1,010	- 26
4	2,608	2,750	+ 5
6	3,957	2,820	- 29
Pool 18 Mar.60	3,309	5,750	+ 74
Pool 18 Mar.60	True N = 3,953	5,750	+ 46

2.434 Discussion.

For small populations, the Method of Removal Sampling provides a reliable estimate of the size of the population. However, it involved too much work to be used for routine estimates of the absolute numbers of larvae in the whole creek at Hackham. Its main use is for detailed studies in single pools.

In addition to using the method to estimate the accuracy of random sampling estimates, it was used to estimate the size of the populations of larvae and pupae in pools 8 and 9 for an experiment on rearing Ae. alboannulatus in outdoor cages (section 2.372).

One disadvantage of the method is that at least about half the population has to be caught and counted in order to get a reasonably precise estimate of N.

2.5 Predation by the dytiscid beetle Nectersoma dispar Germ.

On several occasions in the field I saw the small dytiscid beetle Nectersoma dispar Germ. kill the larvae of Ae. alboannulatus. I recorded N. dispar from nearly every pool in the creek, both from permanent and transient pools, and adults were present throughout the year. When transient pools first filled the larvae of Ae. alboannulatus were the only common aquatic insect, but N. dispar usually appeared less than one month after the pool filled. There were other, larger dytiscids, e.g. Platynectus decempunctatus and Copelatus extensus, but these other species were neither as uniformly distributed nor as abundant as N. dispar. From these observations it seemed likely that N. dispar was the most important predator of Ae. alboannulatus larvae; accordingly some experiments were done to estimate the influence of N. dispar on the numbers of mosquito larvae.

2.51 Methods.

Two replicates of 1 experiment were done in the field. Five trenches 28 x 7 inches and 10 inches deep were dug in swampy ground and allowed to fill. Then 100 third-instar Aedes larvae were put into each pool. Two pools were left as controls and 5, 15 and 30 beetles put into the third, fourth and fifth pools respectively. At the end of 1 week, all the larvae and beetles remaining in each pool were caught and counted. One replicate, (a), was put near pool 3 and the other, (b), near D2.

A similar experiment with the same number of beetles and larvae was set up in the laboratory in glass jars 4 inches in diameter and 8 inches deep.

2.52 Results.

In the field, replicate (a) failed because the pools became

stagnant; some of the beetles either died or left the pools, and many larvae in 1 control died. Replicate (b) was satisfactory; 5, 15 and 30 beetles were recovered at the end of the week. In the laboratory, the beetles killed the larvae so rapidly that the results after only one day are given. The results of the 2 experiments are given in table 2.62 as the numbers of larvae killed.

Table 2.26: Predation by Nectersoma dispar on Aedes larvae.

Experiment	Duration	Number of beetles				
		0	0	5	15	30
In the field ((a)	7 days	3	27	27	17	19
In the field ((b)	7 days	7	1	17	41	69
In the laboratory	1 day	0	0	43	73	91

The results for replicate (b) and for the experiment in the laboratory are given in table 2.63 as the number of larvae killed per beetle. The results for replicate (b) were corrected for the mean number of deaths in the 2 controls by subtracting 4.

Table 2.63: Comparison of results on predation in the field and in the laboratory.

Experiment	Duration	Number of beetles		
		5	15	30
In the field	7 days	2.60	2.47	2.16
In the laboratory	1 day	8.60	4.87	3.03

2.53 Discussion.

The most striking difference between the 2 experiments in table 2.52 is the much greater mortality rate in the glass jars. It seems that experiments of this kind in the laboratory have little relevance to predation in natural pools. Another interesting effect in the laboratory is that the number of larvae killed per beetle decreases when the beetles are crowded. The beetles swam about incessantly, frequently colliding with each other, and this mutual interference probably reduced the rate at which larvae were killed. The results from the field show the same trend, although the effect is practically negligible.

Probably the main difference between the experiments in the laboratory and in the field was the smaller volume of the glass jars. Similarly it would be expected that the mortality rate would be greater in the small holes dug in the field than in the larger, natural pools. Thus the influence of Nectersoma on the numbers of Aedes larvae would be related to the size of the pool and the relative abundance of the 2 species. To get some idea of these factors, 2 typical transient pools were selected and the numbers of Aedes larvae and Nectersoma estimated by taking random samples. In pool 18 there were an estimated 2668 Aedes larvae and 23 Nectersoma. In pool 400 there were an estimated 360 Aedes larvae and 6 Nectersoma.

The areas of the pools and the abundance of Nectersoma relative to the abundance of Aedes larvae are given in table 2.64.

Table 2.64: Abundance of Nectersoma relative to the abundance of Aedes larvae.

No. of pool	Area sq.ft.	Number of Nectersoma per 100 Aedes larvae.
18	21	0.9
400	35	1.7
Expt. in field	1.4	5 15 30

The values for area and abundance of Nectersoma relative to Aedes larvae in table 2.64 indicate that Nectersoma would have little effect on the numbers of Aedes larvae in pools 18 and 400. Occasionally, Nectersoma dispar was observed at higher densities than recorded in pools 18 and 400, usually in transient pools which had been full for many months. In section 2.4 it was shown that the distribution of Aedes larvae changes markedly as the creek fills, many of the larvae being in pools which have recently filled.

Thus, a large proportion of the Aedes larvae occur in pools with a low density of Nectersoma, and it seems likely that predation has little influence on the absolute numbers of larvae in the creek.

3. AN. ANNULIPES.

3.1 Natural History.

3.11 The life-cycle in relation to temperature.

The life-cycle of An. annulipes resembles that of other species of Anopheles. The eggs are laid singly on the surface of the water; the few eggs that I found in the field were floating amongst emergent aquatic plants. In the laboratory the eggs hatched after 2 days at 27°C.

I was unable to estimate the duration of the four larval instars in the field because breeding was continuous, with overlapping generations. In the laboratory, I had much difficulty in rearing the larvae, but the minimum duration of the larval stages was about 16 days at 25°C. The speed of development of the larvae living in natural pools was probably more rapid.

The duration of the pupal stage was measured more precisely. At 25°C, an experiment with 20 pupae gave a mean and its standard error of 2.1 ± 0.086 days.

The speed of development of the ovaries was measured with unfertilized females reared in the laboratory. Several mosquitoes were fed on blood and then periodically dissected until mature eggs were found. Eggs were assumed mature when the "floats" were differentiated. At 21°C, mature eggs were found on the 6th day and at 27°C on the 3rd day. The ovaries of a control series of mosquitoes fed on raisins remained undeveloped. The results must be interpreted cautiously; Muirhead-Thomson (1951) described some tropical species of Anopheles in which the unfertilized females took several meals of blood without developing eggs, whereas fertilized females laid eggs after only 1 meal of blood. Since the ovaries of An. annulipes developed promptly and completely after only 1 meal of

blood, the results are probably valid.

Both at Hackham and along the River Murray, An. annulipes larvae were more abundant during summer than in winter. At Hackham, during the winters of 1958 and 1960, I was unable to find any larvae or pupae. I expected that the population overwintered as adults and consequently I searched for them during winter. Adults were flushed from crevices and rabbit burrows with smoke from an apiarist's smoker, and caught with a net or an aspirator. The numbers of adults caught at Hackham during 1958 and 1960 are combined and given month by month in table 3.01. The results for 1959 were omitted because pupae were present throughout winter and could have metamorphosed to adults.

Table 3.01: Number of adults caught at dusk at Hackham.

Month	No. of catches	Mean no. of adults per catch
Feb.	1	3
Mar.	1	6
Apr.	2	13
May	2	6.5
Jun.	3	8
Jul.	2	1.5
Aug.	1	0
Sep.	1	2

By October of each year, first-instar larvae appeared, indicating that eggs were being laid.

At Teal Flat, on the River Murray, An. annulipes adults were found in rabbit burrows in July and November. Also An. annulipes adults were

caught biting at dusk, these results are given in table 3.02.

Table 3.02: Number of adults caught at dusk at Teal Flat.

Month	No. of catches at dusk	Mean no. of adults per catch.
Jan.	1	1
Feb.	1	5
Apr.	2	1.5
May	1	0
Jul.	2	3
Oct.	2	23

Again the results show that An. annulipes adults were present throughout winter.

The adult, fertilized, females of some species of Anopheles enter diapause during winter in which occasional meals of blood are followed by development of fat-body, the ovaries remaining undeveloped. This happens even when females caught in winter are put at a summer temperature in the laboratory (Guelmino, 1951). No evidence of a winter diapause was found for An. annulipes. When suitable pools were available, first-instar larvae were found during winter, indicating that eggs were being laid. Also, 10 females caught at Hackham in May 1958 laid a total of 264 eggs when put at 25°C in the laboratory.

Diapause has also been described in the larval stages of Anopheles, but never in the pupal stage (Bates, 1949). During the winter of 1959, An. annulipes pupae were found in each monthly count at Hackham (section 3.33), indicating that development continued throughout winter without

larval diapause. Also, larvae collected at Hackham on 29 May, 1958 pupated normally in the laboratory when placed at 20°C.

3.12 Breeding-places at Hackham.

Usually larvae were found amongst the emergent aquatic plants in permanent pools. Chara globularis* related to the green algae, grew in every permanent pool and often was the only aquatic plant in the pool. Two other plants which grew in the water were Typha augustifolia L., a bulrush, and Eleocharis acuta R.Br., a small reed. Juncus bufonius L., a small rush up to 20 cm. high, and Polypogon monspeliensis (L), a grass, grew round the margins of the permanent pools.

Occasionally, larvae were found in pools intermediate between the permanent and transient types. These pools filled in spring, often flooding Polypogon and other grasses which were growing in the bed of the creek. Usually, the Polypogon survived as an emergent plant. Callitriche verna L., a true aquatic plant, was found in these pools with the flaccid, upper parts of the stems floating on the water. Again, the larvae were found amongst the emergent Polypogon and Callitriche.

At Hackham, the distribution of An. annulipes larvae virtually coincided with the distribution of emergent Chara. This observation was interesting because there has been considerable controversy over a supposed larvicidal property of Chara. For example, Matheson and Hinman (1931) claimed that Chara was inimical to larvae, whereas Hamlyn-Harris (1928) found An. annulipes larvae developing normally in pools containing Chara fragilis (= Chara globularis).

* The taxonomy of the Australian Characeae is poorly known. Dr. R.D. Wood, of the University of Rhode Island, designated a speciment "C. globularis intermediate cortex (2-3)".

Hamlyn-Harris (1928, 1932) also reared An. annulipes and the larvae of other species in aquaria containing Chara and Nitella (Characeae). In a review of the Characeae, Wood (1952) concluded that any toxic influence was not related specifically to the Characeae but rather to the general properties of the pools in which certain Characeae grew. My observations at Hackham support Wood's conclusion; provided the pools did not stagnate, the larvae developed normally and metamorphosed to adults. Also, in the laboratory, I successfully reared larvae in pondwater containing Chara globularis.

3.13 Breeding-places along the River Murray.

Surveys of breeding-places along the River Murray were made on both sides of the river between Mannum and Purnong, which are 21 miles apart. Larvae were found only among emergent or floating plants, or amongst floating fragments of plants, mainly Valisneria spiralis L. Occasionally larvae were found in the main stream of the river, but more often the larvae were in the extensive swamps beside the river.

The larvae did not appear to be associated with particular species of plants. They were found amongst almost any vegetation which broke up the surface of the water. The most abundant plants in the swamps were Myriophyllum verrucosum Lindl. and M. elatinoides Gaudich., both with emergent, leafy, stems, Valisneria spiralis and Potamogeton crispus L., both with floating leaves. Azolla filicoides L., a small floating plant, often occurred in dense mats covering the water without a break. Larvae were never found in the mat, although they were often around the edges, or amongst scattered clumps of Azolla.

Most of the plants found in the swamps were also found along the

edges of the river, often in sheltered backwaters behind reeds or fallen trees. However, there was usually a far greater area of emergent plants in the swamps than along the river.

Most of the swamps in which mosquito larvae were found were shallow (1 ft. to 6 ins.) and the amount of water in them depended on the level of the river. The level of the river was influenced by the amount of water flowing down it and also by wind. The wind caused erratic, daily fluctuations of up to 6 inches, which were superimposed on more regular, seasonal changes of several feet. These quantitative aspects of breeding-places are discussed below in section 3.35 under the heading of "resources".

3.21 The relationship between larvae and food in natural pools.

In section 2.35 a model was discussed which related the growth of Ae. alboannulatus larvae to the amount of food in natural pools. The experiments in this section were designed to test the same model for An. annulipes larvae.

The feeding behaviour of anopheline larvae differs from that of culicine larvae in that anopheline larvae feed mainly at the surface of the water (Bates, 1949). Similarly, Russell et al. (1946) stated that floating animal and vegetable life make up the bulk of anopheline larval food. My observations on An. annulipes larvae indicated that they also fed mainly at the surface, amongst emergent aquatic plants. Consequently, the experimental methods for An. annulipes were modified slightly to allow for the difference in behaviour.

3.211 Materials.

All larvae were collected from permanent pools at Dry Creek, about 8 miles north-east of Adelaide. Where possible, third-instar larvae were used, but when larvae were scarce, late second-instar and early fourth-instar larvae were sometimes used.

The pondwater used for all but the last experiment was also collected from Dry Creek. The surface layer of water, in the zone of emergent plants, was collected by skimming with a large shallow tray. The water was filtered through 60-mesh bolting-silk into 2-gallon glass jars and brought to the laboratory as quickly as possible. In the laboratory, the water was thoroughly mixed and 500 ml. aliquots put into polythene bags and frozen. Two batches of pondwater were collected, 1 in February 1961 (pondwater a), and the other in April 1961 (pondwater b).

3.212 Methods.

Growth-rate was measured in the same way as for Ae. alboannulatus, by weighing individual larvae on a torsion balance. Pondwater was concentrated either by evaporation, as for Ae. alboannulatus, or by centrifuging for 20 minutes at 1500 *g* in a refrigerated centrifuge. All the experiments were done at a constant temperature of 27°C, in continuous light from a 15 watt frosted globe.

Larvae were placed individually in petri-dishes rather than in tubes because I expected that the large surface-area of the water in a petri-dish would be better suited to the feeding behaviour of An. annulipes larvae. This hypothesis was tested by measuring the growth-rates of 7 larvae in tubes and of 7 larvae in petri-dishes. Twenty ml. of 1x pondwater was put into each tube and petri-dish. The results are given in table 3.03.

Table 3.03: Comparison of growth-rates of larvae in tubes and petri-dishes.

	tubes	petri-dishes
n at end of expt.	7	6
mean $\Delta \log W$	.015	.026
S	.0206	.0113
$100\ s/\bar{x}$ = C.V.	137%	43%

Although the mean gain in weight was greater in petri-dishes than in tubes, the means are not significantly different ($t = 1.23$, $.30 > P > .20$). Also, the standard deviation for tubes was greater than for petri-dishes, indicating greater variability in the tubes. But once again, the standard deviations do not differ significantly, (the ratio of the variances gives $F = 3.32$, P .20). However, although the differences are not significant, the coefficient of variation (C.V.) is considerably smaller in petri-dishes and petri-dishes were therefore preferred.

A second experiment was designed to test the effect of different treatments on the pondwater, in particular, to test whether pondwater that was frozen and thawed differed greatly from fresh pondwater. The following treatments were used:

A Distilled water
B Boiled pondwater
C Frozen pondwater
D Fresh pondwater
E Fresh pondwater plus Chara.

The distilled water was used as a control for no food; it probably also caused some osmotic stress. Boiling the water would kill all

organisms and denature some organic materials. Freezing the water probably killed the larger organisms, but not all the bacteria. In treatment E, a piece of Chara about 5 cm. long, which supported abundant unicellular epiphytic algae, was added to the pondwater. Ten larvae were used per treatment. The results are given in table 3.04.

Table 3.04: Growth-rates of larvae in different sorts of pondwater.

Treatment	n	Mean $\Delta \log W / \Delta t$
A Distilled water	9	- .062
B Boiled pondwater	10	+ .001
C Frozen pondwater	10	+ .002
D Fresh pondwater	10	+ .002
E Fresh pondwater plus Chara	9	+ .014

In this, and all other experiments in this section, analyses were calculated from the original values of $\Delta \log W$ to eliminate errors of rounding from dividing by the constant, Δt. Where necessary, the results of an analysis were coded to $\Delta \log W / \Delta t$, e.g. in calculating $s\bar{x}$, and for regression equations. The analysis of variance is given in table 3.05.

