The physiology and ecology of the immature stages of the salt marsh mosquito Aedes vigilax Skus (Diptera: Culicidae)

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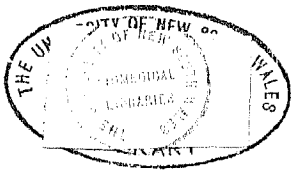
The Physiology and Ecology of the Immature Stages of the Salt Marsh Mosquito *Aedes vigilax* Skuse (Diptera : Culicidae)

Thesis submitted to the
University of New South Wales
for the
Degree of Master of Science
by

Judith Louise Reynolds



School of Biological Sciences.

December 1961.

DECLARATION

The candidate hereby declares that the work reported in this thesis has not previously been submitted to any other university or institute for the award of a higher degree.

Judith Louise Reynolds

December 29th, 1961.

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INTRODUCTION

1.1 General Considerations on Mosquito Ecology

In any species of mosquito, the oviposition site chosen by the gravid female is determined by its behaviour to certain environmental stimuli (O'Gower, 1955, 1957a, 1957b, 1958b; Woodhill, 1941b, and others). However, when environmental conditions are suitable and the eggs laid in a particular site hatch, the resulting aquatic immature stages are obliged to occupy a certain ecological habitat. Their survival then depends upon their physiological and behavioural adaptations to the environmental factors associated with that habitat. Thus, before the control of any species of mosquito can be planned, knowledge of the ecology of the immature stages is essential.

1.2 Ecology of Aedes vigilax

Because the salt marsh mosquito Aedes (Ochlerotatus) vigilax Skuse is a vicious biter and a pest of man, cattle and horses, and because it is medically important as a potential vector of Murray Valley Encephalitis (Lee, Clinton and O'Gower, 1954), Myxomatosis (Lee, Dyce and O'Gower, 1957), and Dengue fever (O'Gower, 1960), an investigation of its ecology was undertaken. A knowledge of the ecology of this species would be essential in determining a means of its economic control.

The method of approach to this investigation was to study the environment factors associated with a typical breeding place of A.vigilax, and to determine which of these factors limited the survival of the immature stages. Certain of these limiting factors were then selected, and the physiological and behavioural responses of the immature stages to them were studied in the laboratory, to correlate these responses with survival.

1.21 Ecology in the Field

The widespread distribution of immature stages of A.vigilax in coastal salt marshes, brackish water, and occasionally in clear and stagnant fresh water throughout regions of Australia, New Guinea, New Caledonia, Fiji, East Oriental Region and Dutch East India has been reported by many authors (Brug, 1924; Cooling, 1924a, 1924b; Edwards, 1924; Hamlyn-Harris, 1927, 1933; Hill, 1917; Knight, Bohart and Bohart, 1944; Lee, 1944, 1951; Mackerras, 1926; O'Gower, 1958a; Paine, 1943; Taylor, 1914; Woodhill, 1936; and Woodhill and Pasfield, 1941). In freshwater habitats larvae of Culex annulirostris (Skuse), and Anopheles annulipes (Walk) have been found in association with A.vigilax (Hamlyn-Harris, 1933), whilst in saline habitats the associated species are Aedes (Finlaya) alboannulatus (Macq.), and the predaceous Aedes (Mucidus) alternans (Westw.), (Cooling, 1924; Hamlyn-Harris, 1927, 1933; and Woodhill, 1936).

Although literature on species of mosquitoes breeding in saline waters is voluminous, (Aarons, 1954; Balfour, 1921; Barroud, 1931; Bates, 1949; Bick, 1951; Bidlingmayer and Schoof, 1956; Bonnett and Chapman, 1958; Bruce-Chwatt and Fitz-John, 1951; Brug, 1924; Clerc, 1909; Connell, 1940 Cooling, 1924; Fellton, 1944; Foley and Yvernault, 1908; Frohne, 1953; Gholap, 1910; Gjullen, Yates and Stage, 1950; Horsfall and Morris, 1952; Komp, 1955; Lee, 1944; Legendre, 1934; Marshall, 1938; Mathis, 1934; Muirhead-Thomson, 1951; Muspratt, 1956; Rageau and Vervent, 1959; Russell and Rao, 1940, de Vogel, 1907; Woodhill, 1936; and Worth, de Sousa and Weinbren, 1961), there have been only limited studies on the ranges of salinity encountered in these habitats (Aedes australis (Erichson) (= concolor Taylor), in rock pools from rainwater to 24% salinity, (O'Gower, 1960);

Anopheles sundaicus (Rodenwaldt) in water of NaCl content (1005-1713 p.p.m), (Sen, 1958;) Anopheles gambiae (Giles) in water of 2.5-170 Cl/100,000 (or 0.13% - 8.95% Cl of seawater), (Smith and Vail, 1959;) Anopheles vagus (Doenitz) in coastal pools of 2.5% - 3.0% NaCl, (de Vogel, 1907), and still fewer investigations have been made of the other environmental factors, or of the effects of these factors on the breeding of the associated species of mosquitoes.

In these investigations, the greatest emphasis has been made on the determination of oviposition sites in salt marshes. These sites have been determined from soil samples from which eggs were either mechanically separated (Horsfall, 1956a), or hatched by flooding the samples, and the resulting first instar larvae scored (Elmore and Fay, 1958). Bradley and Travis (1942, 1943), by transect soil sampling across a marsh, followed by flooding of the samples showed that the distribution of the eggs of Aedes sollicitans (Walk.) and Aedes taeniorhynchus (Wied.) were correlated with marsh elevation, whilst in addition, Elmore and Fay (1958) determined that for these species, temperatures before, and at the time of flooding of the samples, and season of the year, also influenced estimates of the production potential of the areas studied. This would seem to indicate that a diapause mechanism operated. Bodman and Gannon (1950) found that eggs of Aedes vexans (Meig) were deposited in areas subjected to inundation, but exact locations were modified by other environmental factors, for example, heavier soils were more suitable that light soils; eggs were only deposited in places with some shade to retain moisture, and where the margin of transient pools were steep, the egg layer was narrower. In addition to confirming these authors' results, Horsfall (1961) observed that eggs of

A. vexans were laid on the soil proper, under the detritus layer, and that eggs of this and other species of floodwater mosquitoes were deposited in the areas mentioned above, usually at the time when the water table was falling.

The distributions and variations of larvae in salt marshes with respect to environmental factors selected singly have been determined by several authors. Renn (1943) showed that there was a positive correlation between population densities of larvae of Anopheles quadrimaculatus (Say), and the index of "intersection line" (that is, the line of intersection between the three interfaces, viz., water-air, air-plant, and plant-water), and thus confirmed results obtained by Hess and Hall (1943), with other Anopheles spp. Senior-White (1926) found mosquito larvae in water of pH 5.8 - 8.6, Barbour (1928) determined that pH did not have any effect on the development of larvae of Culex quinquefasciatus (Say), and the results of MacGregor (1929) confirmed that pH did not directly influence mosquito larvae, but that it indicated the favourable or unfavourable associations of chemical and biological factors in the breeding places, upon which the successful or unsuccessful development of larvae depended. Later observations by Fellton (1944) on the toleration of a pH of 2.7 by A. sollicitans were in agreement with those observations by the preceding authors of pH tolerance limits of the other mosquito species. Temperature limits associated with the habitats of certain species have also been studied by Horsfall and Morris (1952), and others.

However, there are few detailed studies of climatic factors associated with mosquito breeding in salt marshes. Connell (1940) determined the effects of tides, rainfall, water salinity, water table, soil moisture, soil pH, soil temperature, and predators on larval densities of Aedes cantator

(Coq.), A.sollicitans, A.taeniorhynchus, Anopheles cruciens (Wied.), and Culex salinarius (Coq.); Ribbands (1944b), studied the effects of tide action and rainfall on populations of An.gambiae and An.gambiae var.melas, and was able to correlate the emergence and prevalence of adults with the time elapsed since spring tides which flooded the breeding area; and Marchal (1959) similarly investigated the effects of monthly high tide heights, together with salinity and rainfall on those two populations, (An.gambiae and An.gambiae var. melas), and found that immature stages of each were associated with different salinity ranges.

In the investigation of the ecology of the immature stages of A.vigilax, the topography of the breeding area was surveyed, and seasonal variations in the environmental factors of rainfall, height of tides, depth of water table, depth of pool, water temperature, water salinity, and water pH were studied. The technique of transect soil sampling across pools, followed by flooding and scoring of the resulting first instar larvae, was employed in an attempt to determine site of oviposition, whilst the seasonal abundance and distribution of the immature stages were determined by scoring numbers dipped from quadrats selected at random in a selected pool.

Associations between environmental factors, age distribution, dispersal and population densities of immature stages were determined by statistical analyses.

1.22 Laboratory Experiments on Selected Aspects of Ecology

The factors of salinity, temperature, light, mechanical shock and gravity, which from field investigations were thought to influence survival of immature stages of A.vigilax, were selected for study in the

laboratory. The physiological survival and behavioural responses to these factors of A.vigilax, A.australis, Aedes aegypti (L) and Culex fatigans (Wied.) (the latter three species were chosen as being representative of a range of habitats), were investigated in an attempt to correlate survival and response with ecological distribution.

The immature stages of A.australis inhabit marine rock pools lacking emergent or overhanging vegetation, in the supra-littoral zone ($>$ 6 ft. above mean tide level).These pools are continuously exposed to salt spray, and are flushed by rainwater and by sea water during heavy seas. Heat from the sun and air movement cause evaporation of water in the pools. The degree of exposure of each pool to sunlight, salt spray and wave action may vary considerably. The larvae have been observed to tolerate large hourly (9°F), daily (27°F), and seasonal (54°F) changes in temperature, and a maximum salinity of approximately 24% (that of sea water being 3.5%), with a daily increase of up to 5.2%(O'Gower, 1960). The interaction of the abovementioned physical conditions may affect the survival of the immature stages in rock pools.

In areas with severe winters, the immature stages of A.aegypti will only be found during the winter in collections of water in protected situations, such as water stored underground in tanks and wells and fire buckets in warehouses. But they also occur during milder weather conditions in unprotected bodies of water, e.g. tins and bottles. In areas which have mild winters and in which the annual rainfall is limited to one definite period of the year, e.g. Northern Australia in the dry season, the immature stages of this species are only to be found in large bodies of water, such as rainwater storage tanks attached to habitations, but in the wet season they may also occur in small containers,

e.g. tins, bottles and jars (O'Gower, 1956). Heat from the sun, and air movement may cause evaporation of the water in the small containers, whilst heavy rains may cause overflow. These physical conditions may affect the survival of the immature stages in the small containers, but are less likely to affect those in large tanks.

The immature stages of C.fatigans inhabit open water which is filled with organic matter. The water is dark in colour, is usually deep, and has large diurnal and seasonal fluctuations in temperature (Woodhill and Pasfield, 1941). The depth of the water would tend to minimise temperature fluctuations, and under extreme conditions of evaporation, the organic matter, by retaining moisture for a limited period of time, would maintain a moist habitat for the immature stages until the water was replenished by rainfall. Thus temperature and evaporation would not greatly affect the survival of the immature stages of this species.

Temperature has been shown to influence the rates of development of many species of mosquitoes, notably A.aegypti, (Bar-Zeev, 1958); An.quadrimaculatus, (Hurlbut, 1943); Anopheles stephensi (Liston), and Anopheles subpictus (Grassi), (Lal, 1953); and experiments have proved that in A.aegypti and Culex pipiens (L.), (Headlee, 1940, 1941, 1942); and An.quadrimaculatus (Huffaker, 1944), the metabolic rate is usually greater with a variable temperature than in a constant temperature. In addition, the lethal effects of high and low temperatures have been studied in a number of species: (Bar-Zeev, 1957; Christophers, 1960; Farid, 1949; Lal, 1953; Macfie, 1920; Mellanby, 1960; Muirhead-Thomson, 1940; Pal, 1945; Rees Wright, 1927; Woodhill, 1948).

Studies have also been made on the survival of various species

of mosquitoes which breed in saline habitats, to salinities higher than normally encountered (Bates, 1939b; Beadle, 1939; Muirhead-Thomson,1951; Ribbands, 1944a; Roubaud, Colas-Belcour and Treillard, 1935; Smith amd Vail, 1959, Woodhill, 1941a, 1942). The discovery of morphological and physiological adaptations of certain species (Beadle, 1939, 1957; Dixon,1957 Edney, 1957; Gibbons, 1932; Kettle, 1948; Koch, 1938; Pagast, 1936; Ramsay, 1950, 1951, 1953; Treherne, 1954b; Wigglesworth, 1933a, 1933b, 1938, 1950; Woodhill, 1938; and others) to environmental salinities have added greatly to our knowledge of osmoregulation in mosquitoes, however, few, if any detailed investigations have been made of the influence of previous acclimatization salinities and temperatures on the tolerance of these immature stages to higher salinities.

The survival times of the immature stages of the four species to salinity, and the influence of temperature and acclimatization salinity on these survival times were therefore studied to see whether they could be correlated with the ecological habitats of the four species.

Previous studies of the behavioural responses of the immature stages of mosquitoes to light have been qualitative, and emphasis has been placed on determining phototropic responses: (Folger, 1946; Hocking, 1953; Mellanby, 1958; Muirhead-Thomson, 1940; Omardeen, 1957; Thomas, 1950). Responses to mechanical shock and gravity have similarly been determined on a qualitative basis: (Folger, 1946; Mellanby, 1958; Miller, 1940; and Thomas, 1950).

In the present investigation, in an attempt to correlate behavioural responses of the immature stages of the four species with survival in their ecological habitats, analyses were made of their behavioural responses to the environmental factors of light, gravity and

mechanical shock. As all four species were negatively phototropic, this response was used to quantitatively compare difference in behaviour between the species.

A survey of the literature reveals that in some species of mosquitoes the environmental factors associated with the habitats of the immature stages have been determined by field investigations, whilst with other species, studies have been made in the laboratory of their physiological and behavioural responses to certain of these environmental factors.

In this investigation, the environmental factors associated with the ecological habitat of the immature stages of A.vigilax were studied, and those that were thought to limit survival were selected for study in the laboratory. There the physiological survival and behavioural responses of the immature stages of these factors were determined, and these responses were correlated with survival. Such an approach in the investigation of mosquito ecology has not been recorded in literature.

2. FIELD EXPERIMENTS

2.1 Methods

Tidal salt marshes in the Kurnell Area of Botany Bay in N.S.W. forn an extensive breeding area for A.vigilax. Therefore certain environmental factors were studied to try to correlate breeding of A.vigilax with variations in these environmental factors. The topography of the area was studied, and measurements were made of the climatic factors (rainfall, tide, water table and depth of water) which in these marshes determine the incidence of volume of water necessary for the hatching of the eggs of this species. The other climatic factors of salinity, temperature, and pH, which influence the survival of the resulting aquatic immature stages, were studied in four selected pools in this area. The associations between the climatic factors and the influence of these factors on the occurrence of and distribution of the eggs, larvae and pupae in these pools, was determined.

2.11 Topography

The position of the salt marshes with respect to Woolooware and Weeney Bays within Botany Bay, and the position of the selected pools A, B, C and D, were mapped.

2.12 Climate

2.121 Rainfall

Rainfall figures for the Kurnell Area from December 1960 to November 1961 were obtained from the Bureau of Meteorology, Sydney. From these figures, the lowest rainfall necessary to flood the selected pools was calculated.

2.122 Tides

Records of the heights of daily high tides measured at Port

Jackson from December 1960 to November 1961 were obtained from the Maritime Services Board, Sydney. From these records, the heights of the lowest high tides that flooded the area of salt marsh adjacent to the mangroves bordering Weeney Bay, and the area containing the selected pools, were estimated.

2.123 Water Table

Holes $\frac{1}{4}$" diameter were drilled at 6" intervals along the length of two water pipes, 6 ft. long with interval diameter of 1 inch. These pipes were sunk in to the ground in area I (beside pools A, B, C and D) and area II, raised immediately, emptied of mud, and then sunk again to a depth of six feet. (Plates I and II). Water could enter these pipes, through the drilled holes and the open ends, to the height of the water table in the surrounding substratum. This height of water was measured in inches with a calibrated wooden rod. (Plate 10). Seasonal variations in the water table of each area were determined by bi-weekly observations at 8.30 a.m. from December 1960 to November 1961, and diurnal variations were determined during October 1961.

2.124 Depth of Water in Pool C

The seasonal variations in the depth of water in pool C were determined by bi-weekly measurements at 8.30 a.m. from December 1960 to November 1961.

2.125 Salinity

Samples of water from the four selected pools were transported to the laboratory in air-tight jars, and titrated against $\frac{N}{10}$ silver nitrate, using 1% potassium dichromate as an indicator. From the mean of four titrations, the % salinity of each sample was calculated. Diurnal variations in salinity in the pools were studied by monthly samples from June 1959 to June 1960. The seasonal variations in the salinity of these

pools were investigated by bi-weekly samples at 8.30 am. from December 1960 to November 1961. At the time of sampling, the incidence of immature stages of A.vigilax in the four pools was also noted.

2.126 Temperature

The diurnal and seasonal variations in air temperature (four feet above ground level) and water temperature (one inch below the surface) in pools A, B, C and D from June 1959 to June 1960 were measured using a shielded thermometer. The seasonal variations in the water temperature in pool C were determined by bi-weekly measurements at 8.30 a.m.

2.127 pH

The diurnal variations in pH of the water in the four pools were determined with find range B.D.H. indicator papers by monthly measurement from June 1959 to June 1960. At the time of measurement, the incidence of immature stages of A.vigilax in the four pools was also noted.

2.13 Population Sampling

2.131 Egg Sampling

To try to determine the oviposition sites of A.vigilax in the field, samples of soil 6" square x 2" deep were taken on the same day from a strip transect across pool B (Plate 9), and, over a period of months, from several positions in pool D (Plate 6). These samples were transported to the laboratory, allowed to dry out for various periods of time, and then flooded with water from their pools (Plates 12 and 13). The samples were examined every 24 hours for one week, any first instar larvae that hatched were noted, then the soil samples were allowed to dry out again. After varying periods of time, the samples were reflooded, and any further hatching of larvae were noted.

2.132 Sampling for Immature Stages

Pool C was divided into 120 quadrats of one square foot by means of a foot wide transect ladder (Plate 7). The quadrats were allotted consecutive numbers. 20 of these quadrats were selected at random using a table of random numerals (Fisher and Yates, 1948) and the north-east corner of each sample quadrat was marked with a wooden dowel for future reference. (Plate 8). Seasonal variations in the incidence, density, and distribution of the immature stages of A.vigilax in the pool were investigated by means of the following sampling technique. Twice weekly, from December 1960 to November 1961, at 8.30 a.m. each selected quadrat that was flooded was delimited by a square foot sampler. Dips of approximately 500 ml. were taken from the centre and four corners of the quadrat with a plastic container, and the numbers of each larvae instar and pupae present were recorded. From these records, the seasonal variations in the relative density of the immature stages were calculated.

Analyses were done by means of the χ^2 test for goodness of fit to the Poisson distribution to determine whether, at any time of the year, the immature stages in the pool were randomly distributed with respect to presence or absence of emergent vegetation (e.g. Salicornia), and depth of water.