Table 3.05: Analysis of variance of data in table 3.04.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Between classes	.017574	4	.004394	6.61 ***
Within classes	.028607	43	.000665	
Total	.046181	47		

Comparisons for significance among all the means were made by calculating $D = Q\, s_{\bar{x}}$, where Q is the upper 5% point in the Studentized range (Snedecor, table 10.6.1, 1956). An approximate value for $s^2_{\bar{x}}$ was found by dividing the mean square for within classes by the harmonic mean number of replicates (9.57), giving $s^2_{\bar{x}} = (.0118)^2$; whence D = 0.048. Thus, mean A differs from the other 4, but no other comparisons are significant.

There is a trend for increased growth in treatment E, indicating that better growth-rates might be obtained by including pieces of Chara. However, the design of the experiments required different concentrations of food, and the Chara was so heterogeneous that this was technically impossible. Consequently, the experiments were restricted to pondwater that had been stored frozen.

The interesting comparison is between frozen pondwater (C) and fresh pondwater (D). The means indicate no difference in growth-rate, but even though the fresh pondwater was used 4 hours after it was collected, microscopical examination showed that many of the Protozoa had already died. Hence, the larvae would probably find more food in natural pools, where they have access to living organisms in the water and on the Chara.

3.213 Experimental Results.

(a) Pondwater concentrated by evaporation.

Seven concentrations were used, as given in table 3.06, with 10 larvae in each concentration. The mean growth-rates and the number of larvae (n) left in each treatment, after larvae that died or moulted were discarded, are given in table 3.06.

The first 5 treatments resulted in a roughly linear increase in

growth-rate with increasing concentration, and hence a linear regression was calculated. The calculations were made on the original data because there are unequal numbers per treatment. The analysis is given in table 3.07 and 3.08.

Table 3.06: Growth-rates of larvae in different concentrations of pondwater.

Concentration		n	Mean $\Delta \log W / \Delta t$
A	0	8	- .023
B	0.5x	8	.015
C	1x	9	.005
D	2x	7	.045
E	3x	10	.051
F	4x	6	.050
G	5x	6	- .011

Table 3.07: Analysis of variance of data in table 3.06.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Between classes	.012001	4	.003000	23.26 ***
Within classes	.004772	37	.000129	
Total	.016773	41		

Table 3.08: Analysis of regression for treatments A to E in table 3.06.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Linear regression	.010264	1	.010264	17.73 *
Departures from regression	.001737	3	.000579	4.49 **
Total (between classes)	.012001	4		
Within classes		37	.000129	

In table 3.08, the mean square for departures from regression is tested against the mean square for within classes, and the variance ratio indicates significant departures from linearity. However, the sum of squares due to linear regression accounts for 86% of the total (table 3.08), and the mean square for linear regression tested against the mean square for departures from regression is significant at the 5% level of probability. Thus, the main part of the total variation is due to the linear component of growth-rate on concentration. The linear regression is:

$$Y = -.0112 + .0226\ x.$$

The regression is shown as the solid line in figure 3.01. The broken line indicates the trend in the rest of the data.

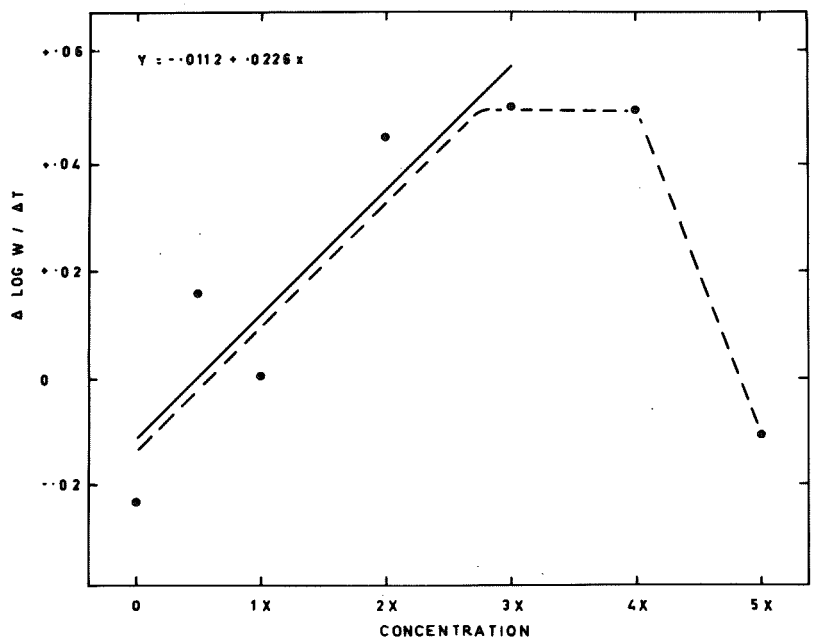


Figure 3.01: Relationship between growth-rate of larvae and concentration of pondwater. The solid line is the linear regression of growth-rate on concentrations 0 to 3x. $Y = -.0112 + .0226\ x$.

If the result for 5x is ignored for the moment, the composite curve conforms to the model, and resembles the curve found for Ae. alboannulatus. Growth-rate increases linearly up to about 2.8x, at which point growth-rate is at a maximum. At concentrations below 2.8 times normal the larvae experienced a relative shortage of food.

The sharp drop at 5x suggested osmotic stress, and consequently the osmotic pressures of the 6 pondwaters were measured. The depressions of the freezing-point measured by the Beckmann Cryoscopic Method, and the equivalent concentrations of NaCl are given in table 3.09.

Table 3.09: Osmotic pressure of concentrated pondwater.

Concentration of pondwater	Δ F	% NaCl
0	0	0
0.5x	0.163	0.28
1x	0.255	0.44
2x	0.419	0.73
3x	0.543	0.94
4x	0.685	1.19
5x	0.916	1.59

To find out if the higher values of osmotic pressure were excessive, the osmotic pressure of the haemolymph of fourth-instar larvae of An. annulipes was measured. A larva was placed on a waxed slide, pierced with a needle, and the haemolymph drawn into a capillary tube, with a drop of NaCl of known concentration on either side of it, separated by an air-space (Barger Method, Prosser, 1950). The changes in the lengths of the columns of haemolymph are given in table 3.10, where - represents a decrease, 0 no change and + an increase. One larva was used for each determination; duplicate determinations being made at each concentration.

Table 3.10: Estimation of osmotic pressure of haemolymph of larvae.

Concentration NaCl	tube a	tube b
1.5%	-	-
1.0%	-	+
0.9%	0	+
0.8%	+	+
0.7%	+	+
0.6%	+	+
0.5%	+	+

The results indicate that the osmotic pressure of the haemolymph of An. annulipes is equivalent to 0.9% to 1.0% NaCl.

Wigglesworth (1938) found that in Ae. aegypti, the total osmotic pressure of the haemolymph was 0.75% to 0.89% NaCl. In various concentrations of artificial sea-water above a concentration equivalent to 0.75% NaCl, the osmotic pressure of the haemolymph increased slightly in excess of the external medium. At an external concentration equivalent to 1.6% NaCl, the larvae died rapidly.

The osmotic pressure of the haemolymph of An. annulipes, and the osmotic stress at a concentration equivalent to 1.59% NaCl in 5x pondwater, are in reasonable agreement with Wigglesworth's results.

As a final check, the influence of osmotic pressure on the larvae was measured under the conditions of an experiment on growth-rates. Five treatments were used:

A distilled water
B 1x pondwater
C 3x pondwater
D 3x pondwater + NaCl ≡ 4x pondwater
E 3x pondwater + NaCl ≡ 5x pondwater

In treatment D and E, NaCl was added to make the concentrations equivalent to those given in table 3.09 for 4x and 5x respectively. Treatments A and B were included as controls, designed to check that growth-rate increased with increasing concentration of food. Ten larvae were placed at each treatment. The means of the treatments and the number of larvae that survived are given in table 3.11.

Table 3.11: Growth-rates of larvae in various pondwaters.

Concentration		n	Mean $\Delta \log W / \Delta t$
A	0	9	- .0230
B	1x	9	+ .0189
C	3x	9	+ .0435
D	3x + NaCl ≡ 4x	6	- .0472
E	3x + NaCl ≡ 5x	2	- .1242

The effect of osmotic pressure on the growth-rates was analysed by fitting a linear regression to treatments C, D and E. Again, the calculations were made on the original data because there were unequal numbers per treatment. The analysis is given in tables 3.12 and 3.13.

Table 3.12: Analysis of variance of data in table 3.11.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Between classes	.030871	2	.015436	15.50 ***
Within classes	.013948	14	.000996	
Total	.044819	16		

Table 3.13: Analysis of regression for treatments C,D,E in table 3.11.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Linear regression	.030226	1	.030226	30.35***
Departures from regression	.000645	1	.000645	0.65
Total (between classes)	.030871	2		
Within classes		14	.000996	

The results in table 3.13 indicate that there are no significant departures from linearity and that the regression of growth-rate on osmotic pressure is highly significant. The regression equation is:

$$Y = .301 - .0861\ x.$$

The calculated regression line is shown in figure 3.02 as a solid line. The broken line indicates that growth-rate increased with increasing concentrations of food, as expected.

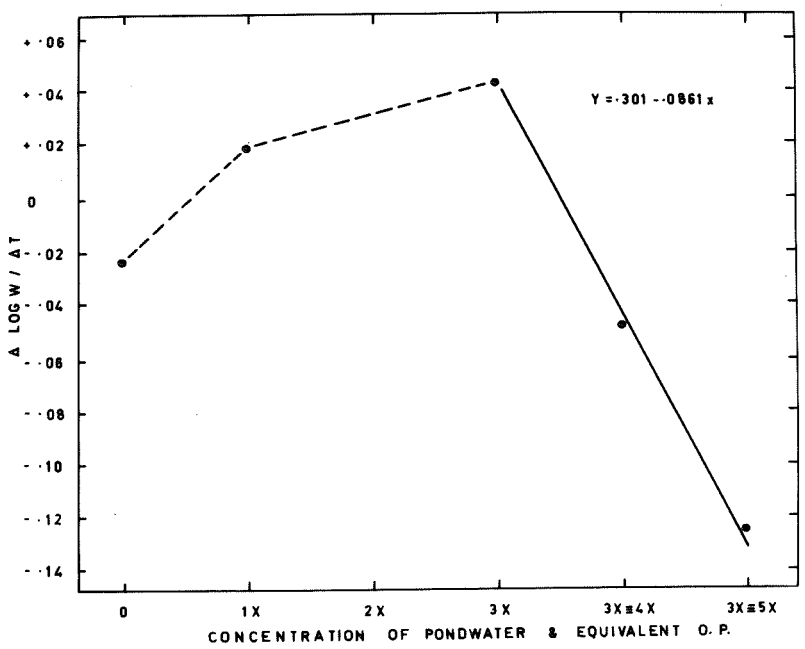


Figure 3.02: The relationship between osmotic pressure and growth-rate. The solid line is the linear regression of growth-rate on the last 3 treatments; it was fitted to the original data. $Y = .301 - .0861\ x$.

Different batches of larvae vary and it is therefore impossible to make a direct comparison with the results given in table 3.06. However, it is clear that treatments D and E in this experiment resulted in a much greater reduction both in weight and in survival than did the corresponding treatments 4x and 5x in table 3.06.

There are probably 2 reasons for this result. Firstly, the concentration of food was held constant at 3x and there may not have been

sufficient food for doing osmotic work in treatments D and E. Secondly, Wigglesworth (1933) found that NaCl was more toxic than equivalent concentrations of artificial sea water. Thus, the mixture of natural salts in pondwater is probably less toxic than NaCl alone. The osmotic pressure of treatment E was about 1.6% NaCl, and after 17 hours, 8 of the 10 larvae had died. Again this result agrees with the result of Wigglesworth (1938) quoted above.

The evidence indicates that concentrations of pondwater exceeding 3x cause osmotic stress, which may mask the effect of increasing the concentration of food. Thus in figure 3.01, the plateau between 3x and 4x may be due to the increased osmotic stress at 4x balancing the increased supply of food. In order to overcome this difficulty, I sought a method for concentrating food without increasing osmotic pressure.

(b) Pondwater concentrated by centrifugation.

Osmotic stress was overcome by concentrating the particulate food only. The particulate food was concentrated by centrifuging the pondwater and decanting the supernatant. In this experiment it is assumed that particulate food is more important than food in solution.

The first experiment was done with frozen pondwater (a) that had been used for the 2 previous experiments. After centrifuging, supernatant was decanted to make the concentration of particles equal to 5x, and aliquots were then diluted with distilled water to give the concentrations in table 3.14. Treatment G, with 1x pondwater that was not centrifuged, was included as a control. Ten larvae were used per treatment. The results of the experiment and the calculated osmotic pressures are given in table 3.14, and the means plotted in figure 3.03.

Table 3.14: Growth-rates of larvae in pondwater concentrated by centrifugation.

Concentration of particles		Calculated O.P. in % NaCl.	n	Mean $\Delta \log W / \Delta t$.
A	0	0	7	- .028
B	1x	.09	9	- .010
C	2x	.18	10	- .002
D	3x	.26	9	- .004
E	4x	.35	10	+ .029
F	5x	.44	9	- .002
G	1x (not centrifuged)	.44	10	+ .013

The analysis of variance is given in table 3.15.

Table 3.15: Analysis of variance of data in table 3.14.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Between classes	.009531	6	.001589	6.16 ***
Within classes	.014264	57	.000250	
Total	.023795	63		

The approximate variance of the means was found by dividing the mean square for within classes by the harmonic mean number of larvae per treatment (9.02). The variance, corrected for Δt is : $s^2_{\bar{x}} = (.007017)^2$, and D = 0.0292 (from table 10.6.1 in Snedecor, 1956). Thus, the only significant comparisons among the means are that E differs from A, B, C, D, F; and G differs from A. The regression of growth-rate on concentration for treatments A to F was calculated, but significant departures from

linearity outweighed the linear component.

It is difficult to interpret the results; the many negative values indicate that increasing the concentration of particles above 1x had little influence on growth. Indeed, treatment B (1x) had the same concentration of particles as the control G, yet the mean was 0.024 units lower than the mean of G. It appears that either centrifuging destroyed some of the food, or the gradient in osmotic pressure or in soluble food outweighed the effect due to particulate food.

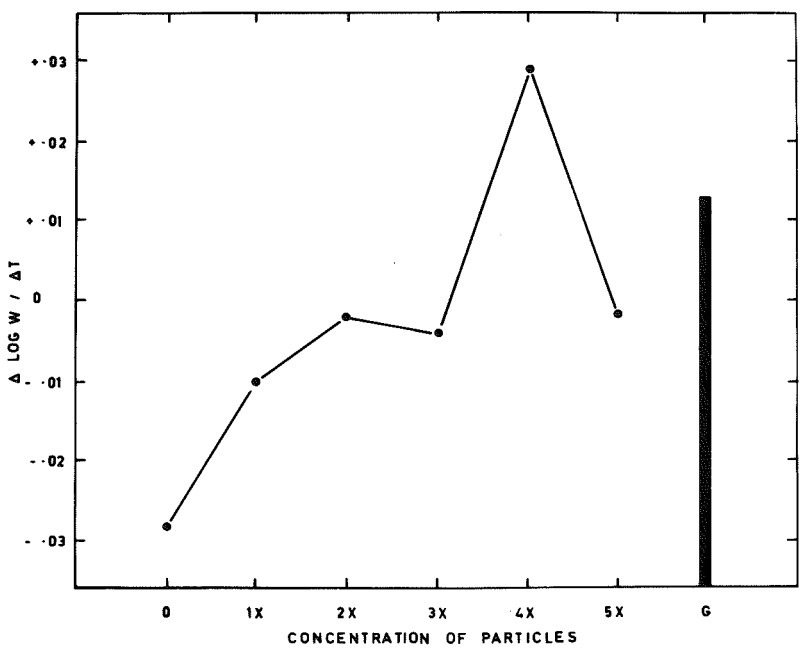


Figure 3.03: Relationship between larval growth-rate and particulate food concentrated by centrifugation.

A second experiment was designed in which the particles were all concentrated to 5x, thoroughly mixed, and aliquots diluted with supernatant pondwater. This procedure kept the osmotic pressure and the concentration of soluble food constant. This experiment was done with the second batch of pondwater from Dry Creek, (pondwater b). Two controls were included; one (F) with the original pondwater (a), which was not centrifuged, and the other (G) with supernatant from pondwater (b).

Larvae were extremely scarce in the field and I was able to find only enough third-instar larvae to use 5 per treatment. I decided to do the experiment in 2 parts, each with 5 larvae per treatment, the second part being done when more third-instar larvae were available. If there was no significant difference between batches of larvae, the 2 halves of the experiment could be pooled to increase replicates to 10 larvae per treatment.

The treatments used and the results of part I are given in table 3.16. Once more, 2 of the mean growth-rates are negative. The approximate regression of growth-rate on treatments A to E was calculated from the means, giving $b_1 = .0033$. The t test for 3 D.F. equalled 0.100, which indicates that b_1 is not significantly different from zero. The means in the 2 controls were nearly equal, indicating that growth in supernatant from pondwater (b) was as great as growth in normal pondwater (a).

Table 3.16: Growth-rates of larvae in centrifuged pondwater. Part I.

Treatment			n	Mean $\Delta \log W / \Delta t$
A	1x		4	+ .049
B	2x		5	- .038
C	3x		4	+ .013
D	4x		5	- .004
E	5x		5	+ .055
F	1x (a)	controls	5	+ .032
G	Supernat.	controls	3	+ .037

The erratic growth-rates in centrifuged pondwater suggested that centrifuging had some effect on the food. After centrifuging the particles were clumped together and I suspected that some of the clumps were too large for the larvae to eat. Hence, instead of duplicating part I, for the second part the particles in all treatments except G were broken up in a "Tosco" blendor.

The particles were concentrated to 5x and blended for 10 minutes. Aliquots were then diluted with supernatant to the same concentrations as in part I. The results are given in table 3.17, and the data for both parts of the experiment are plotted in figure 3.04. The broken line and unshaded columns refer to part I of the experiment; the solid line and shaded columns to part II.

Table 3.17: Growth-rates of larvae in centrifuged pondwater. Part II.

Treatment		n	Mean Δ log W/ Δ t
A	1x	3	+ .092
B	2x	5	+ .093
C	3x	5	+ .068
D	4x	5	+ .111
E	5x	4	+ .069
F	1x (a) } controls	3	+ .105
G	Supernat.)	4	+ .041

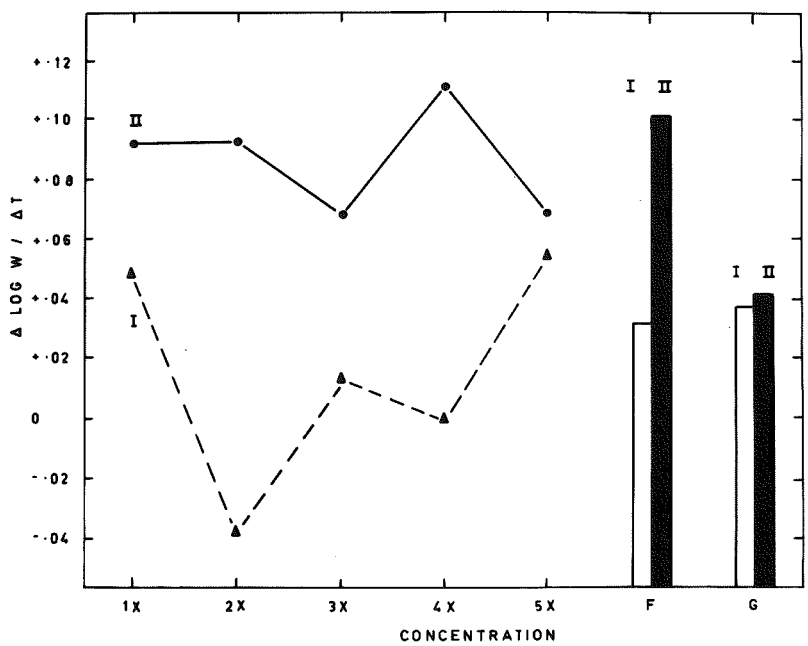


Figure 3.04: Relationship between larval growth-rate and concentration of particulate food. The experiment was done in 2 parts; in part II the particles were broken up with a blendor.

In part II, all the growth-rates were positive, but once more there was no significant regression of growth-rate on treatments A to E (b_2 = - .0021, t = 0.438). It is impossible to do an analysis of variance for the whole experiment because part II included the blending treatment whereas part I did not. However, treatment G (supernatant) was the same in both parts, and the closeness of the means (+ .037 and + .041) indicates that both batches of larvae reacted similarly. The differences between the other treatments in the 2 parts are therefore the result of blending. The results led to 3 conclusions. Firstly, after 10 minutes blending, the growth-rate does not increase when the concentration of the particles is increased from 1x to 5x. Secondly, blending had a similar effect on normal pondwater (a), indicating that freezing may clump particles. Thirdly, supernatant alone supports considerable growth and therefore cannot be disregarded.

Particulate food, soluble food and blending all seemed important and consequently the 3 factors were combined in the one experiment. The design and analysis was suggested by Mr. G.N. Wilkinson of the Division of Mathematical Statistics, C.S.I.R.O. The design consisted of combining various concentrations of supernatant (S) and particles (P), in 3 mixtures, each given at 3 intensities of supply. The actual concentrations, notation, and expected relative growth-rates, are given in table 3.18. The whole table was duplicated with blending times of 2 and 20 minutes.

The expected relative growth-rates are calculated on the assumptions that (a) P and S act independently, and (b) that the logarithms of P and S give additive effects. The expected relative growth-rates are plotted in figure 3.05.

Table 3.18: Design of an experiment with different concentrations of particulate and soluble food.

Actual Concentrations S	P	Relative Concentration S'	P'	Mixture $M = P'/S'$	Intensity of Supply I	Expected growth $\log_2 S' + \log_2 P'$
$\frac{3}{4}x$	$\frac{1}{8}x$	1	1	1		0
$\frac{3}{4}x$	$\frac{1}{4}x$	1	2	2	1	1
$\frac{3}{4}x$	$\frac{1}{2}x$	1	4	4		2
$1\frac{1}{2}x$	$\frac{1}{4}x$	2	2	1		2
$1\frac{1}{2}x$	$\frac{1}{2}x$	2	4	2	2	3
$1\frac{1}{2}x$	$1x$	2	8	4		4
$3x$	$\frac{1}{2}x$	4	4	1		4
$3x$	$1x$	4	8	2	4	5
$3x$	$2x$	4	16	4		6

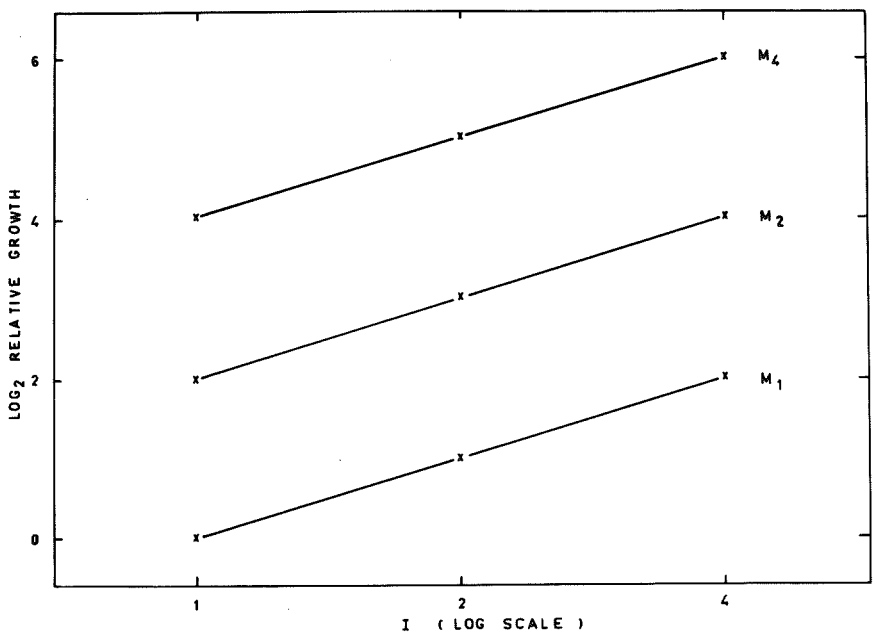


Figure 3.05: The expected growth-rates in 3 mixtures of particulate and soluble food (M) given at 3 intensities of supply (I).

If the lines in figure 3.05 be taken to represent blending for 2 minutes, then the effect of blending for 20 minutes was expected to make more food available and therefore shift the 3 lines up the vertical axis.

The results of the previous experiments guided the choice of concentrations. Thus, supernatant was not concentrated above 3x, to ensure that the effects of osmotic pressure were avoided. Particles were not concentrated above 2x because blended particles in the previous experiment apparently gave maximum growth at 1x. The 9 treatments were duplicated at 2 blending times, and 4 larvae were placed at each treatment, giving a total of 72.

Unfortunately, 27 of the larvae moulted and had to be discarded; leaving 1 treatment with no observations. A value was calculated for the mean of this treatment using the formula:

$$4x = 9\ (I_1\ M_3) + 6\ (I_1\ B_2) + 6\ (M_3\ B_2) - 3I_1 - 3M_3 - 2B_2 + G$$

Where I_1 = sum of means in I_1 class

B_2 = sum of means in B_2 class

M_3 = sum of means in M_3 class

G = sum of all known means

and x = unknown mean = −.048.

The means and number of observations are given in table 3.19.

Table 3.19: Table of mean growth-rates and number of observations for intensity of supply (I), Mixture (M) and blending times (B).

	I					
	1		2		4	
	B_2	B_{20}	B_2	B_{20}	B_2	B_{20}
M_1	.056 3	.003 3	.046 3	.053 2	.034 2	.073 3
M_2	.038 3	.007 2	.019 2	−.004 2	.035 3	.043 3
M_4	.020 3	(−.048) 0	.018 2	.003 2	.038 3	.069 4

The single classification analysis of variance was calculated on the original data and is given in table 3.20.

Table 3.20: Exact analysis of variance of original data.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Between classes	.023007	16	.001438	1.83 almost *
Within classes	.022030	28	.000787	
Total	.045037	44		

An approximate, triple classification, unweighted means analysis of variance was then done, omitting 1 degree of freedom from the second order interaction because a missing value was calculated. The mean square for within classes in table 3.20 was divided by the harmonic mean number of replicates (2.52) to give an estimate of error. The analysis is given in table 3.21.

The analysis indicates that both intensity of supply and mixture were significant, and also the interaction between intensity and blending was significant. The precise variance ratio for the interaction I x B was calculated from the weighted differences in the 3 x 2 table of means (table 3.22). The method is described by Snedecor (p.382, 1956).

Table 3.21: Approximate analysis of variance of means in table 3.19.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
I class	.004155	2	.002078	6.66 **
M class	.002489	2	.001243	3.99 *
B class	.000613	1	.000613	1.96
I x M	.002170	4	.000543	1.74
I x B	.004412	2	.002206	7.07 **
B x M	.000198	2	.000099	0.32
I x B x M	.000648	3	.000216	0.69
Error		28	.000312	

Table 3.22: Table of means for intensity and blending.

		I		
		1	2	4
B	2	.038000	.030143	.035875
	20	.004400	.017167	.062200

The mean square for interaction was .003577 and it was tested against the mean square for within classes in table 3.20 (.000787). $F = 4.54$ * with 2 and 28 D.F. Thus the interaction is significant at the 5% level of probability; the value in table 3.21, found by the approximate method is exaggerated. The differential effect of B with I can be seen in table 3.22. With B_2, I has practically no influence on growth whereas, with B_{20}, growth is slight at I_1, but increases with I_2 and I_3. The same effect can be seen in figure 3.06, in which growth-rate is plotted against I for each M and B. Freehand curves are drawn to show the trends.

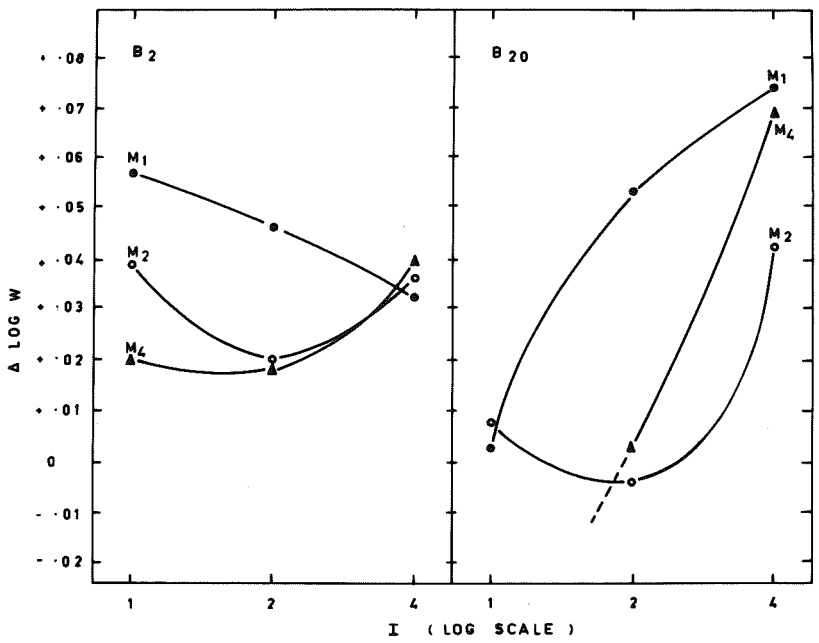


Figure 3.06: Trends of observed growth-rates in 3 mixtures of particulate and soluble food (M), at 3 intensities of supply (I), after blending times of 2 and 20 minutes.

The curved lines in figure 3.06 indicate that the simple additive hypothesis is inadequate to explain the data. However, it appears that M does not influence growth in any systematic way. The unexpected effects of blending are difficult to explain. Possibly, the curves for B_2 indicate that particulate food had little influence on growth-rate, whereas blending for 20 minutes released a toxic substance which affected the larvae more at the lower values of I, than at the higher values.

The results of this experiment suggested further experiments,

e.g. with a series of blending times. But the effects observed are probably entirely due to the way the pondwater was treated in the laboratory, and hence would not be experienced by larvae living in natural pools. The many negative "growth-rates" in treated pondwater suggested that dead food was unsuitable, and that the larvae may require the living food that is abundant in natural pools. I planned an experiment to measure the growth-rates of larvae in cultures of Paramecium at different densities, but it was well into winter before I had sufficient numbers of Paramecium, and I was unable to find any larvae in the creeks.

3.214 Growth-rates in different pools.

The summer of 1960-61 was unusually dry, and by March 1961 An. annulipes larvae at Hackham were found in only 2 pools, each of 1 foot square, originally dug in swampy ground for an experiment with Ae. alboannulatus. The amount of food in these small pools was compared with the amount of food in water from Dry Creek.

Surface water was collected from the 2 small pools, frozen, and an aliquot concentrated. Five treatments were used: distilled water; pondwater (a) from Dry Creek at 1x and 2x; and pondwater from Hackham at 1x and 2x. The larvae were collected from Dry Creek. The results of the experiment, which started with 10 larvae per treatment, are given in table 3.23.

Table 3.23: Growth-rates of larvae in different pondwaters.

Treatment			n	Mean $\Delta \log W/\Delta t$
A	Distilled water	0	9	- .0688
B	Dry Creek (a)	1x	9	-.0064
C	Dry Creek	2x	8	+.0112
D	Hackham	1x	10	-.0176
E	Hackham	2x	9	+.0192

The analysis of variance is given in table 3.24.

Table 3.24: Analysis of variance of data in table 3.23.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Between classes	.016533	4	.004133	14.71 ***
Within classes	.011227	40	.000281	
Total	.027760	44		

The harmonic mean number of replicates is 8.96 and $s_{\bar{x}}$, in $\Delta \log W/\Delta t$ units, is .00896. Hence D (Table 10.6.1, Snedecor, 1956) is .0362. The only significant differences between means are: A differs from B, C, D, E and D differs from E.