2.14 Associations between Population Densities of Immature Stages of A.vigilax and Climatic Factors

In the analyses of the recorded field data χ^2 contingency tables were used to determine the associations between pairs of environmental factors (salinity, water table, depth of pool C, rainfall, tide) and associations between population density of immature stages of A.vigilax and selected environmental factors.

2.2 RESULTS

2.21 Topography

The position of the salt marshes with respect to Woolooware and Weeney Bays may be seen in Plate 1 and Figure 1. The pools studied were located on the landward side of the mangroves (Avicennia officinalis Linn.), in depressions of mud surrounded by dense stands of the succulent-stemmed herb Salicornia australis Sol., amongst which were scattered clumps of Triglochin striata Ruiz and Pav., and Suaeda maritima Dumort. (Plates 1, 2, 3, 4, 5, and 11). Landward from the saltmarsh, beyond the influence of tides, and also in slightly elevated localised areas within the saltmarsh, grasses (Sporobolus virginicus Humb. & Kunth), rushes (Juncus maritimus Lamb.), and sedges (Cladium junceum R.Br.) were dominant. (Plates 1, 4 and 5).

2.22 Climate

2.221 Rainfall

Figures 2 and 3 show the rainfall figures recorded for the Kurnell Area January and February 1961 (summer), and June and July 1961 (winter). Calculations of the rainfall between successive observations December 1960 - November 1961 may be seen in Table 1. The lowest rainfall recorded which flooded the selected pools was 31 points February 11th, 1961, when the water table in that area was 19.25" below soil level.

2.222 Tides

Figures 2 and 3 show daily variations in the heights of the high tides recorded at Port Jackson January and February 1961 (summer), and June and July 1961 (winter). For the heights of the highest high tides recorded between field observations December 1960 - November 1961, see Table 1. The lowest high tide recorded which flooded the area of salt marsh adjacent to the mangroves bordering Weeney Bay was 5'4" (31st January, 1961). The lowest high tide recorded that flooded the area of salt marsh containing the

Figure 1. Map showing relation of study area to Woolooware and Weeney Bays.

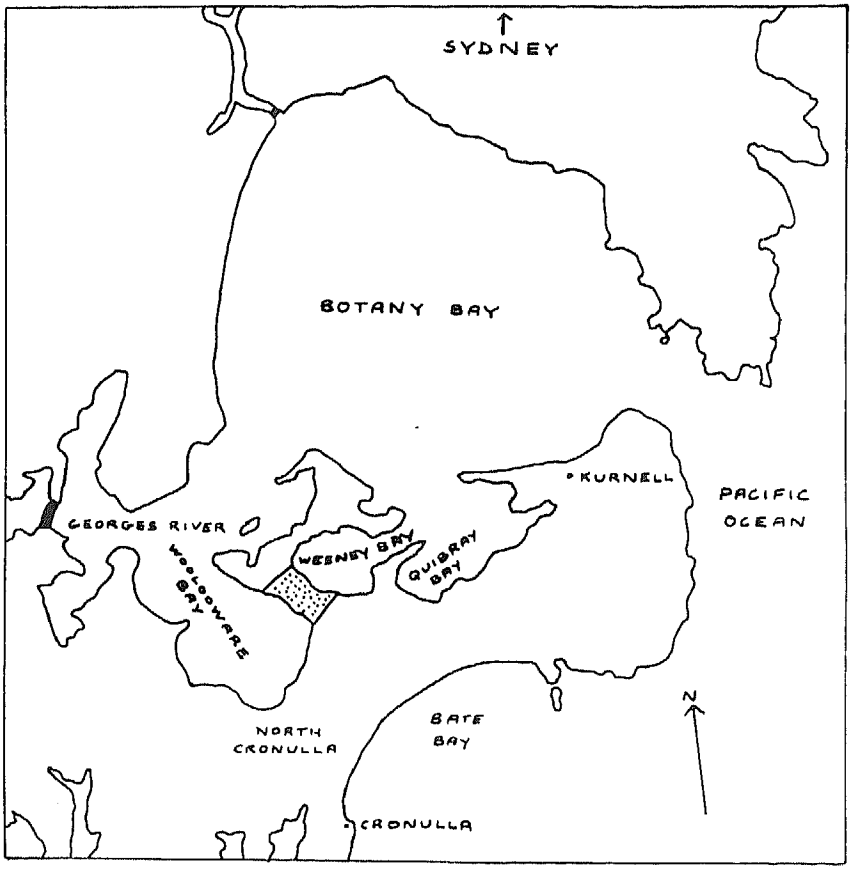
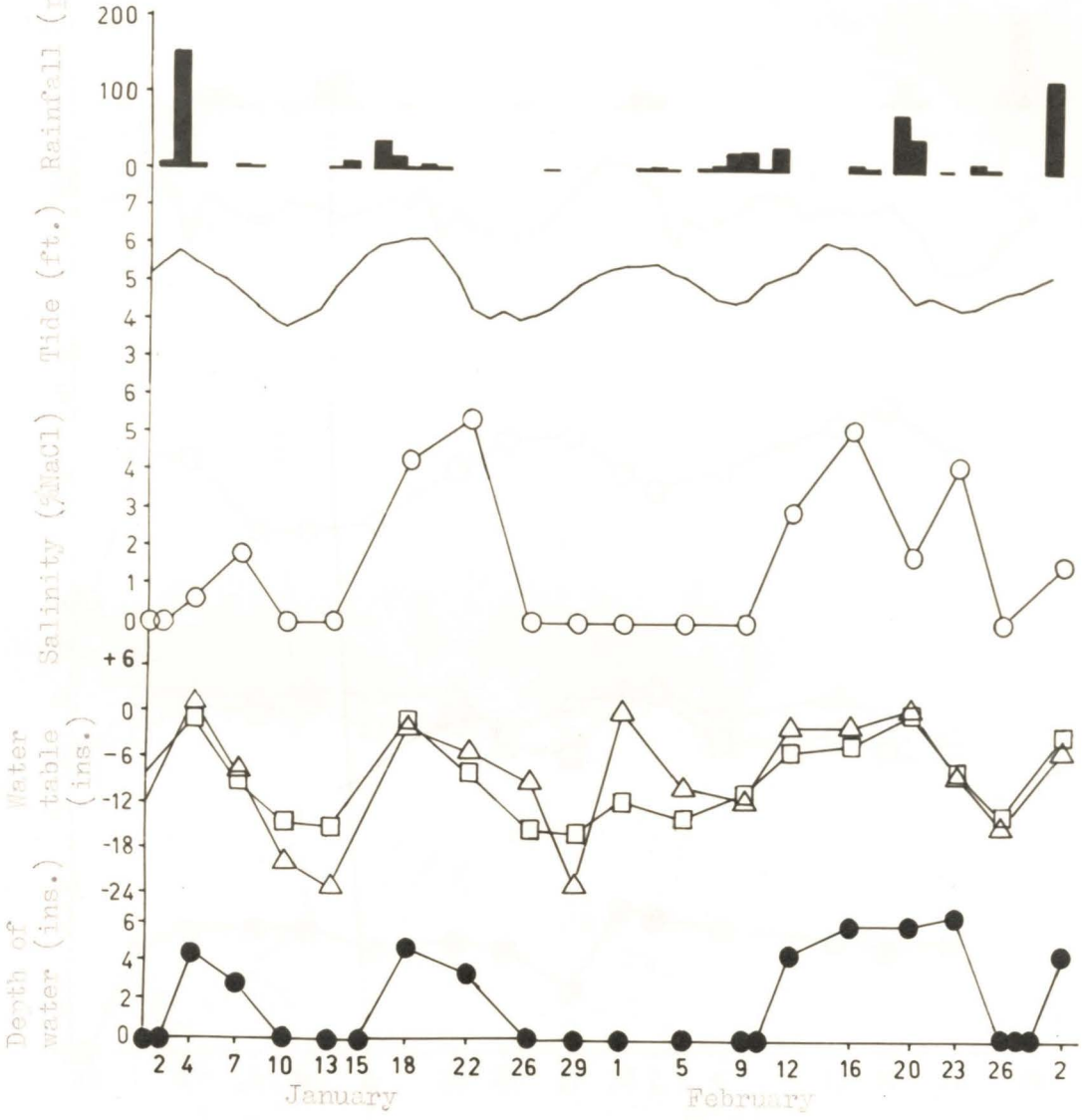


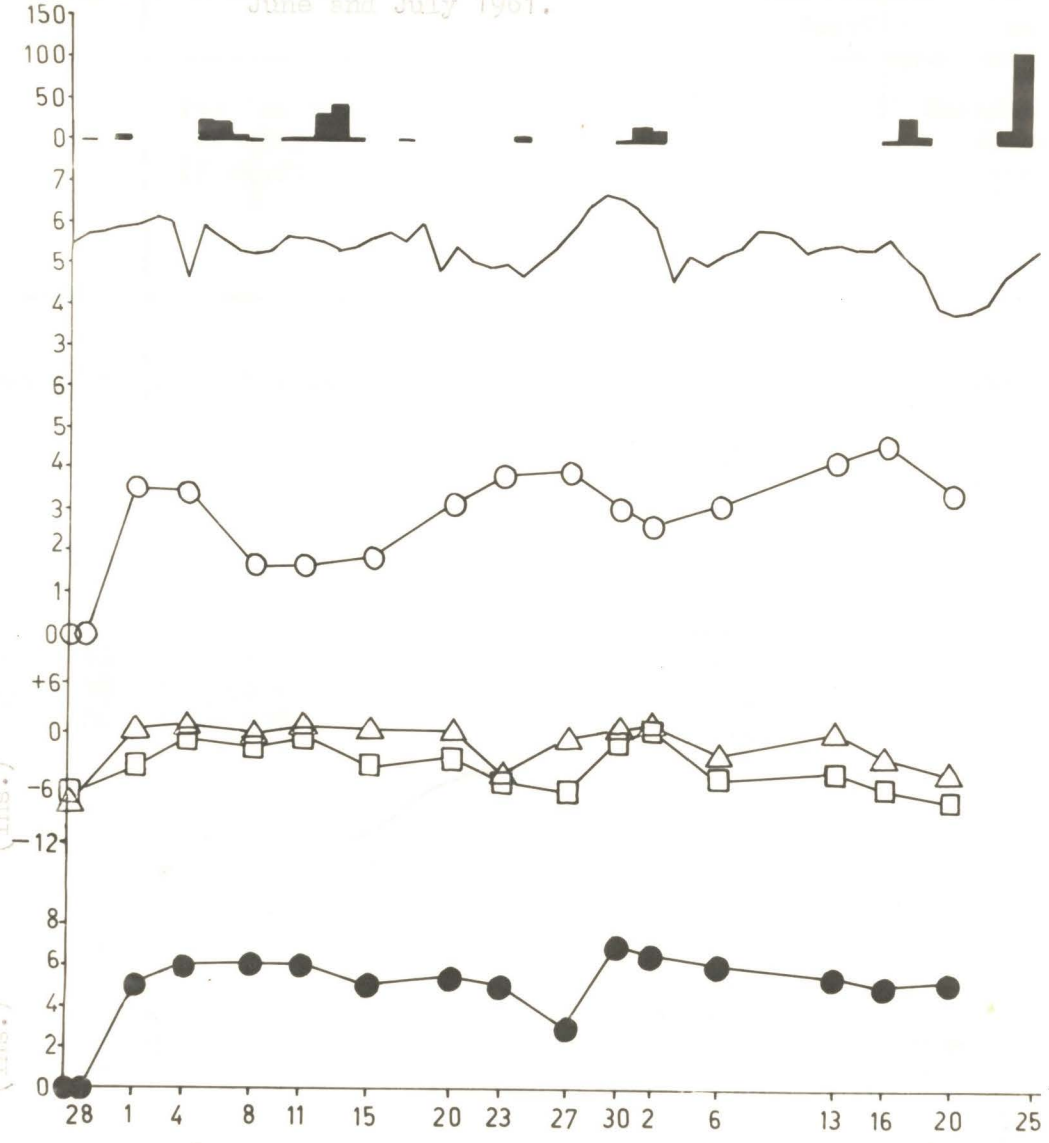
Figure 2. Variation in Salinity and Depth of Water in Pool C, Water Table in Pool and Mangrove Areas at 8.30 a.m., Height of Daily High Tides, and Rainfall Figures for January and February, 1961.



□ Water Table - Pool Area I

△ Water Table - Mangrove Area II

Figure 3. Variation in Salinity and Depth of Water in Pool C, Water Table in Pool and Mangrove Areas at 8.30 a.m., Heights of Daily High Tides, and Rainfall Figures for June and July 1961.



□ Water table - Pool area I

△ Water table - Mangrove area II

TABLE 1

Seasonal Variation in Population Density, Water Temperature, Salinity and Depth of Water in Pool C, and Water Table in the Pool and Mangrove Areas at 8.30 a.m., with Rainfall, Height of Highest High Tide and Maximum Air Temperature Occurring between Observations, and Maximum Air Temperature Immediately Prior to Observations.

Date	At Time of Observation						Between Observations			Prior to Observations
	Population Density (/ cu.ft.)	Water Temperature (°F)	Salinity (% NaCl)	Depth of Water (Inches)	Water Table (0.0 inches at ground level)		Total Rainfall (Points)	Height of Highest High Tide (Ft.-ins.)	Maximum Air Temperature (°F)	Maximum Air Temperature (°F)
					Pool Area	Mangrove Area				
1960										
December 5	Not determined		0.406	6.0	0.0	+0.5	195	6-0	92.4	70.6
9	Not determined		1.392		-7.4	-8.1	96	5-8	67.9	67.9
12	-	-	-	-	-14.5	-21.5	-	4-2	73.4	73.4
15	Not determined		1.537		-5.7	-9.5	43	4-11.5	80.3	80.3
20	3.4		0.667	6.0	0.0	+7.5	564	6-7	72.1	65.0
24	Not determined		2.262	5.0	-3.0	-1.75	41	6-4.5	76.8	76.0
27	Not determined		3.480	1.5	-10.0	-13	-	5-2	86.1	86.1
30	-	-	-	-	-12.5	-21	-	4-9.5	89.5	78.7
1961										
January 4	91.1		0.580	4.5	-0.5	+1.5	165	5-10	80.6	70.7
7	272.0	86.0 (9.30 a.m.)	1.769	3.0	-9.0	-7.5	-	5-3	77.1	75.0
10	-	-	-	-	-14.5	-19.5	9	4-9	77.0	76.8
13	-	-	-	-	-15.0	-23.0	-	4-10.5	82.0	78.8
18	263.3	69.0 (9 a.m.)	4.292	4.75	-1.0	-1.5	58	6-1.5	82.9	73.4
22	114.0	80.0 (9 a.m.)	5.394	3.50	-8.0	-5.0	9	6-2.5	73.6	73.4
26	-	-	-	-	-16.25	-9.0	-	4-4.5	77.6	77.6
29	-	-	-	-	-16.0	-23.0	1	5-0	88.8	88.8
February 1	-	-	-	-	-11.5	+0.5	-	5-4.5	106.9	82.7
5	-	-	-	-	-13.75	-9.75	10	5-6	79.8	77.1
9	-	-	-	-	-10.25	-11.25	33	5-2	87.4	87.4
12	Not determined	76.5	2.987	4.5	-5.0	-0.5	56	5-4.5	73.2	73.2
16	80.8	74.0	5.249	6.0	-4.0	-0.5	-	6-2	78.7	78.7
20	Not determined	75.0	1.798	6.0	0.0	+0.75	91	6-0	78.0	73.8
23	2.0	82.0 (9.15 a.m.)	4.234	6.5	-7.5	-7.75	45	4-8.5	80.6	80.3
26	-	-	-	-	-13.25	-14.75	18	4-9.5	89.9	82.9
March 2	28.4	71.0	1.624	4.5	-2.75	-4.5	121	5-5	79.7	76.7
5	103.8	70.0	2.929	3.0	-9.0	-2.75	7	5-6.5	86.9	74.0
9	-	-	-	-	-13.75	-15.5	15	5-8	81.8	81.8
12	0.0	64.0	0.232	6.0	+1.5	+0.5	60	5-1.5	86.1	70.6
17	346.9	72.0	2.175	6.0	-5.5	-0.75	87	5-9.5	80.9	80.4
21	0.0	66.0	0.667	6.0	+1.0	-4.75	-	5-7.5	79.8	79.8
25	9.5	74.0	1.769	5.0	-6.5	-8.5	24	5-3	80.7	73.4
28	2.6	70.0	1.914	1.5	-7.25	-10.25	2	4-7.5	77.6	76.6
April 1	-	-	-	-	-10.75	-16.75	10	5-4.5	91.8	91.8
5	-	-	-	-	-15.25	-12.0	21	5-4.5	77.4	76.3
8	-	-	-	-	-12.25	-16.5	1	5-4	76.5	75.8
11	-	-	-	-	-16.25	-19.0	18	5-0	75.8	74.8
16	0.0	58.0	5.046	4.5	-3.25	+0.5	47	6-0.5	85.0	74.6
21	9.9	65.0	1.450	6.0	-1.75	+0.5	18	5-9	72.2	71.0
25	1.2	60.0	0.261	6.0	-1.75	+0.75	109	4-7	78.0	69.2
30	2.0	58.0	1.189	5.0	-3.75	-4.75	264	5-1	68.2	68.2
May 3	4.3	60.0	1.595	4.0	+1.0	-4.0	1	6-0.5	71.0	71.0
7	0.7	53.5	2.175	6.0	-0.75	+0.75	123	6-4.5	73.4	62.1
11	1.6	55.0	2.552	3.5	-2.5	+0.75	1	5-7.5	66.8	62.0
15	1.0	50.0	3.161	6.5	-2.75	+0.75	11	6-0	65.4	65.4
18	1.2	51.5	3.306	5.5	-3.75	+0.5	-	5-9.5	73.0	73.0
21	1.0	51.0	3.567	3.5	-6.75	-3.75	2	5-3.5	70.1	68.0
24	1.7	51.0	3.915	2.5	-9.25	-10.0	-	4-7.5	71.2	70.0
28	-	-	-	-	-7.75	-8.5	-	5-4.5	76.0	67.3
June 1	0.0	55.0	3.596	5.0	-4.0	+0.5	8	5-11.5	65.6	65.6
4	0.7	58.0	3.451	6.0	-0.5	+1.0	-	6-2	70.8	70.8
8	1.6	48.0	1.682	6.0	-1.75	0.0	50	5-11.5	65.2	61.6
11	1.6	58.0	1.668	6.0	-0.5	+1.0	3	5-9	66.1	63.1
15	1.2	45.5	1.856	5.0	-4.0	+0.5	82	5-8	66.7	65.3
20	0.4	40.5	3.248	5.5	-3.0	+0.5	1	6-0.5	69.1	61.1
23	0.6	41.0	3.915	5.0	-6.0	-5.0	-	5-1.5	58.9	56.6
27	1.8	44.0	4.046	3.0	-7.0	-0.5	4	5-5	66.3	66.3
30	0.1	47.5	3.147	7.0	-1.0	+0.75	-	6-9	67.1	64.0

TABLE 1 (CONT.)