The comparisons C > B and E > D indicate that concentrating the water increased growth. To compare the amount of food in the 2 pondwaters, the appropriate comparisons are B (Dry Creek) with D (Hackham) at 1x, and similarly C with E at 2x. These comparisons are not significant, and therefore both pondwaters have about the same amount of food.

3.215 General conclusions.

In the first experiment, the pondwater was concentrated by evaporation, and the results from 0 to 4x in figure 3.01, conformed to the model relating larval growth to food.

The experiments with centrifuged pondwater were designed to get the complete curve of growth-rate on concentration; without causing osmotic stress. However, the experiments with centrifuged water showed that there was still considerable food in the supernatant, and that centrifuging apparently clumped the particles. When the clumps were broken up with a blender, the variability between treatments was reduced, and growth-rates increased (figure 3.04). However, the experiment in which the 3 factors were combined showed complicated interactions which were unrelated to what the larvae experienced in natural pools. Thus, the experiments with centrifuged water failed to improve upon the experiment in which the water was concentrated by evaporation.

A second, and fundamental question, is whether the results shown in figure 3.01, which indicated a relative shortage of food, apply to larvae living in natural pools. With Ae. alboannulatus, I was able to relate growth-rates observed in the field with the experiments in the laboratory, but I was unable to make this comparison with An. annulipes. The results in table 3.04 indicate that adding Chara to pondwater increased the growth-rate although the increase was not significant. However, if the larvae were experiencing a relative shortage of food at Dry Creek, it was probably less severe than the relative shortage indicated by figure 3.01.

3.22 The osmotic pressure of natural pools.

In the field, An. annulipes larvae often disappeared from permanent pools that were drying up and becoming stagnant. In the experiments on food, both species of larvae experienced osmotic stress in hypertonic solutions (sections 2.35 and 3.21) and these results led to the hypothesis that as pools dried up, the osmotic pressure increased and killed the larvae.

I was able to test this hypothesis in March 1961. The summer was unusually dry and pools at Hackham that were usually permanent began to dry up. The osmotic pressures in pools that had lost different amounts of water by evaporation were measured by the Beckmann Cryoscopic Method, and the concentrations of NaCl equivalent to the depressions of the freezing point are given in table 3.25. The osmotic pressures of pondwater from transient pools were given in section 2.35; they are repeated in table 3.25 to illustrate the differences between pools.

Assuming that the osmotic pressures of the 5 permanent pools were originally about the same, the results indicate that osmotic pressure is not related to the amount of water lost by evaporation, but that it stays fairly constant. This "buffering" effect is probably due to salts being left in the porous soil around the pool, and to the complex changes that lead to stagnation.

The osmotic pressures in the transient pools tend to be lower than in the permanent pools, because the transient pools are filled by run-off from heavy rains whereas the permanent pools are kept full by seepage. Transient pool 21, with an osmotic pressure equivalent to 0.36% NaCl is situated between the permanent pools 17 and 28, and it probably receives some water from seepage.

Table 3.25: Osmotic pressure of pondwater from permanent and transient pools.

Date	Description of pool	% NaCl
	(a) Permanent pools:	
7 Mar.61	Small pool; full. Larvae present	0.47
"	Pool 16; level fallen 2 inches	0.45
"	Pool 325; ¼ full	0.38
"	Pool 324; nearly dry	0.44
"	Pool D2; nearly dry	0.40
10 Feb.61	Dry Creek, pondwater (a)	0.44
	(b) Transient pools:	
26 Oct.60	Pool 400; half-full, drying up.	0.067
22 Jun.61	Pool 21; full since April	0.36
"	Pool 207; recently filled	0.15
28 Aug.61	Pool 43; recently filled	0.075

It appears that in the pools at Hackham, larvae would not experience osmotic stress. Larval mortality in drying pools is probably associated with the rather indefinable changes leading to stagnation.

3.23 An. annulipes biting at dusk in relation to light intensity.

The data for An. annulipes biting at dusk and light intensity were analysed in the same way as the data for Aedes. Again only 5 counts were sufficiently complete to be analysed.

The light intensities at the lower quartiles for each of the 5 counts were estimated graphically and are given in table 3.26. The 2 dates with asterisks were also used in the calculations for Aedes.

Table 3.26: Number of mosquitoes caught at dusk and light-intensity at lower quartile.

Date	No. of mosquitoes	Foot-candles
10 Nov.58*	9	1.0
5 Feb.59	10	0.1
21 Feb.59	7	0.5
11 Mar.59*	19	0.5
6 Mar.61	10	0.1
	Mean	0.44

For the combined data, the light intensity when 25% of the mosquitoes had been caught was 0.1 foot-candles. Similarly, the period after catching 25% of the mosquitoes until 75% were caught, i.e. the inter-quartile value, was found by iteration to be 22 minutes.

The data for both species of mosquitoes are compared in table 3.27.

Table 3.27: Comparison of Anopheles and Aedes biting at dusk in relation to light intensity.

	Anopheles	Aedes
Mean light int. at lower Q	0.44 f.c.	2.7 f.c.
Light int. at lower Q - combined	0.10 f.c.	2.4 f.c.
Interquartile period	22 mins.	17.6 mins.

The estimates of the mean light intensities for Anopheles are not accurate enough to justify Confidence Limits. However, the range of 0.1 to 1.0 foot-candles does not overlap the 95% Confidence Limits for Aedes of 1.7 to 3.8 foot-candles, suggesting that there is a significant difference between the 2 species. I noticed a similar difference in the

behaviour of the 2 species in daylight. During the day I was frequently bitten by Ae. alboannulatus, but only once by An. annulipes.

The higher light intensities for Aedes in table 3.27 and the slightly shorter interquartile period indicate that Aedes starts biting slightly earlier than Anopheles and also stops earlier. The differences are probably too small to have any effect on the transmission of myxomatosis. However, the different behaviour in daylight might have more important consequences.

3.24 Experiments on rearing An. annulipes.

The first attempts at rearing An. annulipes were made by placing pupae in a cage 14 x 9 inches and 9 inches high. The adults emerged normally and fed from my arm or from a mouse, but did not mate.

Two experiments were then done in a large indoor cage 4 feet x 6 feet and 11.5 feet high. In the first experiment, the temperature and relative humidity were held constant at 25°C and 80% respectively and a "daylight", 20w. fluorescent tube was switched on for 16 hours each day. At the end of the 16 hours of light, a small torch globe was switched on, providing about 1 foot-candle for half an hour, to simulate dusk. An aquarium with a surface area of 1.75 sq. ft., filled with pondwater and emergent Chara, was placed on the floor of the cage. On 18 February, 1958, 187 adults were released in the cage. During the period of "dusk" 1 female engorged from my arm and 1 other female was seen flying about the cage. By 28 February, 1958 all the adults were dead. No eggs were laid.

In the second experiment 124 adults were released in the cage on 9 March, 1958. Larvae and pupae were added to the aquarium and the

number of pupal exuviae counted each day. A further 147 adults emerged fairly evenly over the next 7 days. A mouse was put in the cage during the "dusk" period. I tried to stimulate mating by various changes in light intensity combined with a current of air from a fan. I also tried blowing air through a wire basket containing ice to give a current of cool air such as the mosquitoes might experience at dusk in nature. I saw no mating behaviour, and no eggs were laid. On the 17 March, 1958 I found only 2 females alive; both were dissected but had no sperm in their spermathecae.

A final experiment was done in a cage 6 feet square by 8 feet high built over pool 8 in the creek at Hackham. On 18 May, 1959 there were about 100 adults in the cage. A rabbit was put into the cage periodically. On 26 April, 1959, I could find only 10 adults alive; 5 females were caught and dissected, but all had empty spermathecae. I examined the pool carefully but did not find any eggs or first-instar larvae.

Conclusions.

An. annulipes resemble Ae. alboannulatus in their failure to mate in cages. Mackerris and Lemerle (1949) tried to rear An. annulipes from Queensland in cages 2 feet square by 3 feet high, but they also found that the adults did not mate.

When mosquitoes do not mate in cages, it is often assumed that they mate only in swarms and hence need considerable space. However, Nielsen and Haeger (1960) have presented evidence that little mating occurs in swarms and they argued that swarming is not a necessary condition for mating. They pointed out that despite intensive studies, many species of Anopheles have never been seen swarming, yet females caught in the field are nearly always fertilized. As far as I know there has

been only 1 report of swarming by An. annulipes. Minter (1950) observed funnel-shaped swarms at dusk composed of several species, including An. annulipes, in central western New South Wales. The observations were made in May and August of 1950 following the unusually extensive floods earlier in the year. He recorded that the density of mosquitoes was so great "that when they settled on men or animals it would have been difficult to put a pin between them without touching them". Minter's observation seems to agree with Nielsen and Haeger's conclusion that swarms form when mosquitoes reach high densities.

3.3 The numbers of An. annulipes at Hackham.

The absolute numbers of larvae and pupae in the creek at Hackham were estimated on the same dates as for Ae. alboannulatus. For each count the absolute numbers of larvae and pupae were found by summing the estimated totals for every pool.

3.31 Technique and accuracy of sampling.

Samples of water were scooped up in a rectangular tray 6 inches wide by 8 inches long. As the longer edge of the tray was slid under the water, the tray was moved through 9 inches, thus sampling about half a square foot. The larvae and pupae in each dip were counted and then returned to the pool.

Preliminary sampling showed that the larvae and pupae were virtually confined to emergent plants and consequently sampling was restricted to the zone of emergent plants. The mean number of larvae per square foot was multiplied by the surface area of emergent plants to give the absolute numbers of larvae and pupae in the pool.

As described for Ae. alboannulatus, samples were taken in a random manner, rather than strictly at random. The number of samples collected from each pool depended on the area of emergent plants because once a sample was taken, the larvae immediately around it swam down from the surface. Accordingly, samples were spaced at least 2 feet apart.

Theoretically, Fiducial Limits for the estimates of absolute numbers in the creek could be derived from the variances for individual pools. In practice, however, the actual numbers of larvae counted in the smaller pools were too low to yield meaningfull variances. I expected sampling errors to be greatest when the population of larvae was small. Consequently, in the winter of 1959, when the population of all instars

was low, I estimated the accuracy of the counting method. Eight separate estimates of the absolute numbers (N) of larvae in 4 pools were made on 1 day. Assuming that the population was stable over 1 day, the variability between estimates of N measures the accuracy of the method. The 8 estimates of N for larvae and pupae are given in table 3.28 together with the mean N, the sample standard deviations and coefficients of variation.

Table 3.28: Eight estimates of the absolute numbers of larvae and pupae in 4 pools.

No. of count	1st-2nd larvae	3rd-4th larvae	Pupae
1	4	22	18
2	0	63	0
3	10	112	19
4	3	112	8
5	17	116	27
6	27	140	35
7	2	187	41
8	19	100	33
Mean	10.25	106.5	22.625
S.D.	± 9.765	± 49.06	±14.1
Co-eff.of var.	95%	46%	62%

The coefficients of variation show that the precision of the estimates is low when N issmall, but increases as N increases. Even when N is as low as 100, the method seems reasonably precise. Thus, providing that sampling is representative of the whole population, the estimated values of N are reliable measures of the abundance of larvae and pupae in the creek.

3.32 The spatial distribution of An. annulipes larvae and pupae.

The spatial distribution of animals can be described by comparing the observed numbers of individuals per quadrat with various mathematical models.

In order to get enough observations for statistical analysis, counts of all instars of An. annulipes per dip (quadrat) from 4 pools at Hackham were combined, giving a total of 396 dips. The expected frequencies of a Poisson distribution and a negative binomial distribution are given beside the observed frequencies in table 3.29. The negative binomial distribution was calculated by method (2) given by Bliss (1953).

χ^2 for the Poisson distribution is 25.23 with 2 degrees of freedom and $P > .001$. Thus the larvae and pupae were not distributed at random with respect to dips. The negative binomial distribution gives $\chi^2 = 5.074$ with 4 degrees of freedom and $.20 > P > .10$, and hence it adequately describes the data.

Table 3.29: Comparison of observed distribution of larvae and pupae with the Poisson distribution and negative binomial distribution.

Larvae per dip	Expected Poisson f.	Observed f.	Expected Neg.B.D. f.	Contrib. of Neg.B.D. to χ^2
0	269.02	309	309.00	0
1	103.95	60	52.11	1.195
2	20.08	11	19.08	3.422
3	2.59	7	8.24	0.187
4	0.25	3	3.83	)
5	0.02	3	1.86	)
6	)	0	0.92	) 0.270
7	)	2	0.47	)
8	) 0.09	0	0.24	)
9	)	1	0.25	)
	396	396	396	5.074

A negative binomial distribution may arise either from summing a series of Poisson distributions whose means are distributed as χ^2, or because the presence of 1 unit in a quadrat increases the probability of a second unit occurring in that quadrat. If the larvae and pupae in each of the 4 pools were distributed as Poisson distributions, combining the results could theoretically generate a negative binomial distribution. However, when the undisturbed pools were examined carefully, the larvae and pupae could be seen in definite aggregations, and this is the more likely explanation for the negative binomial distribution.

The aggregations seemed to result from the behaviour of the larvae when feeding at the surface. If a larva swims up to the surface in a clear space, it may make a few feeding movements, but soon moves across the surface by rapidly flexing its body. This movement is repeated intermittently until the larva bumps into some object, such as the stem of a plant. The larva then orientates in a characteristic way, usually perpendicular to the object with a "tripod" of 3 large posterior bristles pressing against the object.

This pattern of behaviour seems to be characteristic of Anopheles larvae and was discussed at length by Bates (1949). He stated that the behaviour has generally been called a thigmotaxis, i.e. directed movement towards an object until the larva is in contact with it. Bates also quoted Renn's idea that the orientation is due purely to forces of surface tension. My own observations in the field indicate that neither explanation is correct. The behaviour is better described as a thigmokinesis, in which the larva shows increased movement until it contacts an object. It is then held in place by forces of surface tension, but the forces of surface tension around a floating stem are surely not adequate to move a

larva 6 to 12 inches, as required by Renn's hypothesis.

Larvae were often aggregated around small areas of open water in the middle of a dense mat of Chara or around floating twigs and reeds. The uneven distribution of such places contributed to the patchy distribution of larvae. If it were possible to recognise areas that were equally attractive to the larvae, stratified sampling would reduce the variability in the estimated means. However, in practice it was impossible to do so.

3.33 Results and analysis of the regular counts of larvae and pupae in the creek.

The estimated absolute numbers of larvae and pupae for the 2 years 1959 to 1960 are given in table 3.30. The totals for all instars are rounded to the nearest tens. The data for the 3 age groups are plotted in figure 3.07 and freehand curves drawn to show the trends.

Table 3.30: Estimated absolute numbers of larvae and pupae at Hackham.