Date	At Time of Observation						Between Observations			Prior to Observations
	Population Density (/ cu.ft.)	Water Temperature (°F)	Salinity (% NaCl)	Depth of Water (Inches)	Water Table (0.0 inches at ground level)		Total Rainfall (Points)	Height of Highest High Tide (Ft.-ins.)	Maximum Air Temperature (°F)	Maximum Air Temperature (°F)
					Pool Area	Mangrove Area				
1961										
July 2	0.0	53.0	2.697	6.5	+0.25	+0.75	16	6-7	62.9	60.2
6	0.2	45.0	3.190	6.0	-5.5	-2.5	13	5-11	68.4	68.4
13	0.1	43.5	4.263	5.5	-4.25	+0.5	-	5-11	65.0	65.0
16	0.1	43.5	4.713	5.0	-6.5	+0.25	-	5-7	64.2	57.6
20	0.1	51.0	3.553	5.25	-8.0	-4.5	44	5-8	59.9	58.1
25				(6.0)			127	5-0.5		
August 7	0.1	45.5	3.596	4.5	-9.5	-10.75	141	7-0	64.1	61.9
11	0.0	56.0	0.682	6.5	-1.5	+0.5	7	5-5	65.1	59.8
20	0.3	57.0 (9.30 a.m.)	1.059	5.5	-5.75	-5.5	274	5-2.5	70.0	70.0
24	0.3	50.0	0.986	5.25	-3.0	+0.5	41	5-8.5	67.8	65.0
31	1.3	54.0	0.812	5.5	-2.5	-2.25	502	6-8.5	68.6	66.4
September 3	1.4	54.0	1.001	5.5	-3.5	-5.0	2	4-11.5	63.5	63.5
7	4.8	59.0	1.117	5.5	-5.5	-9.25	43	5-0	70.2	63.1
14	2.8	55.5	0.986	4.5	-7.75	-11.5	43	4-10.5	63.2	62.8
17	1.5	58.0	0.638	6.0	-2.75	-3.0	28+	5-0	70.1	65.2
21	1.5	62.0	0.522	6.25	-4.75	-5.5	88-	4-11.5	69.9	69.9
24	3.2	65.5	0.754	4.5	-3.25	+0.50	-	5-10.5	78.6	74.3
28	-	-	-	-	-6.75	-3.75	1	6-0.5	86.4	64.8
October 2	-	-	-	-	-13.75	-17.25	-	5-0.5	91.6	91.6
5	-	-	-	-	-14.0	-20.5	-	4-7	76.0	76.0
8	-	-	-	-	-10.0	-15.25	3	4-8.5	72.2	72.2
13	-	-	-	-	-14.5	-20.5	24	5-0.5	79.0	79.0
17	-	-	-	-	-9.0	-18.5	-	5-3.5	80.0	64.9
20	0.0	74.5	3.045	2.25	-9.0	-14.5	51	5-1	74.2	74.2
25	-	-	-	-	-6.0	+0.5	17	5-10.5	84.0	84.0
29	17.8	72.0	5.336	4.5	-6.75	-2.75	-	6-2.5	71.3	69.1
November 2	-	-	-	-	-13.5	-16.5	-	5-2.5	85.1	80.0
7	-	-	-	-	-16.5	-22.25	4	4-10	81.0	79.0
12	-	-	-	-	-9.25	-1.25	37	5-9.5	88.6	66.1
16	-	-	-	-	-10.0	-8.75	31	5-6	86.9	86.9
23	0.9	70.0	0.464	6.75	+0.5	+0.5	1609	5-6.5	76.8	76.8
28	0.4	72.5	0.377	6.50	-1.25	-1.75	310	5-6.5	73.3	70.1

four pools was 5'11½" (May 31st, 1961). Whenever the latter area was flooded, (Plate 2), the water came through the drainage channel from the Weeney Bay area (Position X, Plate 1).

2.223 Water Table

Seasonal variations in the water table of the pool (1) and mangrove areas (11) (Positions 1 and 11, Plate 1) from December 1960 to November 1961 are shown in Table 1. Figures 2 and 3 show the variations in water table during January and February 1961 (summer), and June and July 1961 (winter). The lowest water table recorded below soil surface in the pool and mangrove areas were respectively 16.5" (November 7th, 1961) and 23.0" (January 4th and 29th, 1961). The diurnal variations in these water tables are shown in Table 2.

2.224 Depth of Water in Pool C

Seasonal variations in the depth of water in pool C are shown in Table 1. Figures 2 and 3 show the variations in January and February 1961 (summer) and in June and July 1961 (winter). The maximum depth of water recorded in the pool was 7.0" June 30th, 1961, after it had been flooded by a spring tide (6ft.9").

2.225 Salinity

The seasonal diurnal variations in salinity of the four pools A, B, C and D may be seen in Table 3. The greatest diurnal variation in salinity was recorded in pool D, 2nd May 1960 (1.673% NaCl). The highest salinity recorded was in pool D, 2nd May 1960 (9.019% NaCl, Table 3), and the lowest was recorded in pools A, B, C and D 12th March, 1961 (0.232% NaCl, Table 4). Immature stages of A.vigilax were present in the pools when both extreme values of salinity were recorded (Tables 3 and 4).

2.226 Temperature

The diurnal variations in temperature for February 1960 (summer),

TABLE 2

Diurnal Variation in Water Table of
Pool and Mangrove Areas

Date	Time of High Tide	Height of High Tide (Ft. - ins.)	Time of Day	Water Table Depth Below Ground Level (inches)	
				Pool Area	Mangrove Area
27.10.61	10.17 am.	5 - 9			
	11.00 pm.	4 - 1.5	6.00 pm.	5.00	+ 0.50
28.10.61			6.00 am.	5.00	+ 0.50
			7.00	5.00	+ 0.50
			8.00	5.25	+ 0.25
			9.00	5.50	+ 0.25
			10.00	5.50	0.00
	11.12 am.	5 - 4	11.00	4.75	0.00
			12.00 noon	4.25	0.25
			1.00 pm.	4.75	0.25
			2.00	5.25	0.50
			3.00	5.75	0.75
			4.00	6.25	1.00
			5.00	6.50	1.25
			6.00	6.50	1.50
	11.57 pm.	3 - 11			

TABLE 2

Diurnal Variation in Water Table of
Pool and Mangrove Areas

Date	Time of High Tide	Height of High Tide (Ft. - ins.)	Time of Day	Water Table Depth Below Ground Level (inches)	
				Pool Area	Mangrove Area
27.10.61	10.17 am.	5 - 9			
	11.00 pm.	4 - 1.5	6.00 pm.	5.00	+ 0.50
28.10.61			6.00 am.	5.00	+ 0.50
			7.00	5.00	+ 0.50
			8.00	5.25	+ 0.25
			9.00	5.50	+ 0.25
			10.00	5.50	0.00
	11.12 am.	5 - 4	11.00	4.75	0.00
			12.00 noon	4.25	0.25
			1.00 pm.	4.75	0.25
			2.00	5.25	0.50
			3.00	5.75	0.75
			4.00	6.25	1.00
			5.00	6.50	1.25
			6.00	6.50	1.50
	11.57 pm.	3 - 11			

TABLE 3

Seasonal Diurnal Variation in Salinity, pH, and Incidence of Immature Stages of A. vigilax in Pools A, B, C and D

Date	Salinity (‰ NaCl)												pH												Incidence of Immature Stages (+ or -)			
	Pool A			Pool B			Pool C			Pool D			Pool A			Pool B			Pool C			Pool D						
	10 a.m.	5 p.m.	Variation in Salinity	10 a.m.	5 p.m.	Variation in Salinity	10 a.m.	5 p.m.	Variation in Salinity	10 a.m.	5 p.m.	Variation in Salinity	10 a.m.	5 p.m.	Variation in pH	10 a.m.	5 p.m.	Variation in pH	10 a.m.	5 p.m.	Variation in pH	10 a.m.	5 p.m.	Variation in pH	Pool A	Pool B	Pool C	Pool D
7. 6.59	1.856	0.812	1.044	1.769	1.276	0.493	1.740	1.392	0.348	1.276	1.160	0.116	6.8	6.8	0.0	6.8	6.8	0.0	6.8	6.8	0.0	6.8	6.8	0.0		-	+	+
12. 7.59	-	-	-	1.276	1.392	0.116	1.508	1.624	0.116	1.102	1.044	0.058	-	-	-	7.2	7.6	0.4	7.3	7.6	0.3	6.8	6.8	0.0		-	-	-
11. 8.59	2.726	2.842	0.116	2.784	2.842	0.058	2.842	2.900	0.058	2.842	2.900	0.058	7.6	7.6	0.0	7.4	7.4	0.0	7.5	7.5	0.0	7.4	7.3	0.1	-	-	-	-
15. 9.59	-	-	-	1.276	-	-	1.218	1.160	0.058	0.754	0.754	0.000	-	-	-	7.6	-	-	7.4	7.5	0.1	7.4	7.3	0.1		+	+	+
15.10.59	-	-	-	-	-	-	1.711	-	-	1.566	1.740	0.174	-	-	-	-	-	-	7.3	-	-	7.2	7.2	0.0			+	+
18.11.59	0.348	0.406	0.058	0.348	0.377	0.029	0.348	0.435	0.087	0.319	0.406	0.087	7.3	7.3	0.0	7.2	7.3	0.1	7.2	7.3	0.1	7.3	7.2	0.1	+	+	+	+
28.12.59	-	-	-	-	-	-	-	-	-	4.292	5.046	0.754	-	-	-	-	-	-	-	-	-	7.2	7.2	0.0				-
4. 2.60	2.958	-	-	2.726	3.016	0.290	2.755	3.045	0.290	2.639	2.813	0.174	7.4	-	-	7.3	7.1	0.2	7.3	7.3	0.0	7.1	7.2	0.1	+	+	+	+
1. 3.60	3.654	-	-	4.437	4.843	0.406	4.089	4.466	0.377	4.292	4.350	0.058	7.3	-	-	7.3	7.3	0.0	7.2	7.2	0.0	7.3	7.3	0.0				+
2. 5.60	-	-	-	-	-	-	-	-	-	7.366	9.019	1.653	-	-	-	-	-	-	-	-	-	7.3	7.3	0.0				+
9. 6.60	1.740	1.672	0.068	1.972	2.030	0.058	1.798	1.672	0.126	1.682	1.740	0.058	6.9	6.9	0.0	6.9	6.9	0.0	6.5	6.5	0.0	6.5	6.1	0.4	-	-	-	-

TABLE 4

Seasonal variation in salinity and incidence of immature stages of A. vigilax in Pools A, B, C and D.

Date		Salinity (% NaCl)				Incidence of immature stages (Present +, absent -)			
		Pool A	Pool B	Pool C	Pool D	Pool A	Pool B	Pool C	Pool D
1960									
December	5	0.493	0.435	0.406	0.377	-	-	-	-
	9	-	1.740	1.392	0.638		+	+	-
	12	-	-	-	1.015				-
	15	1.827	1.247	1.537	0.783	+	+	+	-
	20	0.609	0.638	0.667	0.870	+	+	+	-
	24	2.204	2.088	2.262	2.349	+	+	+	+
	27	-	-	3.480	3.103			+	+
1961									
January	4	0.870	0.754	0.580	0.435	+	+	+	+
	7	-	-	1.769	1.044			+	+
	10	-	-	-	1.653				+
	18	6.438	4.533	4.292	3.770	+	+	+	+
	22	-	6.148	5.394	4.553		+	+	+
February	12	-	3.422	2.987	4.495		+	+	+
	16	6.467	5.655	5.249	4.553	+	+	+	+
	20	1.740	2.523	1.798	2.059	+	+	+	+
	23	-	5.046	4.234	3.306		+	+	-
	26	-	-	-	4.263				+
March	2	2.610	1.885	1.624	1.914	+	+	+	+
	5	-	-	2.929	2.842			+	+
	12	0.232	0.232	0.232	0.232	-	-	-	-
	17	-	3.045	2.175	1.189		+	+	+
	21	0.725	0.696	0.667	0.522	-	-	-	-
	25	-	2.494	1.769	0.899		+	+	+
	28	-	-	1.914	0.986			+	+
April	16	5.800	5.307	5.046	4.089	-	-	-	-
	21	2.204	1.653	1.450	2.639	+	+	+	+
	25	0.319	0.261	0.261	0.261	+	+	+	+
	30	-	1.624	1.189	0.638		+	+	+
May	3	-	2.494	1.595	0.841		+	+	+
	7	2.175	2.175	2.175	2.059	+	+	+	+
	11	-	2.610	2.552	2.465		+	+	+
	15	3.335	3.161	3.161	3.277	+	+	+	+
	18	-	3.422	3.306	3.393		+	+	+
	21	-	3.712	3.567	3.480		+	+	+
	24	-	-	3.915	3.741			+	+
	28	-	-	-	4.147				+
June	1	-	3.596	3.596	3.480		-	-	-
	4	3.509	3.451	3.451	3.451	+	+	+	+
	8	1.885	1.682	1.682	1.711	+	+	+	+
	11	1.305	1.668	1.668	1.682	+	+	+	+
	15	2.639	2.059	1.856	1.682	+	+	+	+
	20	3.248	3.248	3.248	3.132	+	+	+	+
	23	-	3.596	3.915	3.364		+	+	+
	27	-	-	4.046	3.712			+	+
	30	3.147	3.147	3.147	3.147	+	+	+	+
July	2	2.697	2.697	2.697	2.697	-	-	-	-
	6	-	3.219	3.190	3.060		+	+	+
	13	-	4.356	4.263	3.944		+	+	+
	16	-	4.945	4.731	4.234		+	+	+
	20	-	3.814	3.553	3.654		+	+	+
August	7	-	3.712	3.596	3.509		+	+	+
	11	0.682	0.682	0.682	0.682	-	-	-	-
	20	-	1.160	1.059	0.754		+	+	+
	24	-	1.131	0.986	8.255		+	+	+
	31	0.754	0.812	0.812	0.798	+	+	+	+
September	3	0.798	1.001	1.001	0.986	+	+	+	+
	7	-	1.145	1.117	1.102		+	+	+
	14	-	0.812	0.986	1.145		+	+	+
	17	0.493	0.638	0.638	0.696	+	+	+	+
	21	0.406	0.435	0.522	0.348	+	+	+	+
	24	-	0.870	0.754	0.464		+	+	+
	28	-	-	-	1.073				+
October	2	-	-	-	2.204				-
	20	-	-	3.045	-			-	
	29	-	5.279	5.336	4.524		+	+	+
November	23	0.464	0.464	0.464	0.464	+	+	+	+
	28	0.377	0.377	0.377	0.377	+	+	+	+

and August 1959 (winter) of the water in pools A, B, C and D, may be seen in Figures 3, 4, 5 and 6 & 7 respectively. Figure 8 shows the seasonal diurnal variation in temperature of the water in pool C in comparison with that of the air temperature. The seasonal variations in water temperature in pool C and the maximum air temperature recorded in Sydney on the day prior to, and between, field observations are shown in Table 1. Hatching of larvae of A.vigilax in the field did not occur when daily maximum air temperatures were below 65 - 70°F (Tables 1 and 7).

2.227 pH

Table 3 shows the seasonal diurnal variation in pH in the four pools A, B, C and D. The lowest pH recorded was 6.1 in pool D - 9th June, 1960. The highest pH recorded was 7.6 in pools B and C, 12th July, 1959; pool A on 11th August, 1959; and pool B on 15th September, 1959. The greatest recorded diurnal variations in pH were 7.2 - 7.6 in pool B on 12th July, 1959, and 6.5 - 6.1 in pool D on 9th June, 1960. Immature stages of A.vigilax were present in the pool irrespective of pH.

2.23 Population Sampling

2.231 Egg Sampling

Data from the sampling for the determination of the oviposition sites of A.vigilax in the field are presented in Tables 5 and 6. Because an apparent diapause mechanism operated in the egg stage, egg sampling was discontinued. However, it would appear from the results obtained that soil partly covered by vegetation (e.g. Salicornia) is probably preferred to bare soil for oviposition.

2.232 Sampling for Immature Stages

The results of the seasonal sampling for relative population densities of the immature stages of A.vigilax are shown in Table 7. The

Figure 4. Summer and Winter Diurnal Variation in Water Temperature in Pool A.

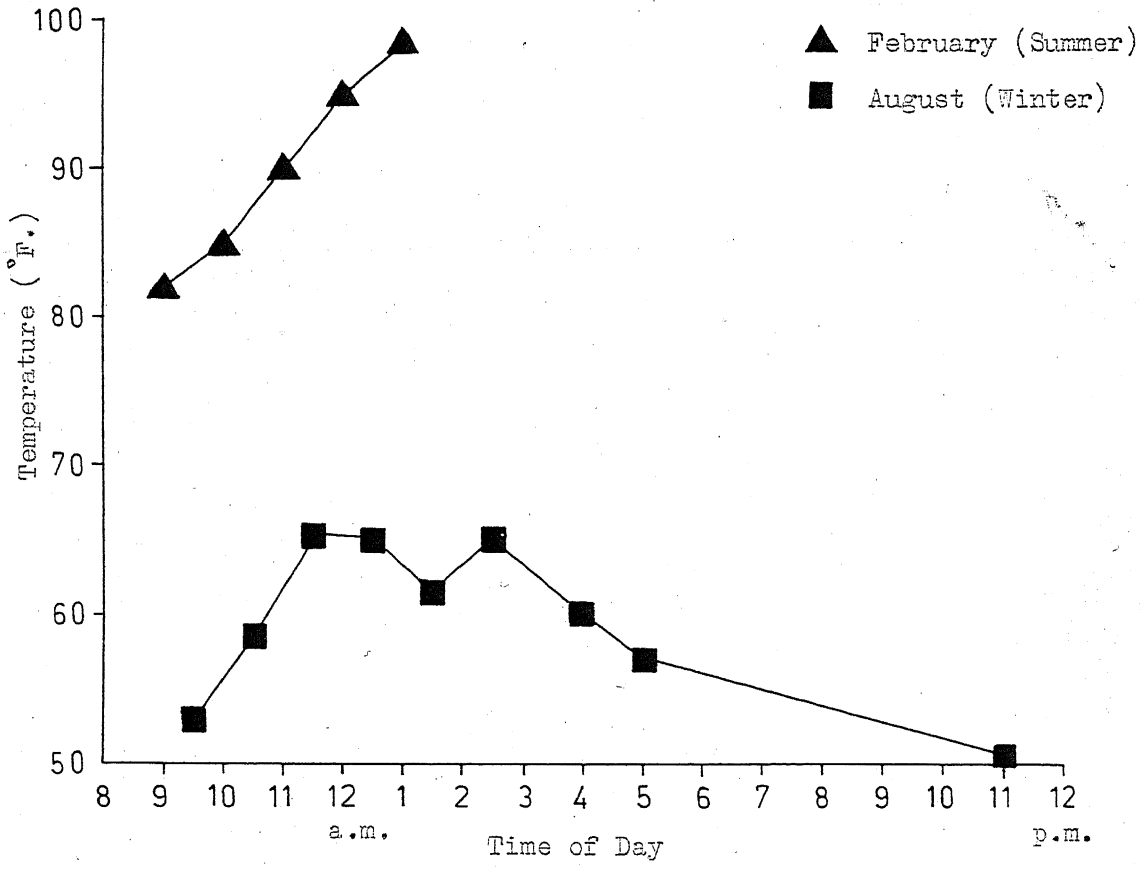


Figure 5. Summer and Winter Diurnal Variation in Water Temperature in Pool B.

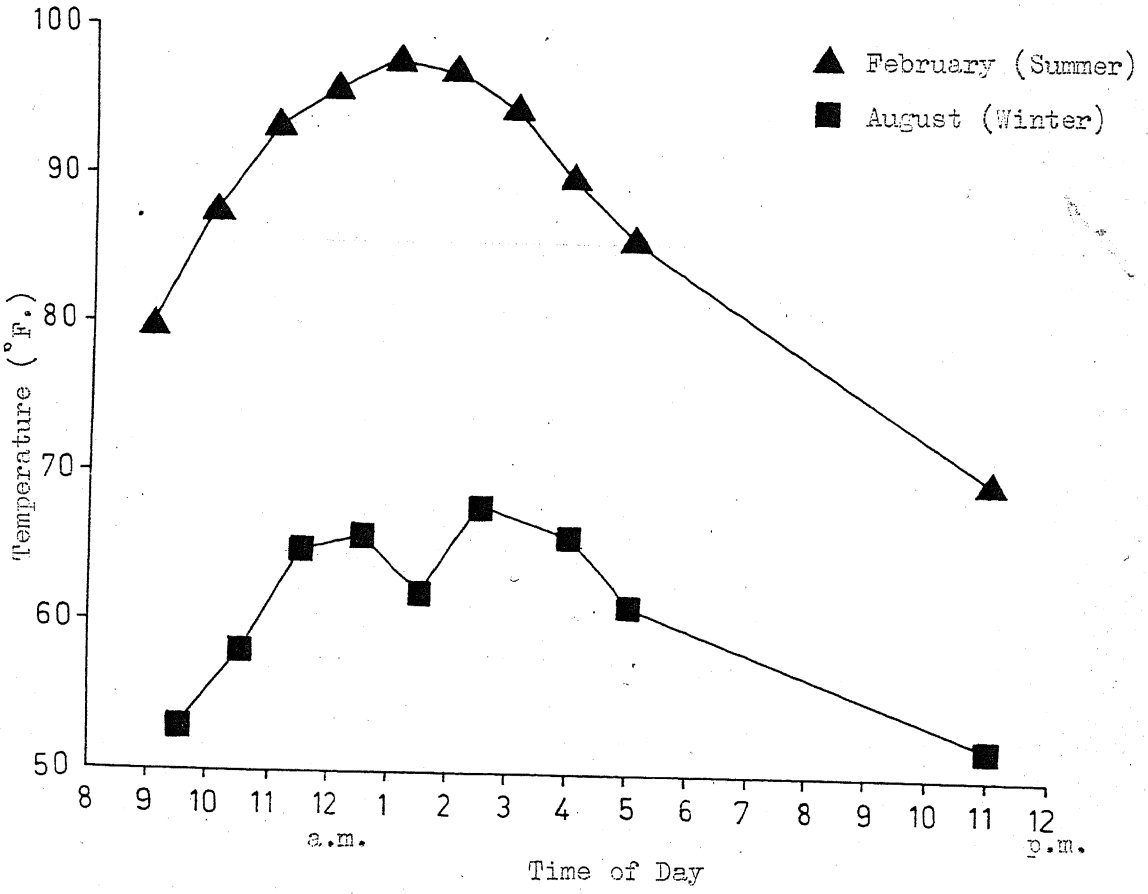


Figure 6. Summer and Winter Diurnal Variation in Water Temperature in Pool C.

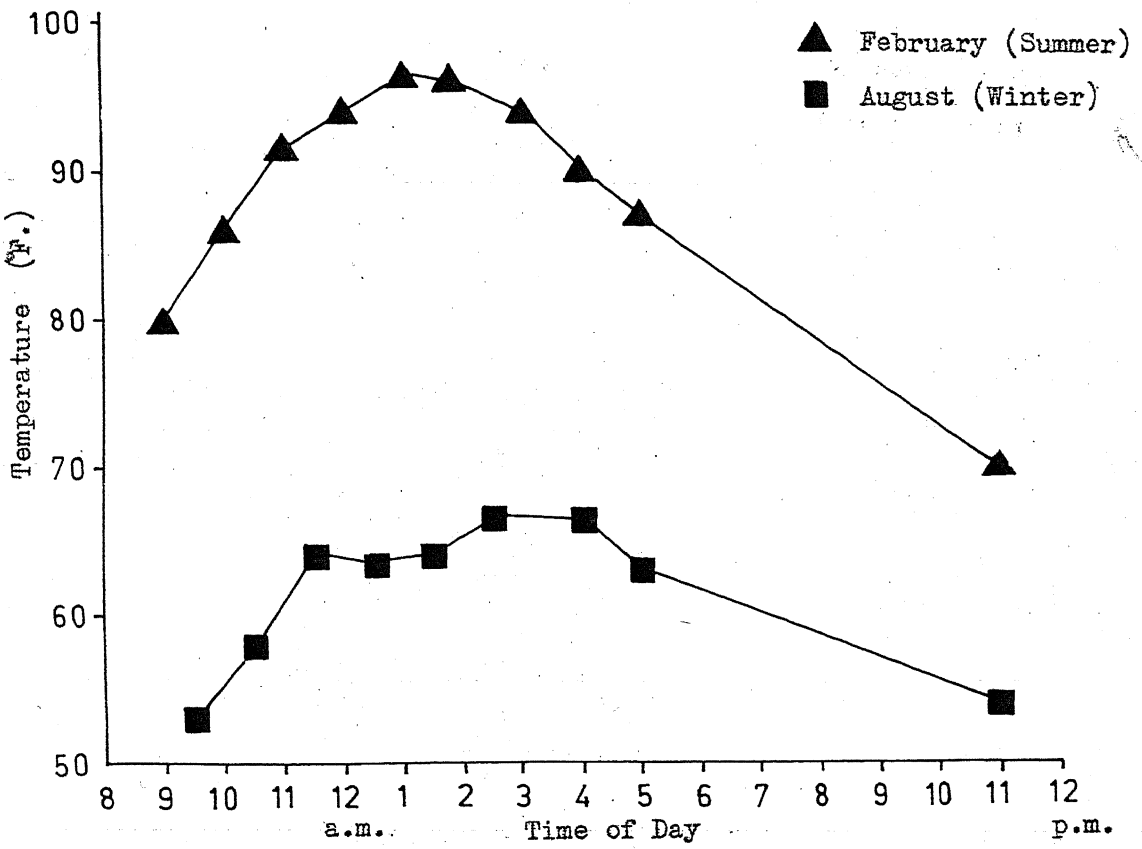


Figure 7. Summer and Winter Diurnal Variation in Water Temperature in Pool D.

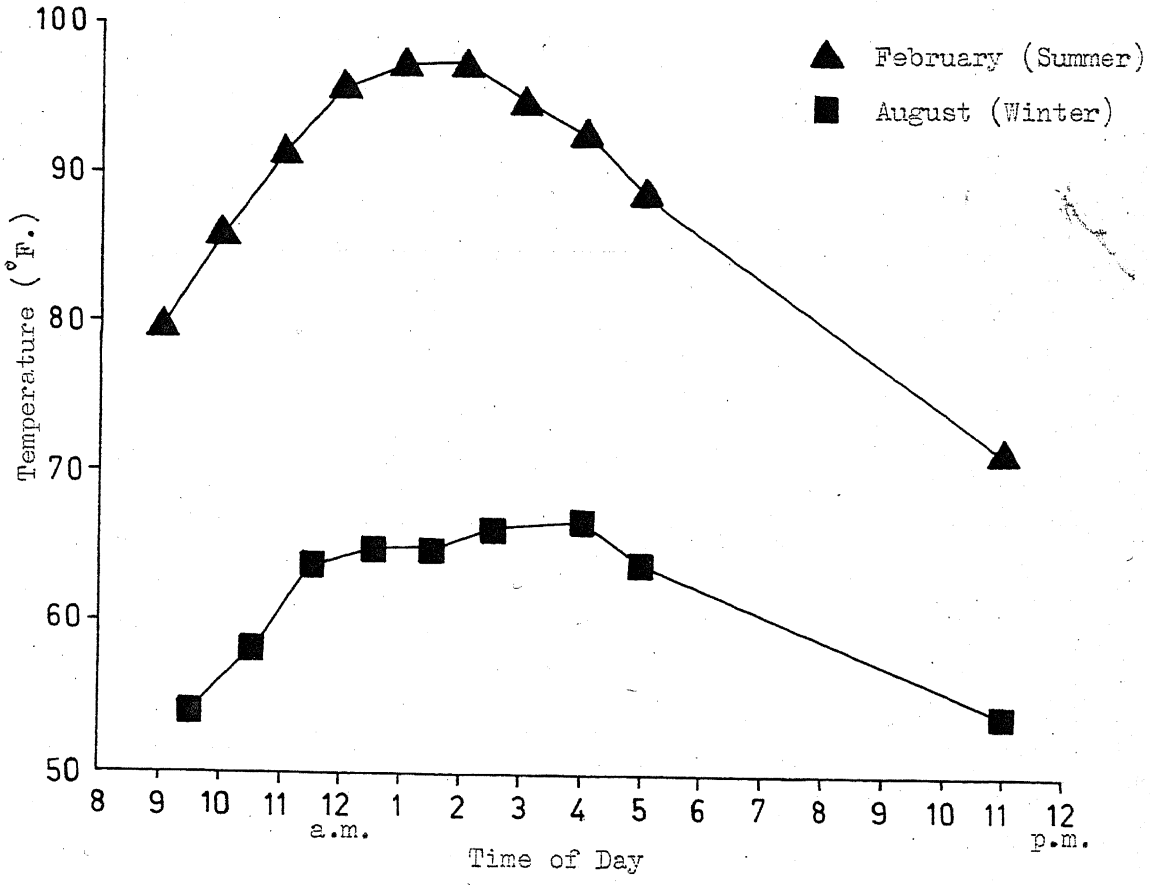


Figure 8. Seasonal Diurnal Variation in Air Temperature and Water Temperature in Pool C.

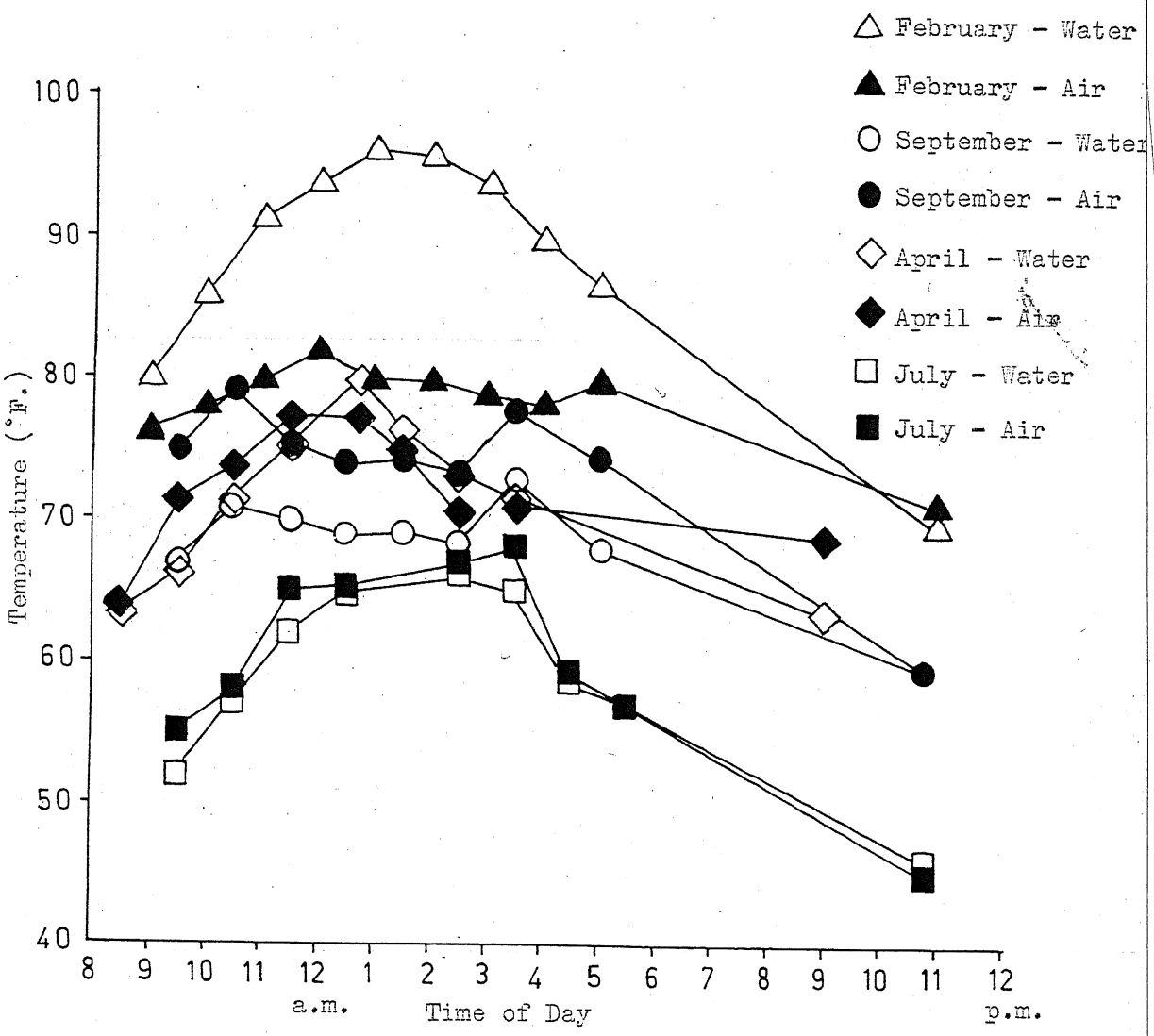


TABLE 5

Data on Egg Sampling in Pool D

Date of Sampling	Position of Soil Sample in Pool D	Time Elapsed between Sampling and 1st Flooding (days)	Number of larvae that hatched after 1st Flooding	Time Elapsed between Drying out and 2nd Flooding (days)	Number of Larvae that hatched after 2nd Flooding
8.1.60	1st 6" Bare mud	2	2		0
8.1.60	1st 6" Salicornia	1	1		0
8.1.60	3rd 6" Salicornia	3	7		0
8.1.60	4th 6" Salicornia	3	6		0
24.3.60	2nd 6" Salicornia	14	369	25	1
24.3.60	2nd 6" Bare mud	14	9	25	0
24.3.60	3rd 6" Bare Mud	14	0	25	0
7.4.60	2nd 6" Salicornia	19	3	14	0
7.4.60	3rd 6" Salicornia	19	6	14	0
7.7.60	1st 6" Salicornia	1	0	-	-
7.7.60	2nd 6" Salicornia	1	0	-	-

TABLE 6

Data on Egg Transect Sampling
across Pool B

Date of Sampling	Position of Soil Sample in Pool B	Time Elapsed between Sampling and Flooding (days)	Number of larvae hatched after flooding
26.2.61	Salicornia	2	9
	Salicornia		0
	Salicornia		0
	Bare mud		0
	Bare mud		0
	Bare mud		0
	Bare mud		0
	Bare mud		0
	Bare mud		0
	Salicornia		0
	Salicornia		1
	Salicornia		0

TABLE 7

Data on Seasonal Sampling in Pool C for Immature Stages of A. vigilax.

Date of Sampling		Number of Quadrats Sampled			Populations of Sampled Quadrats								
					Larvae								
		Vegetation Present	Vegetation Absent	Total	1st Instar	2nd Instar	3rd Instar	4th Instar	1:4	Total	Pupae	Total Population	Population Dens per Quadrat
1960													
December	20	8	12	20	31	28	6	3	10.3:1	68	0	68	3.4
	27	0	4	4	0	✓	✓	✓✓			✓✓✓		
1961													
January	4	8	12	20	1805	51	0	0	1771:0	1856	0	1856	92.8
	7	1	12	13	0	←	3536	→	0:3536	3536	0	3536	272.0
	18	8	12	20	5245	20	0	0	5245:0	5265	0	5265	263.3
	22	1	12	13	137	←	1345	→	1:<9.8	1482	1170	2652	204
February	16	8	12	20	40	1570	5	0	40:0	1615	0	1615	80.8
	23	3	12	15	0	0	0	40	0:40	40	5	45	3.0
March	2	8	12	20	557	10	0	0	557:0	567	0	567	28.4
	5	0	12	12	0	←	1245	→	0:<1245	1245	0	1245	103.8
	12	8	12	20	0	0	0	0	0	0	0	0	0
	17	1	12	13	0	←	4515	→	0:<4515	4515	5	4510	346.9
	21	8	12	20	0	0	0	0	0	0	0	0	0
	25	2	12	14	0	1	8	133	0:133	142	0	142	9.5
	28	0	8	8	0	0	0	4	0:4	4	17	21	2.6
April	16	0	12	12	0	0	0	0	0	0	0	0	0
	21	8	12	20	1	7	178	12	1:12	198	0	198	9.9
	25	8	12	20	0	3	1	17	0:17	21	3	24	1.2
	30	8	12	20	0	9	16	3	0:3	28	11	39	2.0
May	3	2	12	14	1	0	0	50	1:50	55	5	60	4.3
	7	8	12	20	0	0	0	13	0:13	13	0	13	0.7
	11	8	12	20	8	1	0	14	1:1.75	23	8	31	1.6
	15	8	12	20	4	3	0	4	1:1	11	8	19	1.0
	18	8	12	20	2	16	1	1	2:1	20	4	24	1.2
	21	1	12	13	1	7	4	0	1:0	12	1	13	1.0
	24	0	3	3	0	1	2	2	0:2	5	0	5	1.7
June	1	8	12	20	0	0	0	0	0	0	0	0	0
	4	8	12	20	14	0	0	0	14:0	14	0	14	0.7
	8	8	12	20	12	13	7	0	12:0	32	0	32	1.6
	11	8	12	20	2	21	4	5	1:2.5	32	0	32	1.6
	15	8	12	20	0	0	5	19	0:19	24	0	24	1.2
	20	8	12	20	0	0	0	8	0:8	8	0	8	0.4
	23	5	12	17	0	0	5	6	0:6	11	0	11	0.6
	27	0	9	9	0	0	5	11	0:11	16	0	16	1.8
	30	8	12	20	0	0	1	1	0:1	2	0	2	0.1
July	2	8	12	20	0	0	0	0	0	0	0	0	0
	6	8	12	20	0	0	2	0	0	2	1	3	0.2
	13	8	12	20	0	0	1	0	0	1	0	1	0.1
	16	3	12	15	1	0	0	0	1:0	1	0	1	0.1
	20	3	12	15	0	0	0	2	0:2	2	0	2	0.1
August	7	1	12	13	0	1	0	0	0	1	0	1	0.1
	11	8	12	20	0	0	0	0	0	0	0	0	0
	20	8	12	20	0	5	0	0	0	5	0	5	0.3
	24	8	12	20	0	3	1	2	0:2	6	0	6	0.3
	31	8	12	20	20	2	2	0	20:0	24	1	25	1.3
September	3	8	12	20	23	1	1	3	7.6:1	28	0	28	1.4
	7	4	12	16	66	9	0	2	33:1	77	0	77	4.8
	14	0	12	12	1	13	7	7	1:7	28	6	34	2.8
	17	8	12	20	1	4	8	14	1:14	27	3	30	1.5
	21	8	12	20	1	0	1	16	1:16	18	11	29	1.5
	24	0	12	12	6	1	0	0	6:0	7	31	38	3.2
October	20	0	4	4	0	0	0	0	0	0	0	0	0
	29	4	12	16	62	282	1	0	62:0	285	0	285	17.8
November	23	8	12	20	0	2	2	14	0:18	18	0	18	0.9
	28	8	12	20	0	0	0	1	0:1	1	7	8	0.4

population densities and rates of development of those that did, were very low. The distributions of the immature stages in the pool were not at any time of the year random: but were clumped in certain areas. Table 8 shows the seasonal variations in association of relative density of the immature stages with presence or absence of vegetation. The immature stages usually aggregated in the quadrats lacking vegetation. The majority of these quadrats were in the deepest areas of the pool (Figure 9).