Date	First to second instar	Third to fourth instar	Pupae	Total instars
1959				
Feb. 12	3,643	476	132	4,250
Feb. 22	295	135	26	460
Apr. 5	415	332	56	800
May 6	4,130	740	84	4,950
Jun. 7	1,387	622	30	2,040
Jul. 8	554	568	19	1,140
Jul. 29	210	414	46	670
Sep. 2	441	796	67	1,300
Oct. 16	392	683	47	1,120
Nov. 1	150	558	138	850
Nov. 18	752	525	67	1,340
Dec. 10	120	284	64	470
1960				
Jan. 15	2,348	1,194	44	3,590
Feb. 12	339	331	27	700
Mar. 11	1,938	353	31	2,320
Apr. 6	2,513	807	120	3,440
May 11	364	397	15	780
Jun. 2	0	125	39	160
Jul. 11	0	0	0	0
Aug. 15	0	0	0	0
Sep. 15	0	0	0	0
Oct. 19	27	19	0	40
Nov. 13	5	114	7	130
1961				
Jan. 5	1,499	395	77	1,970

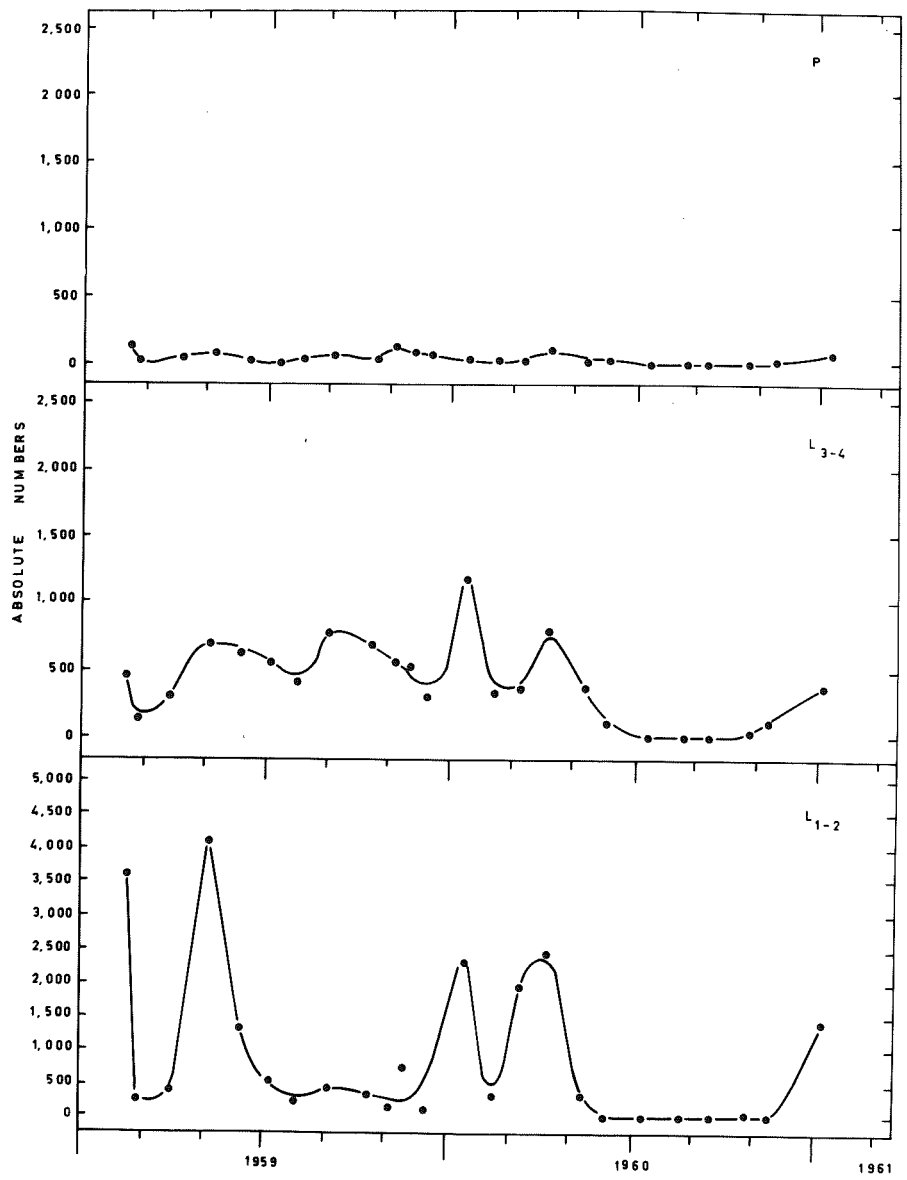


Figure 3.07: The estimated absolute numbers of first to second-instar larvae (L_{1-2}), third to fourth-instar larvae (L_{3-4}), and pupae (P), in the creek at Hackham.

From the column for total instars in table 3.30 it can be seen that An. annulipes was more abundant during the warm summer months, than during the colder, winter months. This fact suggested that abundance was influenced by temperature. Also, An. annulipes was present during the winter of 1959, which was unusually dry, but absent during the wet winter of 1960. This fact suggested that abundance was influenced by rainfall.

Consequently, a partial regression was calculated in which y_i was the total of all instars at the ith count, x_1 was rainfall for the period between the (i-1) and ith counts, and x_2 was the mean of the mean daily air-temperatures for the same period. Data for rainfall were from the Post Office at Morphett Vale and data for temperature from the Waite Agricultural Research Institute.

The data used for calculating the regression equation are given in table 3.31 together with the corrected sums of squares and sums of products. The regression equation is:

$$Y = 1,355 - 2.895763\ (x_1 - 157.7) + 87.34039\ (x_2 - 61.69)$$

and the overall test of significance is given in table 3.32.

Table 3.32: Analysis of variance of partial regression on data in table 3.31.

Source of variation	S.S.	D.F.	Mean S.	Var.Ratio
Due to regression	19,372,012	2	9,686,006	7.62 **
About regression	26,700,988	21	1,271,476	
Total	46,073,000	23		

Table 3.31: Rainfall, mean air-temperature and estimated absolute numbers of all instars at Hackham.

x_1 (points)	x_2 (°F)	y (all instars)
8	75.6	4,250
99	64.7	460
159	69.5	800
13	64.1	4,950
43	56.9	2,040
29	53.3	1,140
152	52.7	670
180	56.3	1,300
190	58.4	1,120
86	61.2	850
24	65.4	1,340
136	69.9	470
158	71.7	3,590
148	70.1	700
193	68.9	2,320
15	70.9	3,440
600	57.8	780
379	53.1	160
265	50.8	0
239	50.2	0
328	52.7	0
194	58.4	40
94	59.9	130
53	68.0	1,970

Corrected sums of squares and products:

	x_1	x_2	y
x_1	431,345	-10,842.2	-2,195,775
x_2		1,346.73	149,013.5
y			46,073,000

The significance of the two independent variables may be tested as in table 3.33 (Snedecor, 1956, p.419).

Table 3.33: Test of significance of the 2 independent variables in the partial regression equation.

Source of variation	D.F.	S.S.	Mean S.	Var.Ratio
x_1 and x_2	2	19,372,012		
x_1 alone	1	11,177,659		
x_2 after x_1	1	8,194,353	8,194,353	6.445 *
x_1 and x_2	2	19,372,102		
x_2 alone	1	16,488,046		
x_1 after x_2	1	2,883,966	2,883,966	2.268
error	21	26,700,988	1,271,476	

The effect due to x_1 and x_2 together is the sum of squares due to regression from table 3.32. The effect of x_1 alone is the sum of squares due to regression for the total regression of Y on x_1. The remainder shows the effect due to x_2 after removing the effect due to x_1 and the mean square is tested against the error mean square from table 3.32. Similarly the effect due to x_1 after removing x_2 is estimated. The variance ratio for 1 and 21 degrees of freedom is equivalent to the square of t, which tests the hypothesis that $b = 0$.

Only x_2 is significant at the 5% level of probability. However, the sum of squares for x_1 after x_2 of about 2.9 million as against 8.2 million for x_2 after x_1, indicates that the partial regression equation will be better for prediction than the total regression of Y on x_2.

The percentage of the total variance accounted for by the regression was calculated from the formula given by Fisher (1925).

$$1 - A = \frac{n - 1}{n - q - 1} (1 - R^2)$$

in which 100A = percent of total variance accounted for by the regression

R^2 = the sum of squares due to regression divided by the total sum of squares

n = number of values of y

q = number of independent variates.

Applying this formula to the data of table 3.32, 100A = 36.9%.

Thus 36.9% of the variance of the population was related to rainfall and mean air temperature. The significant regression indicates that the numbers of larvae are causally related either to the independent variates chosen or else to other factors which are correlated with the independent variates.

A knowledge of the biology of An. annulipes indicates a direct causal relationship between abundance and temperature, in that temperature influences general growth rates and activities.

The inverse relationship between y and rainfall suggests that it is not rainfall as such that is the causal factor, but rather another factor positively correlated with rainfall, namely, the amount of water flowing down the creek. Observations in the field support this idea. In the winter of 1959, less water than usual flowed down the creek. However, An. annulipes larvae were not found in the permanent pools 14, 16 and 17, which are in the main stream, but rather, in small vegetated pools away from the main flow of water.

Usually the number of breeding places during summer is independent of rainfall because the permanent pools are kept full from springs. Occasionally, however, a severe drought in summer dries out the pools and the numbers of larvae would then be positively correlated with rainfall. This

effect was seen in February and March of 1961. Counts of An. annulipes were discontinued because the pools dried out.

3.34 Other factors influencing the numbers of An. annulipes larvae and pupae.

Two other factors that seemed important were the number of breeding places in the creek, which can be regarded as a resource, and the influence of predatory, aquatic insects.

3.341 Resources.

Two sorts of changes were seen in the permanent pools which influenced the numbers of An. annulipes larvae and pupae.

(a) Succession. Even during the short period of 3 years, the succession of different species of plants was observed. The changes in pool 1 are given in table 3.34 as an example of the succession:
Bare soil → Chara globularis → Typha augustifolia .

Table 3.34: The number of larvae and pupae in pool 1 in relation to the succession of aquatic plants.

Date	Description of pool	Total no. of larvae		
		L_{1-2}	L_{3-4}	P
22 Dec.58	Chara: 16 sq.ft. no Typha	610	370	0
23 Jan.59	No plants, water turbid. Sheep drinking from pool.	0	0	0
22 Feb.59	Chara: 6 sq.ins., submerged Water clear	0	0	0
25 Mar.59	Chara: 1 sq.ft., submerged Typha: 4 plants	3	0	0
8 Jul.59	Chara: 2 sq.ft. emergent Typha: 6 plants	2	0	0
16 Oct.59	Chara: 29 sq.ft., half emergent)plate 1 Typha: 8 plants)	5	28	1
11 Mar.60	Chara: throughout the pool. Typha: through half of the pool.	76	76	0
19 Oct.60	Chara) Typha) Pool completely choked. Plate 2.	0	0	0
5 Jan.61	as for 19 Oct. 60	8	8	0
6 Mar.61	Chara: all dead Typha: grazed and trampled, sheep drinking from pool. Plate 3.	0	0	0

Plate 1: Pool 1, 16 October, 1959, showing emergent Chara. Anopheles larvae were present.

Plate 2: Pool 1, 19 October, 1960. Pool completely choked with Typha angustifolia and Chara. No larvae present.

Plate 3: Pool 1, 6 March, 1961. Vegetation grazed and trampled; water turbid after sheep had been drinking from the pool.

The data in table 3.34 indicate that the gradual growth of both Chara and Typha was followed by the presence of larvae. However, when the pool was completely choked with Typha, as in October 1960 (plate 2), only a few larvae were found. Pools 14, 320 and 325 were also invaded by Typha, but not to the same extent.

Another type of succession was seen in pools 2 and 3. In 1959 both pools were about 6 inches deep and larvae were present in emergent Chara. Silt gradually accumulated in the Chara and the reed Eleocharis acuta spread throughout the pools. By the summer of 1960-61, both pools were completely silted up, and no larvae were found.

(b) The effect of sheep drinking from the pools.

Two entries in table 3.34 indicate that sheep drinking from the pool affected the larvae. On 2 January, 1959 an estimated total of 1,556 first to second-instar and 86 third to fourth-instar larvae were in pools 1, 2 and 3. Later, in January, about 200 sheep were enclosed in a paddock in which pools 1, 2 and 3 were the only sources of water. On 23 January, 1959 all the aquatic plants were dead and the water very turbid. I was unable to find any larvae in the pools. The other pools in the creek were undisturbed and several thousand larvae were counted.

A similar situation arose in the summer of 1960-61, which was unusually dry. Again about 200 sheep were drinking from pools in the creek. The course of events is given in table 3.35.

Table 3.35: Effect of sheep drinking from pools during a dry summer.

Date	Observation	Total larvae in creek	
		1st-2nd	3rd-4th
5 Jan. 61	Many pools full - no effect from sheep.	1556	86
7 Feb. 61	Only 10 pools full - sheep drinking from 6 pools.	55	5
6 Mar. 61	Plants destroyed in all pools and water turbid.	0	6

The 6 larvae found on 6 March, 1961 were in a small pool of 1 square foot dug for an experiment with Ae. alboannulatus and protected from sheep with wire-netting.

Thus, in certain circumstances, sheep may profoundly influence the numbers of larvae. In using their resource of drinking water, the sheep walked into the pools and thus destroyed the aquatic plants and clear water which are a resource for An. annulipes larvae. Hence the sheep may be classified as non-predators which do not require to share the same resource (Andrewartha and Birch, 1954).

3.342 Predators.

The permanent pools abounded in aquatic insects which have been listed as predators of mosquito larvae by Bates (1949). The more abundant groups at Hackham were:

Ephemeroptera
Odonata
Hemiptera - Notonectidae
Naucoridae
Coleoptera - Dytiscidae
Gyrinidae
Hydrophilidae

The discrepency between the abundance of first to second-instar and third to fourth-instar larvae shown in figure 3.07 suggests considerable mortality between the 2 age groups, much of which could be due to predation. However, I was unable to find a method for sampling predators from within the mat of aquatic plants without destroying the plants and making the pools unsuitable for An. annulipes.

3.35 The numbers of An. annulipes larvae in relation to breeding-places on the River Murray.

Regular surveys were made of the swamps and river margins between Mannum and Purnong, and the numbers of larvae counted in most of the accessible places. However, the breeding-places were so diverse and the distribution of larvae so patchy that only general trends were discernable.

The actual numbers of larvae and pupae counted in emergent plants both in the swamps and in the main stream of the river are given in table 3.36. The data are the combined results of 10 field-trips.

Table 3.36: Density of larvae in swamps and river-margins along the Murray.

	Swamps		River-margins	
	Actual no.	No. per 100 dips = 50 sq. ft.	Actual no.	No. per 100 dips = 50 sq. ft.
Larvae 1-2	62	3.16	26	7.69
Larvae 3-4	121	6.16	18	5.33
Pupae	15	0.76	1	0.30
No. of dips	1964		338	

It can be seen that the larvae and pupae are at about equal densities in swamp and in the river margins, but the greater area of vegetated swamps supported higher absolute numbers. The low numbers of larvae per 100 dips resulted from the large number of dips in which no larvae were found. The larvae seemed to be quite rare relative to their resources of breeding-places.

The seasonal trends in the amount of water flowing past lock 1 at Blanchetown are shown in figure 3.08.

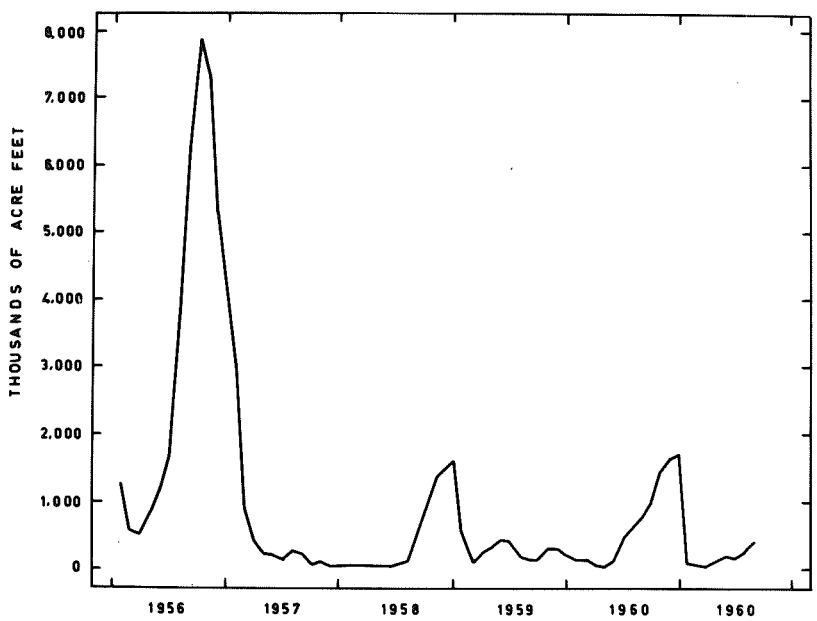


Figure 3.08: The amount of water flowing past lock 1, at Blanchetown on the River Murray.

The peaks in November of 1958 and 1960, represent the usual, annual flood. The more even distribution in 1959 happens occasionally, but both the extremely high peak in 1956 and the very low rate of flow in the summer of 1957-58 are exceptional.

Observations on the swamps, the numbers of larvae and changes in the level of the river are given in table 3.37. The levels are expressed as deviations from the "lower pool level" of 109.5 feet, which is the theoretical level of the river when no water is flowing over the weir at Blanchetown.

Table 3.37: Abundance of larvae in relation to river levels and state of the swamps.