2.24 Associations between Population Densities of Immature Stages of A.vigilax and Climatic Factors

It can be seen from Table 9 that associations exist between the following pairs of environmental factors (water table and depth of pool, water table and tide) and between population density of immature stages and the environmental factors of maximum daily air temperature and depth of pool.

3. LABORATORY EXPERIMENTS

The hatching of the eggs of A.vigilax in the field depends upon suitable environmental conditions, and the survival of the resulting aquatic immature stages is determined by their physiological and behavioural responses to many environmental factors. These responses are governed by previous acclimatization, and intensity and duration of exposure of each factor: Of these many factors, salinity and temperature were selected for physiological survival studies. Salinity, temperature, light, gravity and mechanical shock were the factors selected for behavioural studies. Both series of experiments were designed to determine the effects of all the components of these environmental factors on the physiological and behavioural responses of the immature stages of A.vigilax.

In all experiments, the responses of A.vigilax were compared with those of A.autralis, A.aegypti and C.fatigans, whose immature stages occupy different ecological habitats (see 1.22).

TABLE 8

Seasonal Variation in Associations of Absolute Density of Immature Stages of *A.vigilax* with Presence or Absence of Vegetation in Pool C, determined by χ^2 Contingency Table

Date	Quadrats containing Vegetation (1)		Quadrats lacking Vegetation (2)		P
	Number of quadrats containing water	Number of immature stages in quadrats	Number of quadrats containing water	Number of immature stages in quadrats	
20.12.60	8	61	12	7	< 0.001 (1)
4. 1.61	8	1007	12	849	< 0.001 (1)
18. 1.61	8	1555	12	3710	< 0.001 (2)
16. 2.61	8	715	12	900	< 0.001 (2)
23. 2.61	3	0	12	45	< 0.001 (2)
2. 3.61	8	147	12	420	< 0.001 (2)
17. 3.61	1	160	12	4350	< 0.001 (2)
25.3.61	2	4	12	138	< 0.001 (2)
21.4.61	8	120	12	78	< 0.001 (1)
25. 4.61	8	19	12	5	< 0.001 (1)
30. 4.61	8	21	12	18	0.95-0.05
3. 5.61	2	1	12	54	< 0.001 (2)
11. 5.61	8	2	12	21	0.01-0.001 (2)
18. 5.61	8	4	12	16	0.95-0.05 (2)
8. 6.61	8	11	12	21	0.95-0.05
11. 6.61	8	9	12	23	0.95-0.05
15. 6.61	8	3	12	21	0.01-0.001 (2)
31. 8.61	8	1	12	23	< 0.001 (2)
3. 9.61	8	3	12	25	0.01-0.001 (2)
7. 9.61	4	0	12	77	< 0.001 (2)
17. 9.61	8	5	12	22	0.95-0.05
21. 9.61	8	5	12	24	0.01-0.001 (2)
29.10.61	4	62	12	223	0.95-0.05

Figure 9. Contour Map of Pool C.

• Position of north-east corner of randomly selected quadrat.

TABLE 9

Analyses of Associations between Pairs of Environmental Factors (Salinity, Water Table, Depth of Pool, Rainfall, Tide) and Associations between Population Density of Immature A.vigilax and Selected Environmental Factors (Salinity, Maximum Daily Air Temperature, Depth of Pool). (χ^2 Contingency Tables - see Table 1 for recorded data).

Pairs of Environmental Factors Analysed	P
Water Table/Depth of Pool	< 0.001
Salinity/Depth of Pool	0.8 - 0.7
Salinity/ Rainfall	0.3 - 0.2
Water Table/Rainfall	0.1 - 0.05
Water Table/Tide	< 0.001
Population Density/Maximum Daily Air Temperature	0.01-0.001
Population Density/Salinity	0.2 - 0.1
Population Density/Depth of Pool	< 0.001

Many unsuccessful attempts were made to colonize A.vigilax in the laboratory. Because of this, all larvae used in the experiments were collected from the field as 1st instars. These were reared to the required instar at room temperature in 11" x 11" x 4½" perspex dishes containing water, of salinity similar to that from which they were collected, and food in the form of finely ground "K9" dog biscuits. The water and food were renewed daily. Pupae, when required were separated from the larvae.

The source of the fourth instar larvae of A.aegypti was a laboratory colony maintained in a room of constant temperature 25°C ± 0.5°C in the School of Biological Sciences of the University of New South Wales, whilst fourth instar larvae of C.fatigans were taken from a nearly stagnant manure infusion. Fourth instar larvae of A.australis were collected as required from rock pools at Tamarama, Sydney.

3.1 Methods

3.11 Physiological Survival Experiments

The survival of fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans were determined by exposing them to four solutions of concentrations (by weight) of common salt (NaCl) greater or less than those usually encountered in the field. The influence of temperature on the survival of these four species to those solutions was also determined, while the influence of previous acclimatization was determined only for A.vigilax and A.australis.

In both the acclimatization and experimental solutions, common salt was used to prevent cation deficiency, while food in the form of finely ground "K9" dog biscuits was always present.

In all experiments the larvae were previously acclimatized in the required solutions for 12 hours, duringthe last four of which they were

maintained at the experimental temperature.

In the first series of experiments, batches of 50 larvae of A.vigilax and A.australis were acclimatized in solutions of 0.9% and 3.6% sodium chloride (NaCl), whilst batches of 50 larvae of A.aegypti and C.fatigans were acclimatised in tap water. Then A.vigilax and A.australis larvae were transferred in batches of 5 to 3" x 1" perspex vials containing 25 ml of solutions of distilled water, 10%, 20% and 30% NaCl, and a similar procedure was used for those of 5%, 10%, and 20% and 30% NaCl. (See Plate 14). All experiments were maintained in a controlled temperature and humidity (25° ± 0.5°C, 70 ± 3% R.H.). The larvae were examined at regular intervals, and any that would not respond to a violent mechanical stilulus (tapping the vial, and/or withdrawing them into a pipette) were scored dead. The median lethal exposure (i.e. the period of exposure required to kill 3 of the 5 larvae) for each replicate was observed, and the mean L.E. 50 (period of exposure required to kill 50% of the larvae) for each concentration was calculated. Observations were discontinued after 24 hours, for, beyond this period, growth of lethal fungi in the salt solution would give inaccurate results.

The percentage of chloride ions, and sodium chloride equivalent in the solution of distilled water plus food, and tap water, were determined by titration with N/35.5 silver nitrate using 1% potassium dichromate as an indicator (Piper 1950) to determine the effect of evaporation during the course of the experiments. 25 nl. of each of the 5%, 10%, 20% and 30% NaCl solutions were maintained in vials at 25°C and 30°C (± 0.5°C) with a relative humidity of 70% for 24 hours. The change in concentration of the NaCl solutions over this period were determined by weighing the solutions before and after the exposure period, and these differences were checked by titration for the 5% and 10% solutions. The end point of titration could not

be determined for 20% and 30% NaCl due to dilution factors.

Analyses of variance (Simpson, Rose, and Lewontin, 1960) were performed to determine the intraspecific influence of acclimatization and experimental salinities, and temperature on survival of A.vigilax and A.australis, andthe intraspecific influence of temperature and salinity on survival of A.aegypti and C.fatigans.

"Students t-tests" (Simpson, Roe and Lewontin, 1960) were done to determine whether there were any significant differences in survival to each of the salinities and temperatures between species, viz., A.vigilax and A.australis; A.aegypti and C.fatigans; and A.vigilax and A.aegypti.

Note: The experiment for the determination of the L.E. 50 of A.vigilax in 10% salt at 25°C after acclimatization in 0.9% salt was not completed when performed at the same time of the tear as the other experiments (early Autumn). When this experiment was repeated in the next spring, the results obtained were different from those obtained from the Autumn experiment. This difference was probably due to a seasonal physiological difference between populations, and, although this difference did not affect the significance of any of the conclusions drawn from these experiments, it was necessary to obtain discrete values for the analyses of variance. Those discrete values were interpolated from a graph of the log. of duration of exposure against median lethal exposure.

3.12 Behaviour Experiments

The behavioural responses of the fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans to the selected environmental factors of light, shock, mechanical shock and gravity were studied qualitatively to determine whether these factors had, upon the behaviour of these species, specific effects which could be correlated with the different ecological habitats that the species occupied.

To compare the differences in the behaviour of these larvae to light, their negative phototropic responses were studied by measuring their rates of linear movement under illumination from a parallel beam of light.

To determine the ecological significance of the light behaviour of A.vigilax, a more detailed study was made of the influences of salinity, temperature, instar, and stimulus satiation on the rate of linear movement of the larvae and pupae.

To investigate the responses of the fourth instar larvae of the four species to light, shock, mechanical shock and gravity, the following apparatus was used: A clear perspex container of internal dimensions base 3" square, height 4", blackened on two adjacent sides and marked out in quadrants on the viewing side, was placed in a light-proof box, so that it was equidistant and 5" from three frosted incandescent globes (60 watts, 240 volts, D.C.). These globes were dorsal, ventral and lateral to the perspex container, and were separated from it by sheets of frosted glass. The container was filled to a height of 3" with the solution of required salinity and maintained at $30^{\circ} \pm 0.2^{\circ}C$ (Plates 18 and 19). To eliminate osmotic stress during the experiments, salinities used for A.vigilax and A.australis were 0.9% - 3.6% NaCl, while for A.aegypti and C.fatigans distilled water was used.

Before each experiment, the larvae were acclimatized for one hour under reduced illumination at $30^{\circ} \pm 0.5^{\circ}C$ in the appropriate solution to which food had been added. 50 larvae of each species were used in each experiment, and each larvae was discarded after one shock stimulus. To determine the responses of the larvae to direction of single light shocks (viz. dorsal, ventral and lateral), and to pattern of two light shocks (viz. sequential and simultaneous) from different directions (viz. dorsal, ventral and lateral), the larvae were placed individually under reduced light conditions in the

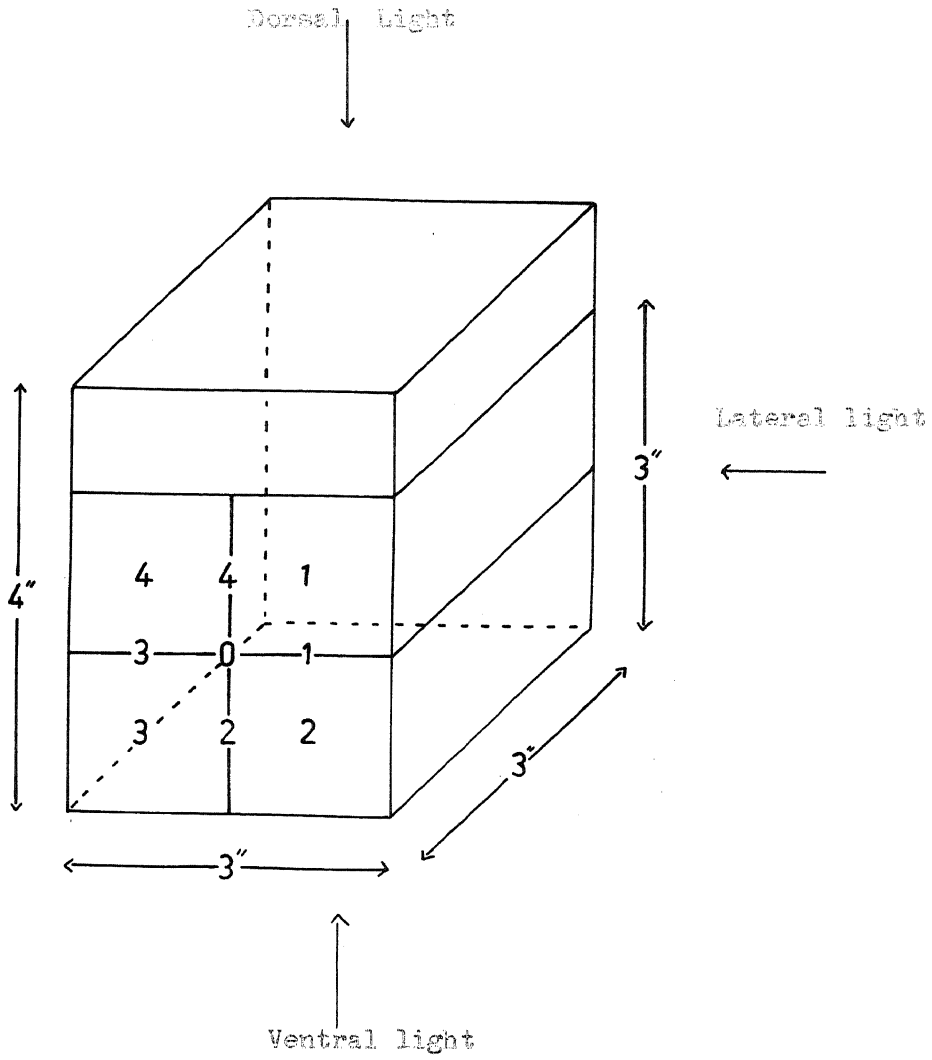
centre of the container. The appropriate light or lights were then switched on, and the responses of the larvae to these light shocks were observed, and their positions in the quadrats noted after five seconds. Figure 15 shows the distribution of the quadrats in the perspex container.

The responses to direction of light shock applied immediately before, or in the case of lateral light only, simultaneously with, mechanical shock, were similarly recorded.

From the results of light shock and mechanical shock experiments, the responses of the larvae to gravity were deduced, and a complete analysis was then made of the responses of the larvae to light shock, mechanical shock and gravity using χ^2 tests. In the comparison of the rates of linear movement of the fourth instar larvae of the four species, dorsal and lateral parallel beams of light were used. The source of illumination, an automotive spotlight (30 watts, 6 volts D.C.) at the end of an internally whitened tube 4" square in cross section and 14" long was placed either above a perspex trough (3" square in cross section, 12.5" high and blackened on three sides) (Plate 16), or at the end of a metal trough (3" square in cross section, 13" long, with glass ends, and blackened on three sides) (Plate 17).

In all the experiments the troughs were filled with the required NaCl solution, which was maintained at the required temperature, and before each experiment, the larvae and pupae were acclimatized for one hour under reduced illumination at the experimental salinity and temperature. The rate of movement of each individual of a random sample of 50 larvae was calculated from the linear distance moved away from the light source during the period of continuous movement. Each larva was discarded after one trial.

Figure 15. Scheme of Quadrants in Perspex Container.



The mean rates of movement were calculated for fourth instar larvae of A.vigilax in solutions of 0.9%, 1.8% and 3.6% NaCl at temperatures of 25°C and 30°C, and for 1st, 2nd, and 3rd instar larvae, and pupae of A.vigilax in 1.8% NaCl at 30°C. The mean rates of movement of fourth instar larvae of the other three species were similarly determined at 30°C, with dorsal and lateral illumination, using 3.6% NaCl for A.australis, and distilled water for A.aegypti and C.fatigans. Students t-tests were done to determine whether there were any significant differences intra-specifically or interspecifically in the mean rates of movement of the four species.

To determine whether stimulus satiation from lateral illumination affected the rate of movement of fourth instar larvae of A.vigilax in 1.8% NaCl at 25°C and 30°C, 10 larvae selected at random for each experiment were maintained at the appropriate salinity and temperature under reduced illumination for one hour prior to the experiment. The mean rate of movement of each larva was then determined over ten consecutive trials at 25°C and 30°C. These mean rates of movement were compared (using Student t-tests) with the mean rates of movement at 30°C for ten non-consecutive trials (15 minutes interval between trials) of another random sample of ten larvae.

3.2 Results

3.21 Physiological Survival Experiments

The influence of acclimatization on L.E. 50 of upper lethal salinites of fourth instar larvae of A.vigilax and A.australis at temperatures of 25°C and 30°C, may be seen in Table 10, and Figures 10 and 11; whilst the analyses of the influence of acclimatization on L.E. 50 of these salinities, are shown in Table 11a and 11b. 0% NaCl (0.033 gm.

TABLE 10

The Influence of Acclimatization on L.E.50 of Upper Lethal Salinities of Fourth Instar Larvae of A.vigilax and A.australis at Temperatures of 25°C and 30°C

Species	Acclimatization Salinity (% NaCl)	Experimental Temperature (°C)	Experimental Salinity (% NaCl)	Mean Duration for L.E.50 (hrs.)	Variance
A.vigilax	0.9	25	0	> 24.0	—
			10	15.4	33.13
			20	4.4	0.93
			30	2.2	0.13
		30	0	> 24.0	—
			10	6.0	2.03
			20	2.5	0.03
			30	1.3	0.04
	3.6	30	0	> 24.0	—
			10	7.0	1.59
			20	2.7	0.11
			30	1.6	0.03
A.australis	0.9	25	0	> 24.0	—
			10	13.1	2.95
			20	5.2	2.16
			30	1.8	0.14
		30	0	> 24.0	—
			10	8.8	4.36
			20	3.1	0.20
			30	1.4	0.04
	3.6	30	0	> 24.0	—
			10	13.0	10.81
			20	4.9	0.96
			30	2.4	0.53

Figure 10. The influence of temperature on L.E. 50 of upper lethal salinities on Fourth instar larvae of A.vigilax and A.australis acclimatized at 0.9% NaCl.