Date	Deviations from pool-level in ft.	Observations on swamps and larvae
21 Feb.58	- 0.37	Swamps dry, larvae absent
1 May 58	- 0.29	Some swamps full, larvae absent
13 Nov.58	+ 2.06	All swamps flooded, few larvae in river
13 Jan.59	+ 0.98	Swamps full, many larvae
29 Apr.59	+ 0.44	Swamps full, many larvae
1 Jul.59	- 0.10	Swamps full, many larvae
30 Nov.59	+ 0.23	Swamps stagnant, few larvae
19 Jan.60	0	Swamps low, larvae absent
1 Mar.60	- 0.10	Swamps dry, larvae absent
25 Aug.60	+ 1.18	Swamps full, few larvae
12 Oct.60	+ 1.50	Swamps full, few larvae
15 Nov.60	+ 1.88	Swamps full, many larvae

As mentioned in section 3.13, the river-level depends mainly on the rate of flow, but wind also causes fluctuations of up to 6 inches. The effect due to wind was eliminated by taking the median of the daily

deviations of level for the 2 weeks around the date on which the larvae were recorded at Mannum.

Three factors that influence the abundance of An. annulipes larvae can be inferred from the data in table 3.37. Firstly, when the river is about 0.1 feet below pool-level as in February 1958 and March 1960, the swamps are dry and the number of breeding-places are at a minimum. Secondly, when the annual flood-peak is low, as in 1959, the swamps may become stagnant and unsuitable for larvae. Thirdly, flooding that raises the river about 2 feet or more above pool-level, as in November 1958, makes the swamps continuous with the river and again reduces the number of breeding-places.

One other factor that influences the quantity and quality of the breeding-places is the annual growth of aquatic plants. Most of the plants die back in winter and then regenerate in spring and summer. This annual cycle is also affected by both daily and seasonal fluctuations in the level of the river. When the cycle of aquatic plants is considered against the seasonal trends in the level of the river, it can be seen that the resources of breeding-places can vary considerably from year to year.

In summary, many An. annulipe larvae were found in the summers of 1958-59 and 1960-61, whereas few larvae were found in the summer of 1959-60. From the low rate of flow in 1957, it can be inferred that few larvae were present in the summer of 1957-58.

4.1 The seasonal incidence of rabbits, myxomatosis, and vectors at Hackham.

4.11 Rabbits and myxomatosis.

Methods. A rough estimate of changes in the numbers of rabbits was made by walking along a standard route and recording the numbers of sick and healthy rabbits seen. The route was 1.5 miles long and was designed to pass the main warrens in the area. The walk was started as the sun dipped below the western hills, and by walking briskly it could be completed while there was still enough light to see clearly. A pair of 7 x 50 binoculars were used to identify myxomatous rabbits. When possible, calm, clear nights were chosen.

In using the method of sight-counts it is assumed that a fairly constant proportion of the population is seen on each occasion. The validity of sight-counts has been studied by officers of the Wildlife Survey Section, Canberra. Myers (1954) found that the method was sufficiently accurate to distinguish between case mortality rates of 99.5% and 95% during outbreaks of myxomatosis. Dunnet (1957) assessed the accuracy of sight-counts by studying the emergence behaviour of rabbits in several warrens. He found considerable variation both between warrens and also between localities. The time of maximum emergence was about 2 hours before sunset in 1 area where the rabbits had not been disturbed by control measures. The time of maximum emergence was about sunset in a second area where the rabbits had been shot and trapped, but after control was stopped the time of maximum emergence became steadily earlier. Dunnet also noted that disturbance by predators (birds and foxes) or by the presence of man, greatly affected the emergence on that night. Rowley (1957) noted similar differences between warrens and also found that the average time of maximum emergence was about 1 hour earlier in summer than in winter.

The discussion in the above paragraph indicates that the results of sight-counts may be quite misleading. However, it was the only practicable method that I could find for estimating trends in the numbers of rabbits at Hackham. In addition to the sorts of errors mentioned above, I expected the results of sight-counts at Hackham to be rather inaccurate because in some places dense stands of trees (Eucalyptus odorata, Behr et Schlechtd.) and bushes (Acacia armata, R.Br.) made it difficult to see the rabbits. Also on week-ends and holidays the rabbits were frequently disturbed by people illegally trapping, shooting, and ferreting. Most of the counts were made on week-days when I was reasonably sure that the rabbits emerged normally.

Table 4.01: The numbers of rabbits counted along a standard route at dusk, Hackham. Figures in brackets refer to myxomatous rabbits.

Date	No. of rabbits
1958	
Mar. 28	12
Apr. 10	7
Jul. 20	9
Sep. 5	15
Dec. 15	15 (1)
Dec. 22	24 (3)
1959	
Jan. 2	28 (2)
Jan. 23	4
Feb. 5	19 (1)
Feb. 12	12
Apr. 9	2
Sep. 15	20
Oct. 16	19
Nov. 18	60

Date	No. of rabbits
1959	
Dec. 22	74
Dec. 29	53
1960	
Jan. 18	64
Feb. 18	121
Feb. 28	67
Mar. 4	30
Mar. 11	30
Mar. 18	60
Apr. 6	13
Apr. 13	36
Oct. 19	12
Dec. 22	28
1961	
Mar. 6	21
Mar. 22	23
Apr. 26	20

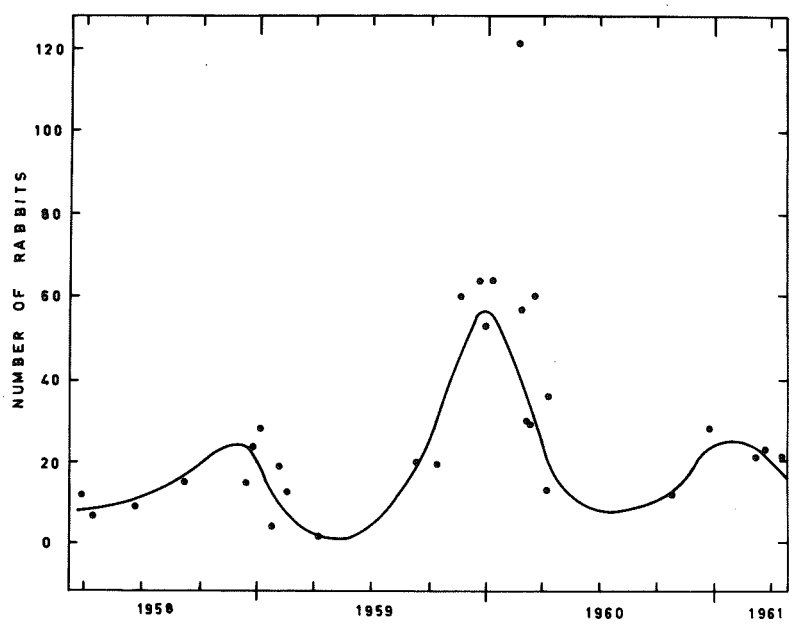


Figure 4.01: The number of rabbits counted along a standard route at dusk, Hackham. A freehand curve is drawn through the points, omitting the count of 121 rabbits on 18 February, 1960, which was an abnormal observation.

Results.

The total numbers of rabbits counted on each occasion are given in table 4.01. The figures in brackets refer to myxomatous rabbits. The data of table 4.01 are plotted in figure 4.01 and a freehand curve drawn through the points to indicate the trend. The 121 rabbits counted on 18 February, 1960 is probably an abnormal observation, and has been omitted in drawing the curve. The weather on that night was calm and sultry, with a strong thundery feeling, and it seemed to affect the behaviour of the rabbits. I was able to walk to within a few yards of

most of the rabbits, whereas usually the nearest I could approach was about 20 yards. Southern (1948), in a study on behaviour of rabbits, reported a similar effect. He stated, "....during normal activity some 30% of the population was usually above ground, and on less frequent occasions, such as just before a thunderstorm, when they were all feeding hard, this proportion might be increased". When the count of 121 is omitted, the free-hand curve reaches a peak of about 70 in January 1960. It seems that the unusual weather resulted in almost double the number of rabbits being counted.

Discussion.

Occasionally, a myxomatous rabbit was seen during the day, both in summer and winter, indicating that myxomatosis was persisting in the area. In January 1959, there was a definite outbreak; in addition to seeing 7 myxomatous rabbits in the routine counts, 11 carcases were found in the area, and some myxomatous rabbits seen during the day. On 21 April, 1959 I shot 6 rabbits in the area at Hackham and tested their sera for myxoma antibodies by the method described below, in section 4.22. One of the 6 rabbits was myxomatous, and the other 5 had antibodies in their sera, indicating that they had recovered from myxomatosis. The sample is small, but the result is consistent with the outbreak observed in the field.

There was no obvious outbreak in the summers of 1957-58, 1959-60 or 1960-61.

The numbers of rabbits counted increased considerably in the summer of 1959-60, and on 22 February, 1960 I tried to start an outbreak by releasing 10 innoculated rabbits in the centre of the area. The rabbits released were susceptible domestic rabbits innoculated intradermally with

0.2 ml. of a suspension of a virulent strain of virus obtained from the Institute of Medical and Vetinary Research, Adelaide. They were released 2 days after they were innoculated.

As can be seen in figure 4.01, the numbers of rabbits decreased in March and April 1960, but there was no evidence of an outbreak of myxomatosis. The decrease was probably due to normal seasonal mortality and extensive poaching and, perhaps, to a change in emergence behaviour.

It is interesting that the greatest seasonal increase I observed occurred in the spring and summer following an outbreak of myxomatosis. September, November and December, in 1959, were unusually wet, resulting in an extensive growing season for plants. The length of the growing season was estimated by calculating the period in which rainfall exceeded one third of the evaporation from a standard meteorological evaporimeter (Trumble, 1937). The calculations were done with daily rainfall from Morphett Vale, and the mean daily evaporation for each month at the Waite Agricultural Research Institute. The results are given in table 4.02.

Table 4.02: The growing season for plants at Hackham in 1959.

Period in which R > .3 E	No. of days
30 Mar. to 29 Apr.	31
18 May to 26 May	9
24 Jun. to 30 Jun.	7
15 Jul. to 3 Jan. 60	175

Poole (1960) considered that rabbits in southern Australia breed mainly in the spring, although they may breed after a well-defined autumnal break of the season. At Hackham, I did not see any kittens during April and May, and the rabbits probably did not start breeding until the well-defined break on 15 July, 1959. Kittens were seen as late as 18 November, 1959 and many juveniles were seen on 22 December, 1959, indicating that the breeding season of the rabbits, like the growing season of the plants, extended into summer.

It is difficult to assess the effectiveness of myxomatosis from the results of the sight-counts. Superficially, it appears that an outbreak of myxomatosis in January 1959 did little to check the increase of the rabbits in the following summer. However, had there not been an outbreak, the population may have increased much more than was observed. Also, there was probably some transmission of myxomatosis each summer at too low a level to be observed by sight-counts.

The sight-counts would have been much more informative had I been able to collect samples, each of about 50 rabbits, and test the sera for myxoma antibodies. I tried shooting at night by spotlight but found that the country was too rough and steep. I also tried using ferrets and traps, but the difficult terrain and my inexperience made the methods lengthy and unrewarding. Consequently, I decided to collect rabbits for serological tests elsewhere at Teal Flat, on the River Murray, where it was possible to shoot by spotlight.

4.12 Vectors.

The outbreak in January 1959 seemed to be associated with An. annulipes. An. annulipes was the only species caught biting at dusk in 5 counts made during December 1958 and February 1959. The estimates of

the absolute numbers of An. annulipes larvae given in section 3.3 unfortunately do not start until February 1959. However, I had fairly regular estimates of the mean number of larvae per dip from 8 pools during 1958. I have calculated the mean number of larvae for the same 8 pools from the counts of absolute numbers so that the relative abundance for the summers of 1958-59 and 1959-60 can be compared. The means are plotted in figure 4.02 with freehand curves to indicate the trends.

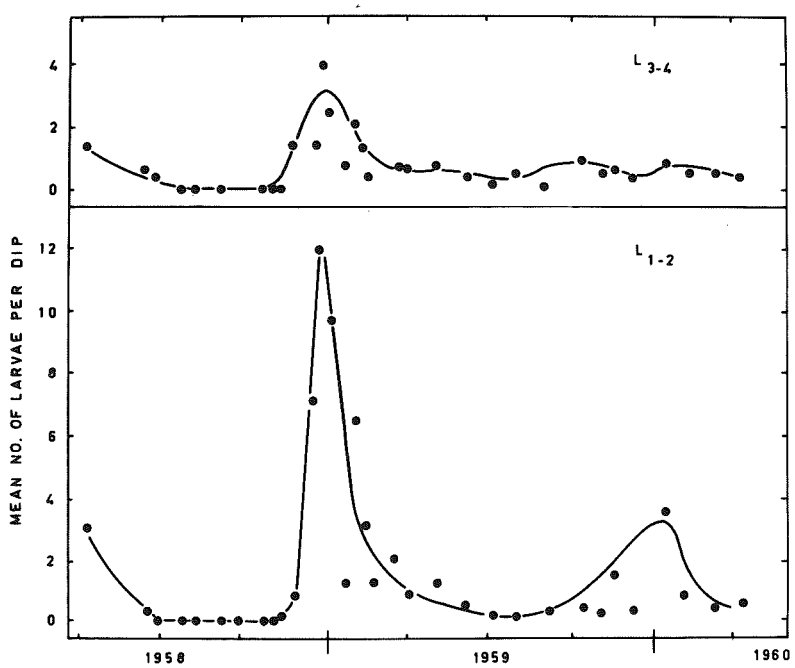


Figure 4.02: The mean number per dip of first to second-instar larvae (L_{1-2}) and third to fourth-instar larvae (L_{3-4}) at Hackham. The points are grand means from 8 pools.

The peaks of both curves for the summer of 1958-59 are 4 times as high as the peaks for the summer of 1959-60, indicating that An. annulipes adults were probably more abundant during the first summer than the second.

Ae. alboannulatus larvae were virtually absent during January and February of 1959, only 1 small transient pool (201) had a few larvae in it. During the summer of 1959-60, Ae. alboannulatus larvae were quite abundant. Also, in 4 counts of mosquitoes biting at dusk during November 1958 to April 1960, a total of 45 Ae. alboannulatus, and 18 An. annulipes were caught.

These figures suggest that vectors may have been numerous enough during the summer of 1959-60 to have caused an outbreak if other circumstances had been as favourable as in 1958-59. I shall suggest below (section 4.24) that the outbreak may have failed to develop because the population of rabbits in 1959-60 included a high proportion of individuals with immunity that had been acquired during the previous outbreak.

4.2 The seasonal incidence of myxomatosis and vectors at Teal Flat, on the River Murray.

4.21 Description of the area at Teal Flat.

Samples of rabbits were collected from a property of 1,500 acres near Teal Flat. The property fronts onto Lake Carlet, a narrow strip of water about 5 miles long which is open to the river at the upper end. The ground slopes slightly up from the banks of the lake and then rises stepwise about 200 feet, with 2 limestone cliffs. The top of the cliffs is about half a mile from the bank of the lake. The rest of the ground back from the cliffs is flat and sandy, with patches of mallee and native pine (Callitris spp.).

Many rabbits were living in the piles of limestone at the bases of the cliffs and in warrens on the narrow strips of land at the bases of the 2 cliffs. Other warrens were found patchily distributed over the flat, upper ground, especially in sandy places.

The rabbits were rather sparse and it did not appear possible to work out a standard route for sight-counts. The rabbits were undisturbed except for some trapping carried out by the manager of the property.

4.22 Methods.

Most of the rabbits were collected by spotlight shooting with a 0.22 rifle. This method probably gave a sample which was fairly random with respect to sex and age. When rabbits were scarce traps and ferrets were used as well as the shooting.

Blood was collected from the rabbit's heart with a pasteur pipette. The tubes of sera were stored frozen and tested by the gel diffusion technique described by Mansi (1957). I am indebted to Professor F. Fenner and Dr. I.D. Marshall of the John Curtin School of Medical Research, Canberra,

for instructing me in the technique. The gel diffusion technique is not as sensitive as the complement fixation or neutralization tests, but for most sera it gave a clear positive or negative reaction.

In Canberra I tested 13 sera by all 3 techniques and hence I was able to compare the weakly positive results of the gel diffusion test with the more precise results of the other 2 tests. In a weakly positive reaction the precipitin line is formed close to the cup containing the unknown serum. By keeping drawings of every test, I was able to judge the tests consistently for the whole series.