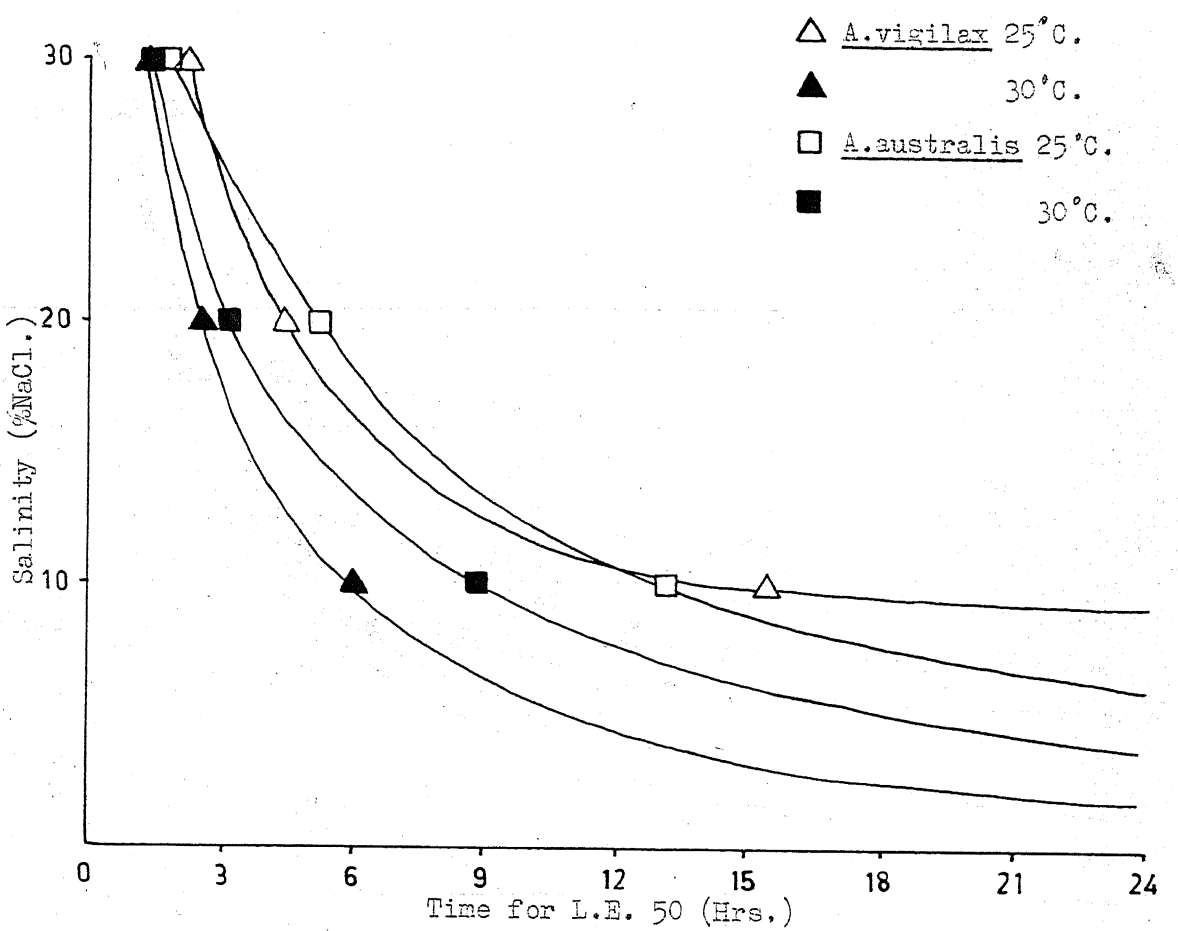


Figure 11. The influence of acclimatization on L.E. 50 of upper lethal salinities of Fourth instar larvae of A.vigilax and A.australis at 30°C.

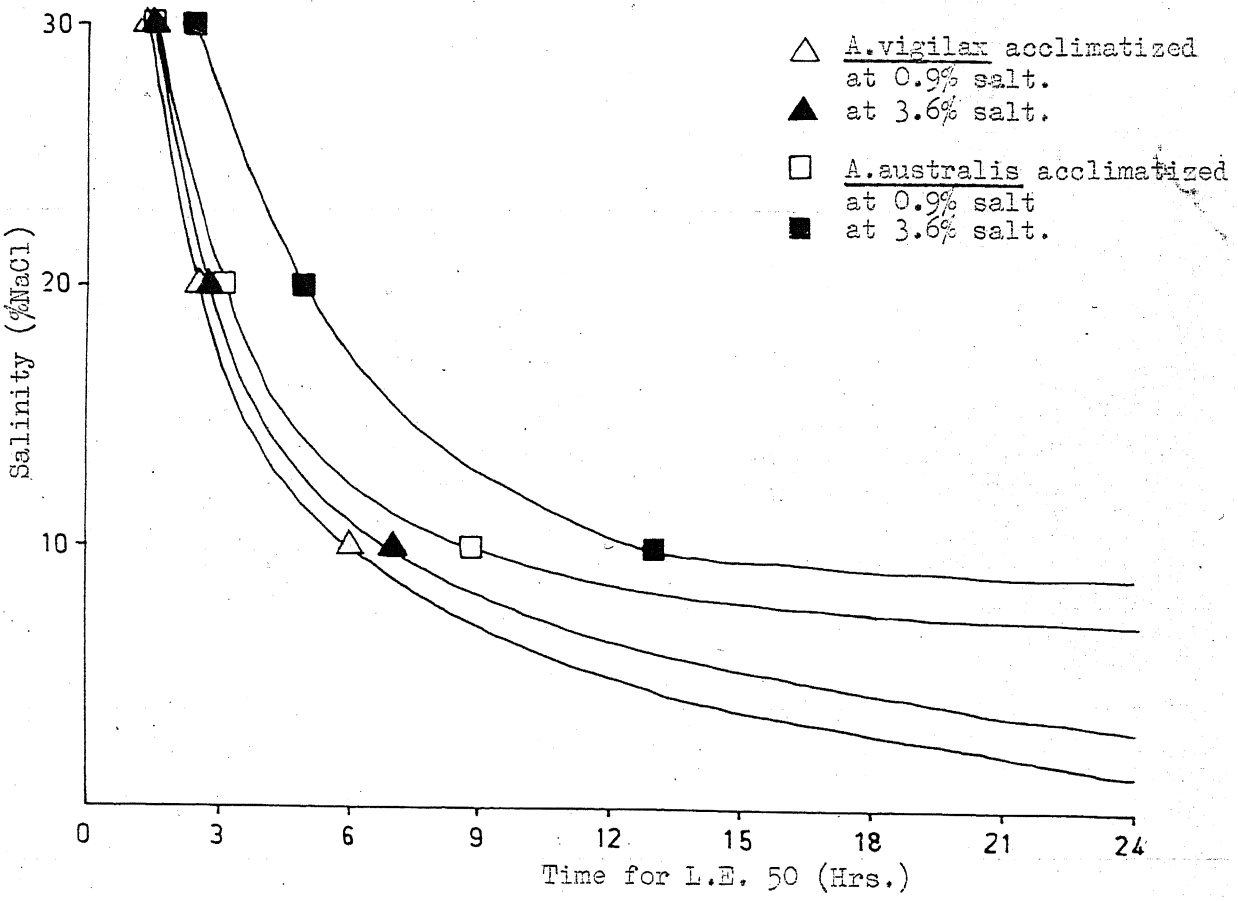


TABLE 11

Intraspecific and Interspecific Analyses of Influence of Acclimatization, Salinity, and Temperature on the L.E.50 of Fourth Instar Larvae of A.vigilax and A.australis

(a) Intraspecific Analyses of Variance

Species	Acclimatization Salinity (% NaCl)			Experimental Salinity (% NaCl)			Experimental Temperature (°C)			Interaction	
	% NaCl	F	p	% NaCl	F	p	°C	F	p	F	p
A.vigilax	0.9			10,20,30	16.340	0.05	25,30	7.243	0.01	3.769	0.05
	0.3,3.6	6.375	0.05	10,20,30	2344.60	< 0.01	30			1.370	> 0.05
A.australis	0.9			10,20,30	242.4	0.01	25,30	41.280	0.01	9.361	0.01
	0.9,3.6	29.360	0.01	10,20,30	159.5	0.01	30			4.807	0.05

(b) Interspecific t-tests

Acclimatization Salinity (% NaCl)	Experimental Temperature (°C)	Experimental Salinity (% NaCl)	t	p
0.9	25	10	1.213	> 0.10
		20	1.440	> 0.10
		30	2.439	0.05-0.02
	30	10	4.223	< 0.001
		20	0.847	> 0.10
		30	1.100	> 0.10
3.6	30	10	5.398	< 0.001
		20	6.739	< 0.001
		30	3.383	0.01-0.001

TABLE 12

The Influence of Temperature on L.E.50 of Upper Lethal Salinities of Fourth Instar Larvae of A.aegypti and C.fatigans

Species	Experimental Temperature (°C)	Experimental Salinity (% NaCl)	Mean Duration for L.E.50 (Hrs.)	Variance
A.aegypti	25	5	1.5	0.16
		10	0.8	0.13
		20	0.4	0.01
		30	0.3	0.00
	30	5	1.1	0.03
		10	0.7	0.04
		20	0.3	0.01
		30	0.2	0.06
C.fatigans	25	5	1.6	0.03
		10	1.0	0.06
		20	0.4	0.00
		30	0.3	0.00
	30	5	1.1	0.03
		10	0.7	0.01
		20	0.4	0.01
		30	0.3	0.00

Figure 12. The influence of temperature on L.E. 50 of upper lethal salinities on Fourth instar larvae of A.aegypti and C.fatigans.

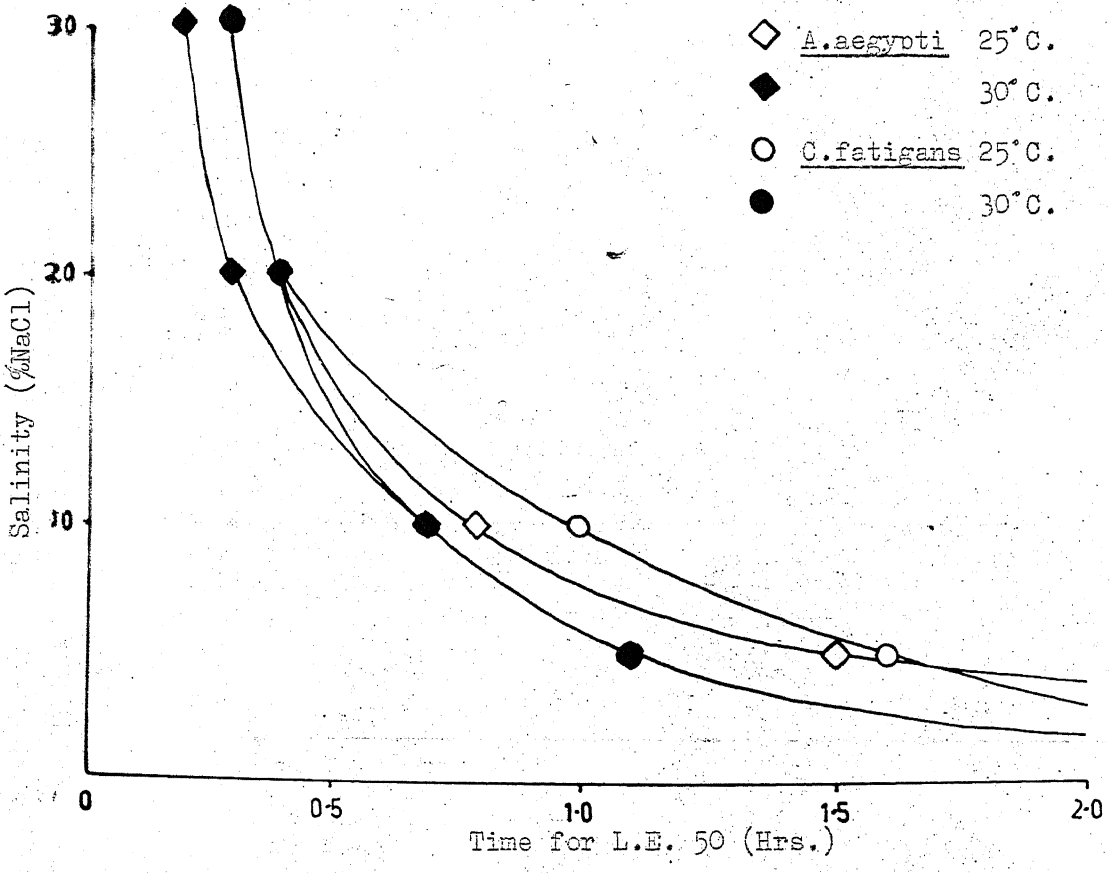


TABLE 13

Intraspecific and Interspecific Analyses of Influence of Salinity and Temperature on the L.E.50 of Fourth Instar Larvae of A.aegypti and C.fatigans

(a) Intraspecific Analyses of Variance

Species	Experimental Salinity (% NaCl)			Experimental Temperature (°C)			Interaction	
	% NaCl	F	p	°C	F	p	F	p
A.aegypti	5,10 20,30	98.0	0.01	25,30	146.0	0.01	5.00	0.01
C.fatigans	5,10 20,30	229.0	0.01	25,30	47.0	0.01	13.5	0.01

(b) Interspecific t-tests

Experimental Temperature (°C)	Experimental Salinity (% NaCl)	t	p
25	5	0.714	> 0.10
	10	1.460	> 0.10
	20	0	∞
	30	0	∞
30	5	0	∞
	10	0	∞
	20	2.24	0.05 - 0.02
	30	1.293	> 0.10

TABLE 14

Interspecific Analyses of Influence of Salinity, and Temperature on the L.E. 50 of Fourth Instar Larvae of A.vigilax and A.aegypti (t - tests)

Experimental Temperature (°C)	Experimental Salinity (% NaCl)	t	p
30°C	10	11.67	<0.001
	20	34.93	<0.001
	30	11.00	<0.001

Figure 13. The influence of upper lethal salinities on L.E. 50 of Fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans at 25°C.

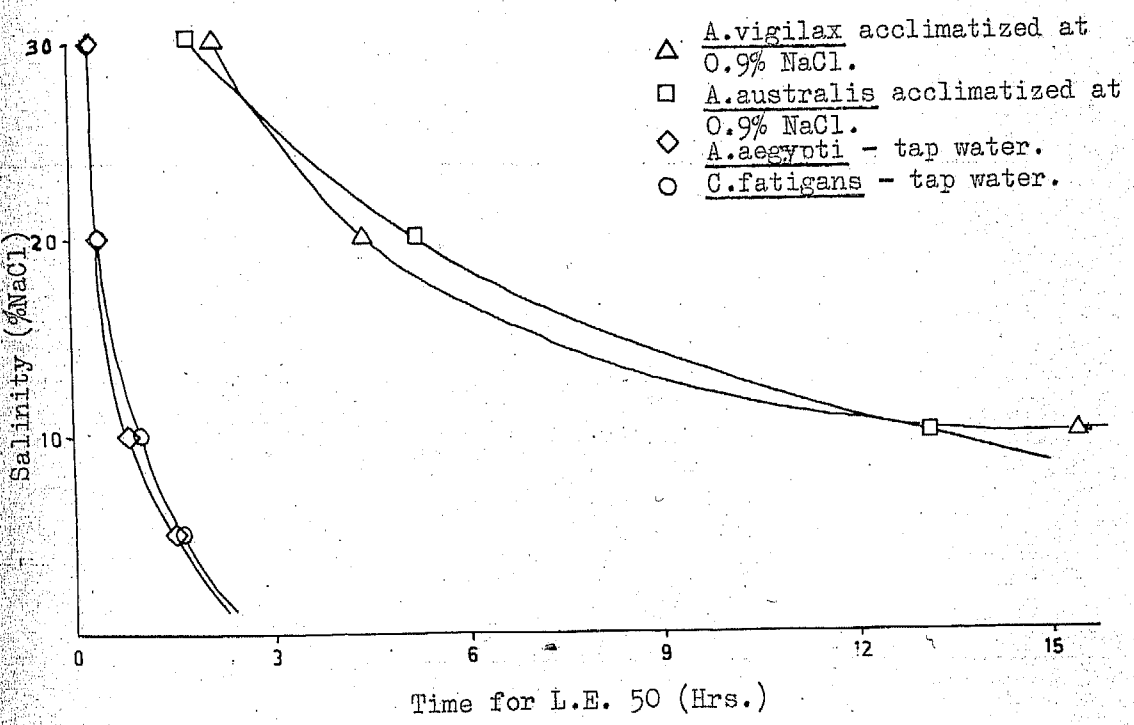


Figure 14. The influence of upper lethal salinities on L.E. 50 of Fourth instar larvae of A.vigilax, A.australis, A.aegypti, and C. fatigans at 30°C.

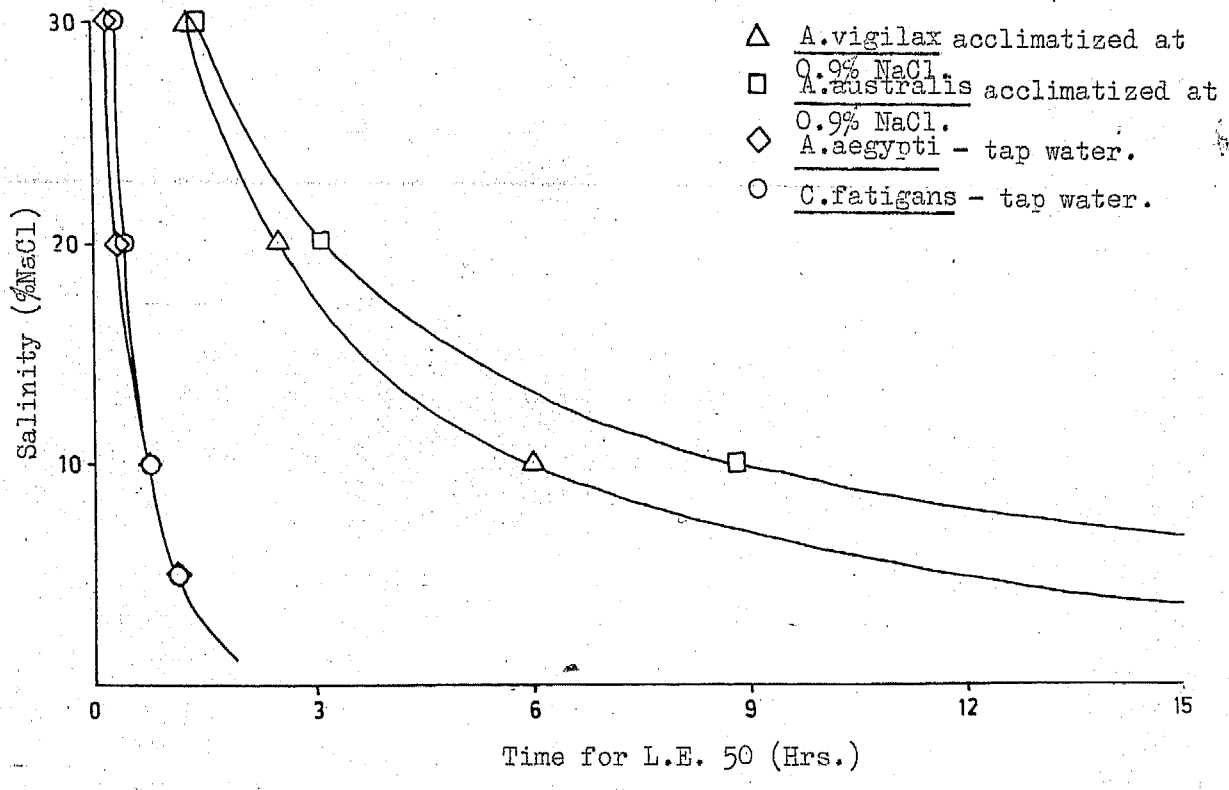


TABLE 15

Increase in Salinity of the Experimental Saline Solutions at Temperatures of 25°C and 30°C, at 70% Relative Humidity, Determined by Titration and Weighing

Experimental Temperature (°C)	Expected Experimental Salinity (% NaCl)	TITRATION				WEIGHING			
		Initial Salinity (% NaCl)	Salinity after 24 hours (% NaCl)	Increase in Salinity (%) /24 hours	/hour	Initial Weight (gms)	Weight loss after 24 hrs. (gms.)	% Loss in Weight /24hours	/hour
25	5	4.669	4.916	0.247	0.010	25.7567	1.1492	4.461	0.186
	10	9.396	9.831	0.435	0.018	26.6690	1.2115	4.540	0.189
	20					27.9170	0.9183	3.289	0.133
	30					29.3089	0.8650	2.951	0.123
	5	4.669	4.988	0.219	0.009	25.7993	1.5913	6.167	0.257
	10	9.396	10.005	0.609	0.025	26.6499	1.5179	5.696	0.237
	20					28.0243	1.3206	4.714	0.196
	30					29.3180	1.1836	4.038	0.168

TABLE 16

The Influence of Direction of Illumination on the on the Phototropic Responses of the Fourth Instar Larvae of A.vigilax, A.australis, A.aegypti and C.fatigans at 30°C

Species	Phototropic Response	Direction of Illumination Shock		
		Dorsal	Ventral	Lateral
A.vigilax	+ ve	2	4	0
	- ve	96	84	100
	Nil	2	12	0
A.australis	+ ve	0	2	4
	- ve	96	90	92
	Nil	4	8	4
A.aegypti	+ ve	0	0	0
	- ve	100	98	100
	Nil	0	2	0
C.fatigans	+ ve	4	2	6
	- ve	96	96	94
	Nil	0	2	0

Phototropic responses of the larvae expressed as the percentages that moved within five seconds.

TABLE 17

The Influence of Direction and Pattern of Illumination Shock on the Direction of Movement of Fourth Instar Larvae of A.vigilax at 30°C

Illumination Shocks		Direction of Movement %				
Direction	Pattern	Quadrant 0	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Dorsal and Lateral	Sequential	2	2	2	90	4
	Simultaneous	0	4	20	64	12
Ventral and Lateral	Sequential	4	8	8	10	70
	Simultaneous	0	4	16	18	62
Expected random Percentage		20	20	20	20	20

Direction of Movement expressed as % of larvae in each quadrant after five seconds.

TABLE 18

Analyses of the Influence of Direction and Pattern of Illumination Shock on the Direction of Movement of Fourth Instar Larvae of A.vigilax at 30°C (χ^2 tests) (see Table 17)

Illumination Shocks		P
Direction	Pattern	
Dorsal and Lateral	Sequential/random	< 0.001
	Simultaneous/random	< 0.001
	Sequential/simultaneous	< 0.001
Ventral and Lateral	Sequential/random	< 0.001
	Simultaneous/random	< 0.001
	Sequential/simultaneous	0.05 - 0.01

TABLE 19

Dominance of Direction of Illumination Stimuli on the Direction of Movement of Fourth Instar Larvae of A.vigilax, resulting from Simultaneous Illumination Shocks. (See Table 17)

Directions of Simultaneous Illumination Shocks	Quadrant	Dominance of Direction of Illumination %		
		L = D	L > D	D > L
Dorsal and Lateral	1	100	0	0
	2	0	0	100
	3	56	31	13
	4	0	100	0
		L = V	L > V	V > L
Ventral and Lateral	1	0	100	0
	2	0	0	100
	3	89	11	0
	4	39	41	19
Expected random percentage		33	33	33

L = Lateral
D = Dorsal
V = Ventral

TABLE 20

Analyses of Dominance of Direction of Illumination Stimuli on the Direction of Movement of Fourth Instar Larvae of A.vigilax, resulting from simultaneous illumination shocks. (χ^2 tests)(See Table 19)

Directions of Simultaneous Illumination Shocks	Quadrant	Movement	p
Dorsal and Lateral	1	Dominant/random	< 0.001
	2	Dominant/random	< 0.001
	3	Dominant/random	< 0.001
	3	L=D/L > D	0.05-0.01
	4	Dominant/random	< 0.001
Ventral and Lateral	1	Dominant/random	< 0.001
	2	Dominant/random	< 0.001
	3	Dominant/random	< 0.001
	4	Dominant/random	< 0.001
	4	L=V/L > V	0.95-0.05

TABLE 21

The Influence of Direction and Pattern of Illumination Shock on the Direction of Movement of Fourth Instar Larvae of A.australis at 30°C

Illumination Shocks		Direction of Movement %				
Direction	Pattern	Quadrant 0	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Dorsal and Lateral	Sequential	0	0	8	92	0
	Simultaneous	0	0	0	98	2
Ventral and Lateral	Sequential	0	0	0	2	98
	Simultaneous	0	0	0	14	86
Expected random Percentage		20	20	20	20	20

Direction of Movement expressed as % of larvae in each quadrant after five seconds.

TABLE 22

Analyses of the Direction and Pattern of Illumination Shock on the Direction of Movement of Fourth Instar Larvae of A.australis at 30°C. (χ^2 tests) (see Table 21)

Illumination Shocks		p
Direction	Pattern	
Dorsal and Lateral	Sequential/random	< 0.001
	Simultaneous/random	< 0.001
	Sequential/simultaneous	0.01-0.001
Ventral and Lateral	Sequential/random	< 0.001
	Simultaneous/random	< 0.001
	Sequential/simultaneous	0.01-0.001

TABLE 23

Dominance of Direction of Illumination Stimuli on the Direction of Movement of Fourth Instar Larvae of A.australis, resulting from Simultaneous Illumination Shocks. (See Table 22)

Directions of Simultaneous Illumination Shocks	Quadrant	Dominance of Direction of Illumination %		
		L = D	L > D	D > L
Dorsal and Lateral	1	0	0	0
	2	0	0	0
	3	59	25	16
	4	100	0	0
		L = V	L > V	V > L
Ventral and Lateral	1	0	0	0
	2	0	0	0
	3	43	57	0
	4	35	58	7
Expected random percentage		33	33	33

L = Lateral
D = Dorsal
V = Ventral

TABLE 24

Analyses of Dominance of Direction of Illumination Stimuli on the Direction of Movement of Fourth Instar Larvae of A.australis, resulting from Simultaneous Illumination Shocks.(χ^2 tests)(See Table 23)

Direction of Simultaneous Illumination Shocks	Quadrant	Movement	p
Dorsal and Lateral	3	Dominant/random	< 0.001
	3	L=D/L> D	< 0.001
	4	Dominant/random	< 0.001
Ventral and Lateral	3	Dominant/random	< 0.001
	3	L > V/L=V	0.95-0.05
	4	Dominant/random	< 0.001
	4	L > V/L=V	0.05-0.01

TABLE 25

The Influence of Direction and Pattern of Illumination Shock on the Direction of Movement of Fourth Instar Larvae of A.aegypti at 30°C.

Illumination Shocks		Direction of Movement (%)				
Direction	Pattern	Quadrant 0	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Dorsal and Lateral	Sequential	2	0	10	86	2
	Simultaneous	4	0	0	96	0
Ventral and Lateral	Sequential	4	0	0	2	94
	Simultaneous	2	0	0	28	70
Expected random Percentage		20	20	20	20	20

Direction of Movement expressed as % of larvae in each quadrant after five seconds.

TABLE 26

Analyses of the Influence of Direction and Pattern of Illumination Shock on the Direction of Movement of Fourth Instar Larvae of A.aegypti at 30°C.(χ^2 tests) (see Table 25)

Illumination Shocks		p
Direction	Pattern	
Dorsal and Lateral	Sequential/random	< 0.001
	Simultaneous/random	< 0.001
	Sequential/simultaneous	0.01-0.001
Ventral and Lateral	Sequential/random	< 0.001
	Simultaneous/random	< 0.001
	Sequential/simultaneous	< 0.001

TABLE 27

Dominance of Direction of Illumination Stimuli on the Direction of Movement of Fourth Instar Larvae of A.aegypti, resulting from Simultaneous Illumination Shocks. (See Table 26)

Directions of Simultaneous Illumination Shocks	Quadrant	Dominance of Direction of Illumination %		
		L = D	L > D	D > L
Dorsal and Lateral	1	0	0	0
	2	0	0	0
	3	50	40	10
	4	0	0	0
		L = V	L > V	V > L
Ventral and Lateral	1	0	0	0
	2	0	0	0
	3	57	43	0
	4	46	43	11
Expected random percentage		33	33	33

L = Lateral
D = Dorsal
V = Ventral

TABLE 28

Analyses of Dominance of Direction of Illumination Stimuli on the Direction of Movement of Fourth Instar Laryae of A.aegypti, resulting from Simultaneous Illumination Shocks. (χ^2 tests)(See Table 27)

Direction of Simultaneous Illumination Shocks	Quadrant	Movement	p
Dorsal and Lateral	3	Dominant/random	< 0.001
	3	L = D/L > D	0.95-0.05
Ventral and Lateral	3	Dominant/random	< 0.001
	3	L=V/L > V	0.95-0.05
	4	Dominant/random	< 0.001
	4	L=V/L > V	0.95-0.05

TABLE 29

The Influence of Direction and Pattern of Illumination Shock on the Direction of Movement of Fourth Instar Larvae of C.fatigans at 30°C

Illumination Shocks		Direction of Movement (%)				
Direction	Pattern	Quadrant 0	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Dorsal and Lateral	Sequential	4	0	56	38	2
	Simultaneous	0	4	4	64	28
Ventral and Lateral	Sequential	0	2	6	4	88
	Simultaneous	0	2	4	22	72
Expected random Percentage		20	20	20	20	20

Direction of Movement expressed as % of larvae in each quadrant after five seconds.

TABLE 30

Analyses of the Influence of Direction and Pattern of Illumination Shock on the Direction of Movement of Fourth Instar Larvae of C.fatigans at 30°C. (χ^2 tests) (see Table 29)

Illumination Shocks		p
Direction	Pattern	
Dorsal and Lateral	Sequential/random	< 0.001
	Simultaneous/random	< 0.001
	Sequential/simultaneous	< 0.001
Ventral and Lateral	Sequential/random	< 0.001
	Simultaneous/random	< 0.001
	Sequential/simultaneous	0.01–0.001

TABLE 31

Dominance of Direction of Illumination Stimuli on the Direction of Movement of Fourth Instar Larvae of C.fatigans, resulting from Simultaneous Illumination Shocks (see Table 30).

Directions of Simultaneous Illumination Shocks	Quadrant	Dominance of Direction of Illumination %		
		L = D	L > D	D > L
Dorsal and Lateral	1	100	0	0
	2	0	50	50
	3	62	31	7
	4	29	29	42
		L = V	L > V	V > L
Ventral and Lateral	1	100	0	0
	2	100	0	0
	3	27	55	18
	4	58	28	14
Expected random percentage		33	33	33

L = Lateral
D = Dorsal
V = Ventral

TABLE 32

Analyses of Dominance of Direction of Illumination Stimuli on the Direction of Movement of Fourth Instar Larvae of C.fatigans, resulting from Simultaneous Illumination Shocks. (χ^2 tests). (See Table 31)

Directions of Simultaneous Illumination Shocks	Quadrant	Movement	p
Dorsal and Lateral	1	Dominant/random	< 0.001
	2	Dominant/random	< 0.001
	2	L > D/D > L	> 0.9
	3	Dominant/random	< 0.001
	3	L=D/L > D	0.01-0.001
	4	Dominant/random	0.95-0.05
	4	D > L/L > D	0.95-0.05
Ventral and Lateral	1	Dominant/random	< 0.001
	2	Dominant/random	< 0.001
	3	Dominant/random	< 0.001
	3	L > V/L=V	0.01-0.001
	4	Dominant/random	< 0.001
	4	L=V/L > V	0.01-0.001

NaCl/litre; C.f. 0.088 gm. NaCl/litre tap water) was not lethal to A.vigilax or A.australis.

The influence of temperature on L.E. 50 of upper lethal salinities of fourth instar larvae of A.aegypti and C.fatigans is shown in Table 12 and Figure 12, and the analyses of the influence of temperature on the L.E. 50 of these salinities may be seen in Table 13. The responses of A.aegypti to the upper lethal salinities were not significantly different from those of C.fatigans (Table 13b).

At 25°C and 30°C the L.E. 50's of the 10%, 20% and 30% NaCl solutions for A.vigilax and A.australis were obviously greater than those for A.aegypti and C.fatigans. (Tables 10, 12, and 14, and Figures 13 and 14). Evaporation from the NaCl solutions during the experiments was negligible. (Table 15).

3.22 Behaviour Experiments

The fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans were negatively phototropic to dorsal, ventral and lateral illumination shocks. (Table 16).

The influence of direction and pattern of illumination shock on the fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans at 30°C shown in tables 17, 21, 25 and 29 respectively, and the analyses of this influence are given in tables 18, 22, 26 and 30.

The dominance of direction of illumination stimuli on direction of movement of these larvae resulting from simultaneous dorsal and lateral and ventral and lateral illumination shocks at 30°C is shown in Tables 19, 23, 27 and 31 respectively, and the analyses of these are given in Tables 20, 24, 28 and 32.

Tables 33, 39, 45 and 51 show the influence of both direction and pattern of dorsal, ventral and lateral illumination shock together with

TABLE 33

The Influence of both Direction and Pattern of Illumination Shock together with Mechanical Shock on the Behaviour of Fourth Instar Larvae of A.vigilax at 30°C

Direction of Illumination Shock	Pattern of Illumination with Mechanical Shock	Behaviour (%)		
		Nil	Undirected movement	Directed movement
Dorsal	Sequential	0	100	0
Ventral	Sequential	6	82	12
Lateral	Sequential	2	26	72
	Simultaneous	0	0	100
Expected random percentage		33	33	33

Reaction of larvae expressed as % that moved during five seconds.

TABLE 34

Analyses of the Influence of both Direction and Pattern of Illumination Shock together with Mechanical Shock on the Behaviour of Fourth Instar Larvae of A.vigilax at 30°C (χ^2 -tests) (See Table 33)

Direction of Illumination Shock	Pattern of Illumination with Mechanical Shocks	Behaviour	p
Dorsal	Sequential	reaction/random	< 0.001
Ventral	Sequential	reaction/random	< 0.001
		directed/undirected movement movement	< 0.001
Lateral	Sequential	reaction/random	< 0.001
		directed/undirected movement movement	< 0.001
	Simultaneous	reaction/random	< 0.001
	Sequential/Simultaneous	Behaviour	< 0.001

TABLE 35

Influence of Sequential and Simultaneous Lateral Illumination Shock together with Mechanical Shock, on the Direction of Movement of Fourth Instar Larvae of A.vigilax at 30°C. (See Table 33).

Sequence of Lateral Illumination with Mechanical Shock	Direction of Movement (%)				
	Quadrant 0	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Sequential	2	0	0	90	8
Simultaneous	0	0	2	80	18
Expected random percentage	20	20	20	20	20

TABLE 36

Analyses of the Influence of Sequential and Simultaneous Illumination Shock together with Mechanical Shock, on the Direction of Movement of Fourth Instar Larvae of A.vigilax at 30°C (χ^2 tests) (see Table 35)

Pattern of Lateral Illumination with Mechanical Shock	Movement	p
Sequential	Directed/random	< 0.001
	Quadrant 3/Quadrant 4	< 0.001
Simultaneous	directed/random	< 0.001
	Quadrant 3/Quadrant 4	< 0.001
Sequential/Simultaneous	Directed movement	0.05-0.01

TABLE 37

Dominance of Light and Gravity Stimuli on the Direction of Movement of Fourth Instar Larvae of A.vigilax at 30°C, resulting from Sequential and Simultaneous Lateral Illumination together with Mechanical Shock. (See Table 35)

Pattern of Lateral Illumination with Mechanical Shock	Dominance of Stimuli (%)			
	Quadrant	L > G	L = G	G > L
Sequential	1	0	0	0
	2	0	0	0
	3	100	0	0
	4	100	0	0
Simultaneous	1	0	0	0
	2	0	0	100
	3	58	35	7
	4	78	12	0
Expected random percentage		33	33	33

L = Light
G = Gravity

TABLE 38

Analyses of Dominance of Light and Gravity Stimuli on the Direction of Movement of Fourth Instar Larvae of A.vigilax at 30°C resulting from Sequential and Simultaneous Lateral Illumination together with Mechanical Shock (χ^2 tests) (See Table 37)

Pattern of Lateral Illumination with Mechanical Shocks	Quadrant	Movement	p
Sequential	3	dominant/random	< 0.001
	4	dominant/random	< 0.001
Simultaneous	2	dominant/random	< 0.001
	3	dominant/random	< 0.001
	3	L > G/L=G	0.05-0.01
	4	dominant/random	< 0.001
	4	L > G/L=G	< 0.001

TABLE 39

The Influence of both Direction and Pattern of Illumination Shock together with Mechanical Shock on the Behaviour of Fourth Instar Larvae of A.australis at 30°C

Direction of Illumination Shock	Pattern of Illumination with Mechanical Shock	Behaviour (%)		
		Nil	Undirected movement	Directed movement
Dorsal	Sequential	78	10	12
Ventral	Sequential	32	14	54
Lateral	Sequential	14	6	80
	Simultaneous	0	2	98
Expected random ratio		33	33	33

Reaction of larvae expressed as % that moved during five seconds.

TABLE 40

Analyses of the Influence of both Direction and Pattern of Illumination Shock together with Mechanical Shock on the Behaviour of Fourth Instar Larvae of A.australis at 30°C. (χ^2 tests) (See Table 39)

Direction of Illumination Shock	Pattern of Illumination with Mechanical Shocks	Behaviour	p
Dorsal	Sequential	reaction/random	<0.001
		nil/directed movement	<0.001
Ventral	Sequential	reaction/random	<0.001
		directed movement/nil	<0.001
Lateral	Sequential	reaction/random	<0.001
		directed movement/nil	<0.001
	Simultaneous	reaction/random	<0.001
		directed/undirected movement/movement	<0.001
	Sequential/Simultaneous	Behaviour	<0.001

TABLE 41

Influence of Sequential and Simultaneous Lateral Illumination Shock together with Mechanical Shock, on the Direction of Movement of Fourth Instar Larvae of A.australis at 30°C. (See Table 39).

Sequence of Lateral Illumination with Mechanical Shock	Direction of Movement (%)				
	Quadrant 0	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Sequential	0	2	2	86	8
Simultaneous	2	2	4	82	10
Expected random percentage	20	20	20	20	20

TABLE 42

Analyses of the Influence of Sequential and Simultaneous Illumination Shock together with Mechanical Shock, on the Direction of Movement of Fourth Instar Larvae of A.australis at 30°C. (χ^2 tests) (See Table 41)

Pattern of Lateral Illumination with Mechanical Shock	Movement	p
Sequential	directed/random	< 0.001
	Quadrant 3/Quadrant 4	< 0.001
Simultaneous	directed/random	< 0.001
	Quadrant 3/Quadrant 4	< 0.001
Sequential/Simultaneous	Directed	0.05-0.01

TABLE 43

Dominance of Light and Gravity Stimuli on the Direction of Movement of Fourth Instar Larvae of A.australis at 30°C, resulting from Sequential and Simultaneous Lateral Illumination together with Mechanical Shock. (See Table 41).