4.23 Results.

The results are given in table 4.03, expressed as the percentage of sera which gave a positive reaction (immune sera), and the number of sera tested. The 95% Confidence Limits were estimated from the binomial variance. When the number of immune sera (a) was less than 15, table F in Davies (1957) was used. When (a) was 15 or greater, the normal approximation to the binomial distribution was used. The results are given in table 4.03.

Table 4.03: The proportion of immune rabbits in the population at Teal Flat during 1959 to 1961.

Date	No. of rabbits	% immune sera	95% Confidence Limits
15 Jan. 59	30	87.5	73.3 to 95.8
1 Jul. 59	32	100	89.1 to 100
1 Dec. 59	65	29.2	18.1 to 40.3
1 Mar. 60	42	33.3	19.6 to 49.7
28 Aug. 60	26	38.5	20.2 to 57.0
15 Nov. 60	56	17.9	8.9 to 30.4
14 Jun. 61	66	54.5	42.5 to 66.5

The data are plotted in figure 4.03 and a freehand curve drawn through the points. The broken line indicates an interpolation which is described in section 4.24.

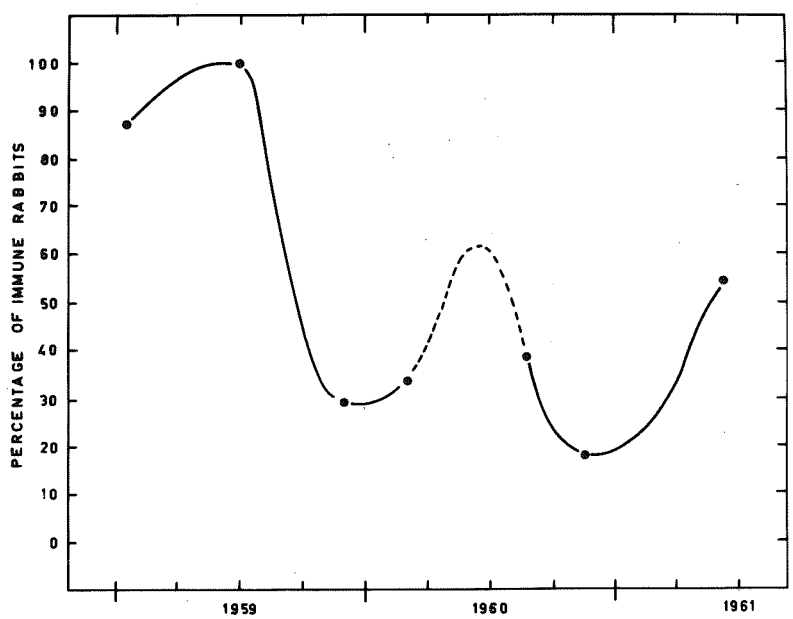


Figure 4.03: The proportion of rabbits with acquired immunity at Teal Flat, on the River Murray. A freehand curve is drawn through the points. The broken part of the line indicates an interpolation described in the text.

4.24 Discussion.

The confidence intervals for some of the samples are rather wide, but the changes in immunity were consistent with independent observations in the field on the incidence of myxomatosis.

There are 2 values of acquired immunity which seem particularly

instructive. One is the proportion of immunity among adults in winter, which indicates the severity of the outbreak they were exposed to in the previous summer. The other is the proportion of acquired immunity in the population at the beginning of the following summer, before transmission of myxomatosis starts. The latter value will usually be less than the former, because susceptible rabbits born in the spring, reduce the proportion of immune rabbits present at the beginning of summer.

Unfortunately, in 1960 I was unable to directly estimate the proportion of old immune rabbits present in winter because the sample collected in August included young rabbits which had not been exposed to the outbreak in the previous summer. An indirect estimate was made by dividing the August sample into old adults and young of the season. Assuming no mortality during winter, the proportion calculated from the old adults indicates the degree of immunity in winter.

In the August sample, each rabbit was weighed just after it was shot, and its age estimated from its weight. Fourteen rabbits weighed more than 1.7 Kg. and hence were all old adults. Nine weighed less than 0.795 Kg. and hence were young rabbits (Dunnet, 1956). This left 3 doubtful cases of 1.30, 1.25 and 1.19 Kg. I am grateful to Mr. K. Myers for access to his unpublished data on growth-rates of rabbits in large, natural enclosures. From Myer's data, the 3 rabbits were probably born in March, April and May respectively. The early breeding was quite likely because unusually heavy rains fell at Mannum in late summer: 152 points in February; 53 points in March; and 142 points in April.

It seems reasonable to include the rabbits born in March and April in the group which was exposed to myxomatosis. Thus, of the 16 rabbits exposed to myxomatosis, 10 had immune sera, which gives 62.5% as the estimate

of the proportion of acquired immunity in the winter of 1960.

From the data in table 4.03 it is possible to check whether this estimate is reasonable. In July 1959, all the rabbits were immune, therefore, assuming no mortality among immune rabbits, the decreased percentage immunity of 29.2% in December 1959, was due entirely to susceptible young rabbits. For every 100 rabbits present in July, let there be (100 + x) rabbits in December, where x represents the young rabbits.

$$\text{Then } \left(\frac{100}{100 + x} \right) .100 = 29.2\%$$

$$\text{That is } x = 242$$

Assuming 50 rabbits in every 100 are females, this gives an average of 4.8 young per female as the actual rate of increase of the population. This seemed rather low, so I calculated the length of the growing season for plants, by the same method as used in section 4.11, using rainfall at Mannum and monthly evaporation at the Waite Agricultural Research Institute, Adelaide. The results are similar to those for 1959 at Hackham, and are given in table 4.04.

Table 4.04: Growing season for plants at Teal Flat in 1960.

Period in which R > .3 E	No. of days
31 Mar. to 14 May	45
18 May to 30 May	13
13 Jun. to 19 Jun.	7
22 Jul. to 28 Aug.	38
14 Sep. to 31 Nov.	79

No young rabbits were collected in the sample of 1 July, so the rabbits probably did not begin breeding until the break of the season on

22 July, 1959. Since the next sample was collected on 15 November, 1959, the rabbits had only about 3 months in which to breed and thus were unlikely to have had more than 3 litters.

The average number of kittens per litter is about 4.6 (Myers, personal communication) which means that one doe could produce about 14 kittens. A survival rate of 4.8 kittens gives a mortality of 9.2 per 14 kittens or 66%. This mortality rate is probably not excessive, the country is dry with only 10 to 11 inches of rain per year, and foxes, hawks and feral cats are prevalent.

If the same assumptions are made for the winter of 1960, it is possible to work back from the percentage of immunity of 17.9 on 15 November, 1960. For every 100 adult rabbits present in winter, let there by w immune rabbits,

$$\text{Then } \frac{100\ w}{(100 + 242)} = 17.9\%$$

$$\text{i.e. } w = 61\%$$

which is in close agreement with the percentage of 62.5, calculated from the adult rabbits in the August sample.

Now, acquired immunity is only 1 of many factors that have to be considered in the epidemiology of a viral disease transmitted by arthropod vectors. But in some circumstances it may be more important than the other factors. It is well known for malaria that a severe epidemic results in a high proportion of acquired immunity, which may eliminate the disease in local areas, and usually it is some years before another severe epidemic (MacDonald, 1957).

This mechanism seems to be working in the Mannum region. After the severe outbreak of myxomatosis in 1958-59, there were only mild out-

breaks in 1959-60 and 1960-61. In December 1959, the percentage of 29.2, or nearly 1 immune rabbit in every three, was probably sufficient to prevent a severe outbreak in that summer. By the next summer the percentage had decreased to 17.9, and again there was no severe outbreak. Probably, the proportion of immune rabbits at the beginning of summer will continue to decrease, until acquired immunity will have little importance.

The proportion of acquired immunity at the start of summer (y) will depend on both the number of young produced per doe (r) and the proportion of immune adults in the previous winter (k). Assuming no mortality among immune adults, for any value of k, the relationship between y and r is given by:

$$y = \frac{k}{1 + .5r}$$

This is the equation for a hyperbola in which $y = k$, when $r = 0$, i.e. if no young survive the proportion of immune rabbits is unaltered. At the other extreme, y asymptotes to zero as r becomes indefinitely great. In practice, r is likely to lie between about 3 and 25.

I have drawn curves for 4 values of k to illustrate the interrelations between r, k and y (figure 4.04). The curve for $k = 1$ represents the case at Teal Flat for 1959, and it can be seen that when $r = 5$, $y = 0.29$. But for the same curve, the results in the high rainfall areas of eastern Australia would be quite different. In a more favourable environment, a rabbit might have up to 7 litters, which might result in values of r of 15 to 20. In these circumstances the proportion of immune rabbits will be as low as 0.12 to 0.08. Thus, the effect of acquired immunity is likely to be far more important in regions like Mannum, than in the higher rainfall regions. The curves for values of $k < 1$ illustrate the decreased effect of acquired immunity to be expected

after less severe outbreaks.

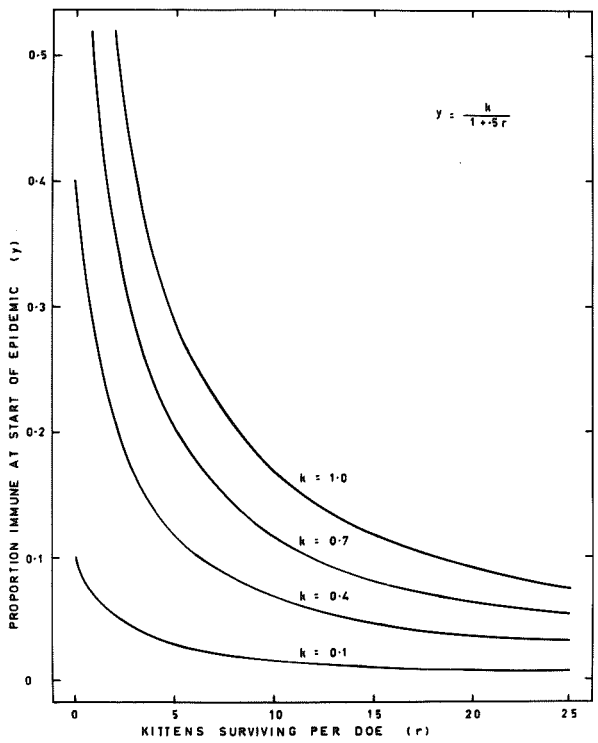


Figure 4.04: Theoretical relationships between the proportion of immune rabbits in winter (k), the proportion of immune rabbits at the beginning of the following summer (y) and the annual rate of increase of the rabbits (r). The curves are $y = \frac{k}{1 + 0.5\ r}$

By 1957, a virus of grade III virulence was predominant in the field, and the genetic resistance of the rabbit had slowly increased (Fenner, 1959a). Provided that these factors do not change markedly,

the course of myxomatosis in the Mannum area is likely to be occasional severe outbreaks with mild outbreaks in between.

Indeed, this has been the history of myxomatosis for several years prior to 1958. Farmers, and a member of the local Board of Rabbit Control told me that the outbreak of 1958-59 was the first severe one for 4 to 5 years. The local Board had been conducting innoculation campaigns, but discontinued them after 1957-58 because they seemed to have no effect. Hence, the severe outbreak of 1958-59 developed without human aid.

4.25 Vectors.

The incidence of An. annulipes larvae was discussed in section 3.35. The best estimate of abundance was to classify larvae as few or many. Thus:

Summer of 1957-58	probably few
1958-59	many
1959-60	few
1960-61	many.

The severe outbreak of 1958-59 coincided with many An. annulipes larvae. Occasionally larvae of Culex annulirostris Skuse, C. pipiens australicus Dob. and Drummond and Ae. alboannulatus were found, but these species were not as wide spread nor as abundant as An. annulipes.

Counts of mosquitoes biting at dusk were made when possible but unfavourable weather and the time entailed in collecting samples of rabbits resulted in only 9 counts covering the full period of dusk activity. The counts were made at 3 localities to determine the dispersion of adults. One location was beside the river at Teal Flat, the second about 1 mile inland near a large warren in mallee scrub, and the third about 2 miles inland in similar scrub near several small warrens. The total numbers

of adults caught each night are given in table 4.05. Counts on 2 successive nights are paired and give some indication of the relative abundance at different locations.

Table 4.05: Mosquitoes caught biting at dusk at different locations near Teal Flat.

Date	Locality	An. annulipes	C. annulirostris
12 Feb. 58	By river	5	0
14 Jan. 59	By river	1	25
29 Apr. 59	By river	1	1
30 Apr. 59	1 mile inland	2	0
2 May 59	2 miles inland	0	0
3 Jul. 59	1 mile inland	0	0
4 Jul. 59	2 miles inland	6	0
13 Oct. 60	By river	36	0
12 Oct. 60	1 mile inland	10	0

Both the number of counts, and the total numbers of mosquitoes caught are too small to draw any rigorous conclusions about the abundance and distribution of adults. However, the data indicate that An. annulipes was present in all 3 localities, suggesting that this species disperses well into the mallee country bordering the river, which is similar to the results found by Myers et al. (1954).

Also, during the severe outbreak of 1958-59, both An. annulipes and C. annulitrostris were caught. Probably both these species were important vectors, although the data collected by Myers et al. (loc cit.) indicate that An. annulipes is the more important vector.

The only other species of mosquito attracted to man at dusk was

Ae. alboannulatus; 6 females were caught on 4 July, 1959, 2 miles inland. The only other abundant blood-sucking arthropod in the area was the stick-fast flea Echidnophaga myrmecobii which was found on nearly every rabbit examined. It has been shown that E. myrmecobii can transmit myxomatosis, but that its restricted mobility makes it an inefficient vector (Bull and Mules, 1944).

It is interesting to compare the results at Mannum with the results from an area at Corowa, N.S.W., which also fronts onto the River Murray (Myers, 1956; Myers et al., 1954). Both places have similar vegetated swamps along the river, but Corowa has a higher rainfall. At both places An. annulipes and C. annulirostris were the most abundant species. Outbreaks of myxomatosis occur regularly each summer at both places, but at Corowa the outbreaks are over by January or February, whereas at Mannum the outbreaks start later and finish in March or April. This difference is related to the annual flood-peak of the River Murray, which passes Corowa in October and reaches Mannum about 2 months later, in December. As at Mannum, in years with a normal, annual flood, the mosquitoes do not breed extensively at Corowa until the flood has passed and the aquatic plants have grown in the swamps, (Myers, 1954).

4.3 Myxomatosis in the Far North of South Australia.

By 1958, in the eastern states of Australia, the virus present in the field was attenuated, and the rabbits had developed considerable genetic resistence. In 2 experiments in N.S.W. a virulent strain of the virus was repeatedly introduced into 2 populations of rabbits, but although outbreaks occurred, they were due mainly to the attenuated field strain of virus (Fenner et al., 1957). Fenner (1959b) concluded that innoculation campaigns should be discontinued, except when circumstances were especially favourable.

In 1958, the South Australian Department of Agriculture reported that rabbits were more abundant in the upper and far north of South Australia than in any other part of the state. Rainfall in the upper part of South Australia is erratic and I expected that mosquitoes were abundant only after heavy rains, which may be 5 or more years apart. Thus, it seemed possible that outbreaks of myxomatosis occurred infrequently and consequently that the rabbits might not have developed much genetic resistance. If this was so, innoculation campaigns following heavy rains might be quite successful.

To look for evidence for this hypothesis, I accompanied an Inspector of Stock from the South Australian Department of Agriculture, on a routine trip from Pt. Augusta in South Australia to Birdsville in Queensland.

4.31 Rabbits.

Rabbits were abundant along our route from Frome Downs Station (near the southern tip of Lake Frome) up to Coopers Creek, and there were many commercial rabbit trappers and freezing depots in the area. Few rabbits were seen north of Coopers Creek, although many old, disused warrens indicated the original extent of the rabbits' distribution.

Thirty rabbits were shot in the dry bed of Coopers Creek near

Etadunna Homestead and their sera tested for myxoma antibodies. Fifteen sera gave a positive reaction, showing that the proportion of immune rabbits was 50%, with 95% Confidence Limits of 32.1% to 67.9%.

4.32 Vectors.

The trip was deliberately made in September because good rains had fallen and many swamps and claypans held water. I expected that aedine mosquitoes utilised these transient collections of water and therefore I examined them carefully for larvae and pupae.

However, I found larvae in only 2 places, both permanent swamps. One large swamp was formed by the overflow of an artesian bore at Lake Harry, the other was a small swamp around a wind-mill at Red Banks bore, near Wirrealpa Homestead. The larvae were brought back to the laboratory and the adults bred out proved to be C. globocoxitus Dob.

Many other extensive permanent swamps were examined. Most of them were vegetated, and Chironomidae larvae and aquatic Heteroptera and Coleoptera were found, but no mosquito larvae. The only adults caught biting at dusk were Ae. tremula (Theo.) near the town of Copley.

4.33 Conclusions.

Since the rainfall at CoopersCreek is less than 5 inches per year, few rabbits are likely to live more than 1 year. Thus, the 50% immune rabbits in the area indicate recent transmission, although no severe outbreak had been reported for several years. However, several professional rabbiters told me that they noticed myxomatous rabbits each summer.

The absence of mosquitoes from the transient collections of water does not support the hypothesis that good rains are automatically followed by many mosquitoes. People living in the area told me that mosquitoes

were prevalent in summer, but not usually a nuisance during the colder months.

Thus, there are probably mild outbreaks of myxomatosis each summer, coinciding with the increased activity of mosquitoes. The large number of rabbits in the area, and the fairly high proportion of immunity suggest that attenuated viruses and genetically resistant rabbits have developed, as in the eastern states. In these circumstances, innoculation campaigns would probably be of little use for controlling rabbits.

4.4 The initial spread of myxomatosis.

Myxomatosis was first tested in Australia by the Department of Agriculture of New South Wales in 1926 (Ratcliffe et al., 1952). The first experimental release was made by Bull and Mules (1944) in a series of experiments lasting from 1937 to 1943 on Yorke Penninsula, South Australia and near Koonamore Station, in the north-east of South Australia. The second experiment release was made by the Wildlife Survey Section of C.S.I.R.O., during May to December, 1950, near Echuca and Albury, on the River Murray (Ratcliffe et al., 1952). The same strain of virus (Moses strain) was used for the two releases (Fenner, et al., 1952).

The results of the first release and of the earlier experiments by the Wildlife Survey Section indicated that although myxomatosis dispersed throughout a warren, it did not disperse from one warren to the next. However, in early December 1950, myxomatosis flared up at a test-site near Albury, and by the end of December it was dispersing throughout the Murray-Darling river system, apparently confined to the watercourses.

The distribution of myxomatosis during 1950-51 and subsequent summers, is shown in figure 4.05, reproduced from Fenner (1959a).

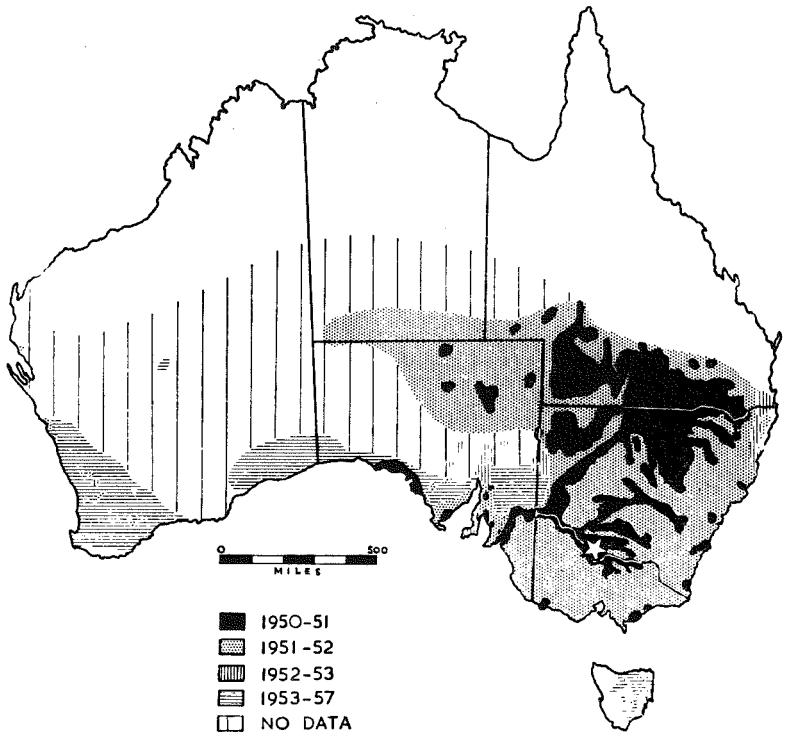


Figure 4.05: The spread of myxomatosis after 1950. The white star is near Echuca. (Reproduced from Fenner, 1959a).

Two hypotheses were proposed to account for the rapid dispersal of myxomatosis over such a vast area. Ratcliffe et al. (1952) considered that myxomatosis probably originated from the test-site near Albury and was dispersed by wind-borne mosquitoes. Brereton (1953) proposed that myxomatosis dispersed from the first release near Echuca, in May 1950, and was spread by the movements of sick rabbits along the watercourses. These two hypotheses are not mutually exclusive; both ways of dispersal almost

certainly occurred, although perhaps not to the extent envisaged in either hypothesis.

I think a third hypothesis is worth considering. Myxomatosis may have persisted in the north of South Australia from the release, in 1943, by Bull and Mules, and started to disperse during the exceptionally wet years of 1949 and 1950.

The 3 hypotheses are discussed in turn below.

4.41 Dispersal by wind-borne vectors.

Ratcliffe et al. (1952) emphasised the vast and rapid increase that took place in the distribution of myxomatosis. In November 1950, they knew of the disease only in the area between Albury and Echuca, where they had been releasing it since the previous May. By December, it was reported from the Darling River, nearly 400 miles away, and by January its distribution had extended to the Far North and West Coast of South Australia (Lines, 1952; and figure 4.05).

Mosquitoes were extremely abundant at this time following the record-breaking floods in Northern New South Wales. For example, Myers (personal communication) counted up to 50 An. annulipes biting a sick rabbit per 10 minutes. Ratcliffe et al. (1952) considered that the unusually great abundance of vectors at this time was sufficient to explain the rapid dispersal of myxomatosis throughout eastern Australia from a single focus near Albury. This explanation was also supported by the discovery of myxomatosis on islands off the coast of Victoria (Myers, K; personal communication). Similarly Ratcliffe (1959) stated that myxomatosis appeared on an island off the coast of Queensland, 200 miles from the nearest population of rabbits.

This hypothesis implies that large numbers of infected mosquitoes

were dispersed by winds, or other agencies over distances of up to 1,000 miles during a period of not more than 5 months.

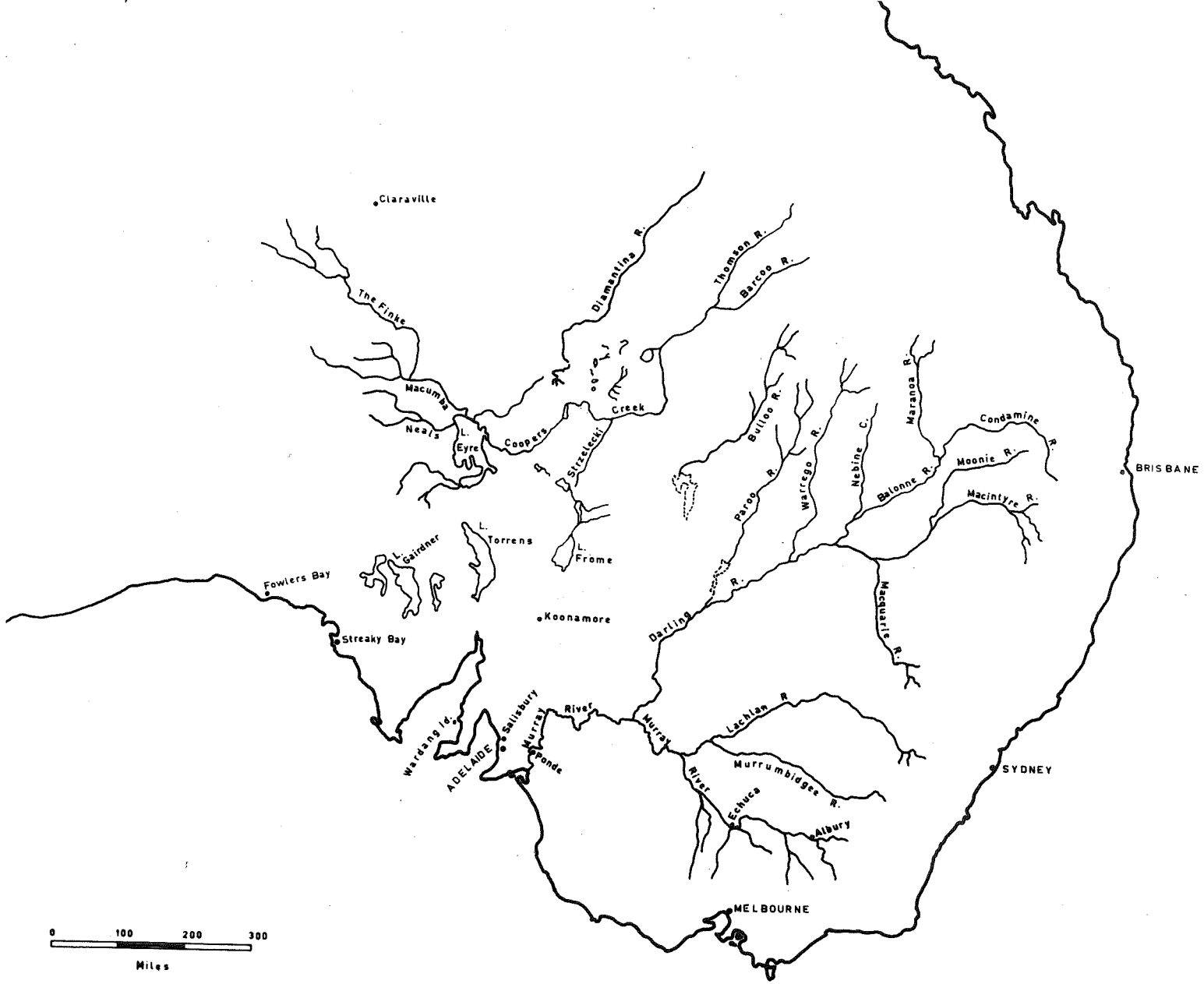
4.42 Dispersal by sick rabbits.

Brereton's hypothesis is based on evidence he collected on the dispersal of myxomatosis up 5 Northern rivers, the Bulloo, Paroo, Warrego, Maranoa and Macquarie (figure 4.06), where he found that myxomatosis moved steadily up the rivers at a rate of about 3 miles per day. He then "tested" his hypothesis by measuring the distance down the Darling and up the Murray to the place where myxomatosis originated. Provided he assumed that it originated from the first release at Echuca, in May 1950, the result of about 3 miles per day supported his hypothesis.

Brereton argued that the outbreak at Claraville, Northern Territory, in August 1951, also supports his hypothesis. He argued that myxomatosis "crept" from the Bulloo marshes on 15 February, 1951 to Coopers Creek and then along watercourses (presumably up the Macumba and Finke rivers) into the Northern Territory.

But, myxomatosis was reported from the Macumba by 17 January, 1951 (Lines, 1952). Similarly, other records for South Australia are not consistent with Brereton's hypothesis. For one thing, there are no major watercourses, although after heavy rains such as fell in 1949 and 1950 much of the Northern area consists of flood plains with chains of billabongs and small watercourses (Andrewartha, 1940). On Brereton's hypothesis, the outbreaks at Streaky Bay, on the West Coast of South Australia, presumably originated from the Murray. The shortest distance around the top of Spencers Gulf between the Murray and Streaky Bay is about 450 miles. Myxomatosis was first reported on the Murray in the second week in January and at Streaky Bay by early March, about 45 days later (Lines, 1952).

Figure 4.06: A map of the watercourses of south-eastern Australia. The map also shows places discussed in the text in relation to the spread of myxomatosis.


Claraville
The Finke
Macumba
Neals
L. Eyre
Coopers Creek
Diamantina R.
Thomson R.
Barcoo R.
Strzelecki
Bulloo R.
Paroo R.
Warrego R.
Nebine C.
Maranoa R.
Balonne R.
Condamine R.
Moonie R.
Macintyre R.
BRISBANE
L. Gairdner
L. Torrens
L. Frome
Fowlers Bay
Streaky Bay
Koonamore
Darling R.
Macquarie R.
Wardang Id.
ADELAIDE
Salisbury
Murray River
Murray Ponde
Lachlan R.
Murrumbidgee R.
River Echuca
Albury
SYDNEY
MELBOURNE
0 100 200 300
Miles

This gives a rate of 10 miles per day; clearly different from the rate postulated by Brereton.

Thus, Brereton's hypothesis must be rejected because it accounts for only part of the observed facts. Furthermore, it seems unlikely that sick rabbits would maintain an <u>average</u> dispersal as great as 3 miles per day over large distances, both in summer and winter.

4.43 <u>Persistence of myxomatosis in South Australia from the the release by Bull and Mules</u>.

Several experiments were done on rabbits living in enclosures on Wardang Island, and nearby on Yorke Penninsula but the more interesting experiments were done near Koonamore, in the North East of South Australia, where the virus was introduced into natural populations of rabbits.

Most of the virus was disseminated by rabbit traps modified to inject virus into the rabbit without otherwise injuring it. The virus was released in 7 areas, and in 6 of these a total of 2,164 traps were sprung. Experiments in enclosures showed that about 75% of traps that were sprung infected rabbits, and hence about 1,623 rabbits were infected by traps. In the seventh area, 103 rabbits were caught and innoculated, making an estimated total of 1,730 rabbits innoculated between 29 November, 1942 and 12 August, 1943. Definite outbreaks were observed in 3 of the 7 areas. In the other 4 areas, sick rabbits were seen, but the density of the rabbit population apparently did not decrease. The last release was made between June and August, 1943. No sick rabbits were seen after 16 August and observations were discontinued on 3 October, 1943.

Experience gained since 1950 has shown that 1 characteristic of myxomatosis is its ability to persist between outbreaks, often at levels

too low to be readily observed. Thus, the sample of rabbits I collected at Coopers Creek in September 1958 (section 4.3) indicated considerable recent transmission although people living in the area reported only mild outbreaks during the past few years.

Even during the winter of 1951, when the virus was still highly virulent, myxomatosis apparently persisted throughout most of its distribution (Ratcliffe et al., 1952). Admittedly this persistence occurred after the virus had dispersed over a vast area, but even so, it is possible that myxomatosis persisted from the experiments of Bull and Mules. That it was not noticed between 1943 and 1950 is understandable, the population of the Far North is sparse and few people knew of the disease.

In 1949, and especially in 1950, exceptional flooding occurred in the Far North, and Coopers Creek flowed into Lake Eyre in June 1949, for the first time in 30 years. During 1950, all the Northern rivers were flooding and Lake Eyre reached its maximum depth in September 1950 (Peake-Jones, 1955). The flooding probably resulted in an unusually high density of mosquitoes, as was observed in Eastern Australia. Hence, if myxomatosis persited for the 7 years following 1943, it probably began to disperse in 1950.

4.44 Conclusions.

There is ample evidence to reject Brereton's hypothesis, but the other 2 hypotheses are speculative. The hypothesis of Ratcliffe et al., (1952) is probably simpler, but is postulates dispersal of mosquitoes on an incredible scale.

My hypothesis is based on 2 assumptions, firstly that myxomatosis released by Bull and Mules persisted, and secondly that the scarcity of

mobile vectors, due to adverse weather, prevented the disease from dispersing widely. The second assumption is probably reasonable, but the first is doubtful. It could equally well be argued that in the absence of mobile vectors the highly virulent virus killed itself along with the rabbits it infected.

It is unlikely that myxomatosis dispersed unobserved through the rabbit population after 1943 and suddenly flared up in 1950. In Eastern Australia, serological data indicated that the rabbits had never before been exposed to myxomatosis (Marshall, I.D., personal communication). Unfortunately, no similar information exists for South Australia and the hypothesis that myxomatosis persisted from the experiments of Bull and Mules cannot be lightly dismissed.

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