Pattern of Lateral Illumination with Mechanical Shock	Dominance of Stimuli (%)			
	Quadrant	L > G	L = G	G > L
Sequential	1	100	0	0
	2	100	0	0
	3	88	0	12
	4	100	0	0
Simultaneous	1	100	0	0
	2	0	0	100
	3	44	49	7
	4	60	40	0
Expected random percentage		33	33	33

L = Light
G = Gravity

TABLE 44

Analyses of Dominance of Light and Gravity Stimuli on the Direction of Movement of Fourth Instar Larvae of A.australis at 30°C, resulting from Sequential and Simultaneous Lateral Illumination together with Mechanical Shock. (χ^2 tests). (See Table 43)

Pattern of Lateral Illumination with Mechanical Shock	Quadrant	Movement	p
Sequential	1	dominant/random	< 0.001
	2	dominant/random	< 0.001
	3	dominant/random	< 0.001
	4	dominant/random	< 0.001
Simultaneous	1	dominant/random	< 0.001
	2	dominant/random	< 0.001
	3	dominant/random	< 0.001
	3	L=G/L > G	0.95-0.05
	4	dominant/random	< 0.001
	4	L>G/L=G	0.05-0.01

TABLE 45

The Influence of both Direction and Pattern of Illumination Shock together with Mechanical Shock on the Behaviour of Fourth Instar Larvae of A.aegypti at 30°C

Direction of Illumination Shock	Pattern of Illumination with Mechanical Shock	Behaviour (%)		
		Nil	Undirected movemrt	Directed movement
Dorsal	Sequential	0	100	0
Ventral	Sequential	0	100	0
Lateral	Sequential	0	22	78
	Simultaneous	0	0	100
Expected random ratio		33	33	33

Reaction of larvae expressed as % that moved during five seconds.

TABLE 46

Analyses of the Influence of both Direction and Pattern of Illumination Shock together with Mechanical Shock on the Behaviour of Fourth Instar Larvae of A.aegypti at 30°C. (χ^2 tests) (See Table 45).

Direction of Illumination Shock	Pattern of Illumination with Mechanical Shocks	Behaviour	p
Dorsal	Sequential	reaction/random	< 0.001
Ventral	Sequential	reaction/random	<0.001
Lateral	Sequential	reaction/random	<0.001
		directed/undirected movement movement	<0.001
	Simultaneous	reaction/random	<0.001
	Sequential/Simultaneous	Behaviour	<0.001

TABLE 47

Influence of Sequential and Simultaneous Lateral Illumination together with Mechanical Shock on the Direction of Movement of Fourth Instar Larvae of A.aegypti at 30°C. (See Table 45).

Sequence of Lateral Illumination with Mechanical Shock	Direction of Movement (%)				
	Quadrant 0	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Sequential	0	0	0	84	16
Simultaneous	0	0	0	74	26
Expected random percentage	20	20	20	20	20

TABLE 48

Analyses of the Influence of Sequential and Simultaneous Illumination Shock together with Mechanical Shock, on the Direction of Movement of Fourth Instar Larvae of A.aegypti at 30°C. (χ^2 tests) (See Table 47)

Pattern of Lateral Illumination with Mechanical Shock	Movement	p
Sequential	directed/random	< 0.001
	Quadrant 3/Quadrant 4	< 0.001
Simultaneous	directed/random	< 0.001
	Quadrant 3/Quadrant 4	< 0.001
Sequential/Simultaneous	Directed	< 0.001

TABLE 49

Dominance of Light and Gravity Stimuli on the Direction of Movement of Fourth Instar Larvae of A.aegypti at 30°C, resulting from Sequential and Simultaneous Lateral Illumination together with Mechanical Shock. (See Table 47).

Pattern of Lateral Illumination with Mechanical Shock	Dominance of Stimuli (%)			
	Quadrant	L > G	L = G	G > L
Sequential	1	0	0	0
	2	0	0	0
	3	100	0	0
	4	100	0	0
Simultaneous	1	0	0	0
	2	0	0	0
	3	51	46	3
	4	100	0	0
Expected random percentage		33	33	33

L = Light
G = Gravity

TABLE 50

Analyses of Dominance of Light and Gravity Stimuli on the Direction of Movement of Fourth Instar Larvae of A.aegypti at 30°C, resulting from Sequential and Simultaneous Lateral Illumination together with Mechanical Shock. (χ^2 tests). (See Table 49).

Pattern of Lateral Illumination with Mechanical Shocks	Quadrant	Movement	p
Sequential	3	dominant/random	< 0.001
	4	dominant/random	< 0.001
Simultaneous	3	dominant/random	< 0.001
	3	L > G/L=G	0.95-0.05
	4	dominant/random	< 0.001

TABLE 51

The Influence of both Direction and Pattern of Illumination Shock together with Mechanical Shock on the Behaviour of Fourth Instar Larvae of C.fatigans at 30°C

Direction of Illumination Shock	Pattern of Illumination with Mechanical Shock	Behaviour (%)		
		Nil	Undirected movement	Directed movement
Dorsal	Sequential	14	80	6
Ventral	Sequential	12	82	6
Lateral	Sequential	0	8	92
	Simultaneous	0	0	100
Expected random ratio		33	33	33

Reaction of larvae expressed as % that moved during five seconds.

TABLE 52

Analyses of the Influence of both Direction and Pattern of Illumination Shock together with Mechanical Shock on the Behaviour of Fourth Instar Larvae of C.fatigans at 30°C. (χ^2 tests) (See Table 51).

Direction of Illumination Shock	Pattern of Illumination with Mechanical Shocks	Behaviour	p
Dorsal	Sequential	reaction/random	<0.001
		undirected/nil movement	<0.001
Ventral	Sequential	reaction/random	<0.001
		undirected/nil movement	<0.001
Lateral	Sequential	reaction/random	<0.001
		directed/undirected movement movement	<0.001
	Simultaneous	reaction/random	<0.001
	Sequential/Simultaneous	Behaviour	0.01-0.001

TABLE 53

Influence of Sequential and Simultaneous Lateral Illumination together with Mechanical Shock on the Direction of Movement of Fourth Instar Larvae of C.fatigans at 30°C. (See Table 51).

Sequence of Lateral Illumination with Mechanical Shock	Direction of Movement (%)				
	Quadrant 0	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Sequential	0	4	0	92	4
Simultaneous	0	0	0	88	12
Expected random percentage	20	20	20	20	20

TABLE 54

Analyses of the Influence of Sequential and Simultaneous Illumination Shock together with Mechanical Shock, on the Direction of Movement of Fourth Instar Larvae of C.fatigans at 30°C. (χ^2 tests) (See Table 53).

Pattern of Lateral Illumination with Mechanical Shock	Movement	p
Sequential	directed/random	<0.001
	Quadrant 3/Quadrant 4	<0.001
Simultaneous	directed/random	<0.001
	Quadrant 3/Quadrant 4	<0.001
Sequential/Simultaneous	Directed	0.05-0.01

TABLE 55

Dominance of Light and Gravity Stimuli on the Direction of Movement of Fourth Instar Larvae of C.fatigans at 30°C, resulting from Sequential and Simultaneous Lateral Illumination together with Mechanical Shock. (See Table 53).

Pattern of Lateral Illumination with Mechanical Shock	Dominance of Stimuli (%)			
	Quadrant	L > G	L = G	G > L
Sequential	1	100	0	0
	2	0	0	0
	3	100	0	0
	4	50	0	50
Simultaneous	1	0	0	0
	2	0	0	0
	3	57	38	5
	4	0	83	17
Expected random percentage		33	33	33

L = Light
G = Gravity

TABLE 56

Analyses of Dominance of Light and Gravity Stimuli on the Direction of Movement of Fourth Instar Larvae of C.fatigans at 30°C, resulting from Sequential and Simultaneous Lateral Illumination together with Mechanical Shock. (χ^2 tests). (See Table 55).

Pattern of Lateral Illumination with Mechanical Shock	Quadrant	Movement	p
Sequential	1	dominant/random	< 0.001
	3	dominant/random	< 0.001
	4	dominant/random	< 0.001
	4	L > G/G > L	> 0.9
Simultaneous	3	dominant/random	< 0.001
	3	L > G/L=G	< 0.001
	4	dominant/random	< 0.001
	4	L=G/G > L	< 0.001

mechanical shock on the behaviour of fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans respectively, whilst the analyses of these results are given in Tables 34, 40, 46 and 52 respectively. For the influence of sequential and simultaneous lateral illumination shock together with mechanical shock on the direction of movement of fourth instar larvae of the four species, see Tables 35, 41, 47 and 53, and for the analyses of these results see Tables 36, 42, 48 and 54. Dominance of light and gravity stimuli on the direction of movement of fourth instar larvae of the four species, resulting from sequential and simultaneous lateral illumination together with mechanical shock are shown in Tables 37, 43, 49 and 55, and the anlayses of this dominance are given in Tables 38, 44, 50 and 56.

For the influence of direction of illumination, salinity and temperature on the rate of movement of fourth instar larvae of A.vigilax see Table 57 and Figures 16, 17, 18 and 19. The analyses of the influences of these factors are seen in Table 59.

The results of the comparison of the rates of movement at 30°C of the fourth instar larvae of the four species are shown in Table 60. Table 61 gives the analyses of these results.

Stimulus satiation to lateral illumination did not occur in fourth instar larvae of A.vigilax (Table 62).

TABLE 57

The Influence of Direction of Illumination, Salinity, Temperature and Age of Larvae and Pupae on the Rate of Movement of A.vigilax

Direction of Illumination	Salinity (% NaCl)	Temperature (°C)	Instar	Mean Rate (ins./sec.)	Variance
Lateral	0.9	25	4	1.99	0.15
		30	4	2.36	0.12
	1.8	25	4	2.00	0.19
		30	1	0.72	0.11
			2	1.25	0.18
			3	1.90	0.06
			4	2.58	0.37
			P	1.74	0.45
	3.6	25	4	2.09	0.09
		30	4	2.19	0.13
Dorsal	0.9	25	4	2.44	0.18
		30	4	2.46	0.15
	1.8	25	4	2.04	0.16
		30	1	0.95	0.11
			2	1.17	0.04
			3	1.97	0.27
			4	2.70	0.24
			P	3.37	0.67
	3.6	25	4	1.67	0.09
		30	4	2.11	0.14

TABLE 58

Analyses of the Direction of Illumination and Age of Larvae and Pupae, on the Rate of Movement of A.vigilax at 30°C and 1.8% NaCl (t-tests)

Direction of Illumination	Instars	p
Lateral	1/2	< 0.001
	2/3	< 0.001
	3/4	< 0.001
	4/P	< 0.001
Dorsal	1/2	< 0.001
	2/3	< 0.001
	3/4	< 0.001
	4/P	< 0.001
L/D	1	0.01-0.001
L/D	2	0.3 -0.2
L/D	3	0.4 -0.3
L/D	4	0.3 -0.2
L/D	P	< 0.001

TABLE 59

Analyses of the Influence of Salinity, Temperature and Direction of Illumination on the Rate of Movement of Fourth Instar Larvae of A.vigilax. (Checkerboard of t values and probabilities).

Direction Of Illumination			Lateral						Dorsal					
Temperature			25°C			30°C			25°C			30°C		
	Salinity		0.9‰NaCl	1.8‰NaCl	3.6‰NaCl	0.9‰NaCl	1.8‰NaCl	3.6‰NaCl	0.9‰NaCl	1.8‰NaCl	3.6‰NaCl	0.9‰NaCl	1.8‰NaCl	3.6‰NaCl
Lateral	25°C	0.9‰NaCl	t p											
		1.8‰NaCl	t p	0.119 > 0.9										
		3.6‰NaCl	t p	1.443 0.2–0.1	1.202 0.3–0.2									
	30°C	0.9‰NaCl	t p	5.035 < 0.001										
		1.8‰NaCl	t p		5.480 < 0.001		1.818 0.1–0.05							
		3.6‰NaCl	t p			1.508 0.2–0.1	2.404 0.05–0.02	3.899 < 0.001						
Dorsal	25°C	0.9‰NaCl	t p	5.540 < 0.001										
		1.8‰NaCl	t p		4.780 < 0.001				4.850 < 0.001					
		3.6‰ NaCl	t p			6.998 < 0.001			10.480 < 0.001	5.232 < 0.001				
	3 0C	0.9‰NaCl	t p				1.081 0.3–0.2		0.246 0.9–0.8					
		1.8‰NaCl	t p					1.086 0.3–0.2		7.377 < 0.001		2.717 0.01–0.001		
		3.6‰NaCl	t p					1.088 0.3–0.2			6.488 < 0.001	2.757 0.01–0.001	6.767 < 0.001	

TABLE 60

The Influence of Direction of Illumination on the Rate of Movement of Fourth Instar Larvae of A.vigilax, A.australis, A.aegypti and C.fatigans at 30°C.

Species	Direction of Illumination	Mean Rate of Movement	Variance
A.vigilax 3.6% NaCl	Lateral	2.19	0.13
	Dorsal	2.11	0.14
A.australis 3.6% NaCl	Lateral	2.75	0.92
	Dorsal	2.06	0.44
A.aegypti Distilled Water	Lateral	1.39	0.18
	Dorsal	1.39	0.04
C.fatigans Distilled Water	Lateral	1.76	0.21
	Dorsal	1.57	0.17

Figure 16. Influence of temperature and salinity on the rate of movement of Fourth instar larvae of A.vigilax under dorsal illumination.

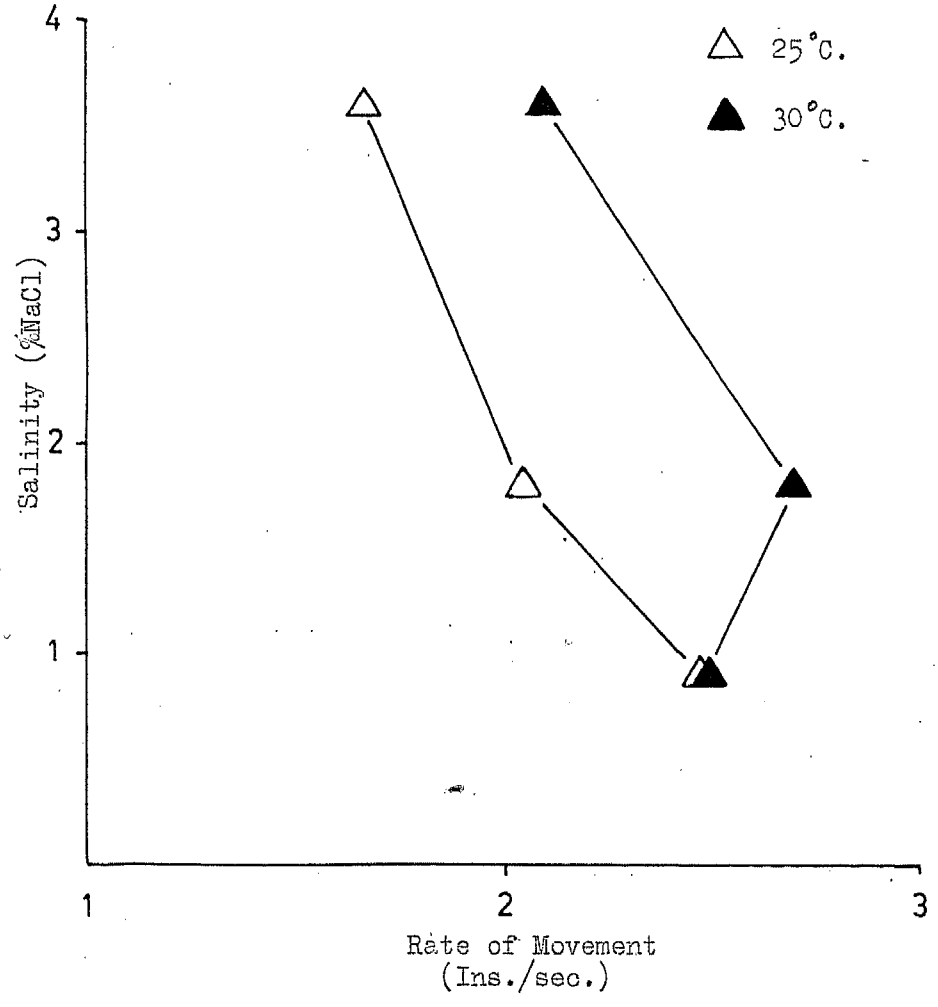


Figure 17. Influence of Temperature and Salinity on the Rate of Movement of Fourth Instar Larvae of A.vigilax under Lateral Illumination.

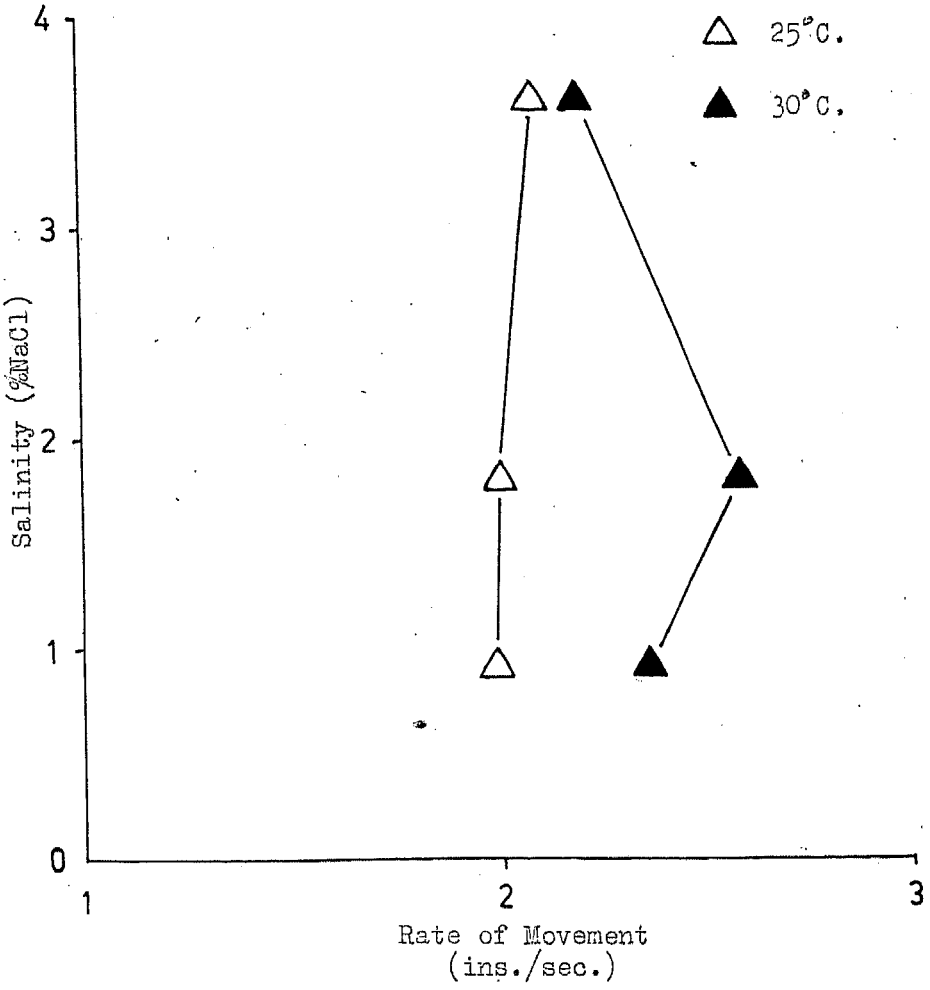


Figure 18. Influence of direction of illumination on the rate of movement of Fourth instar larvae of A.vigilax at 25°C.

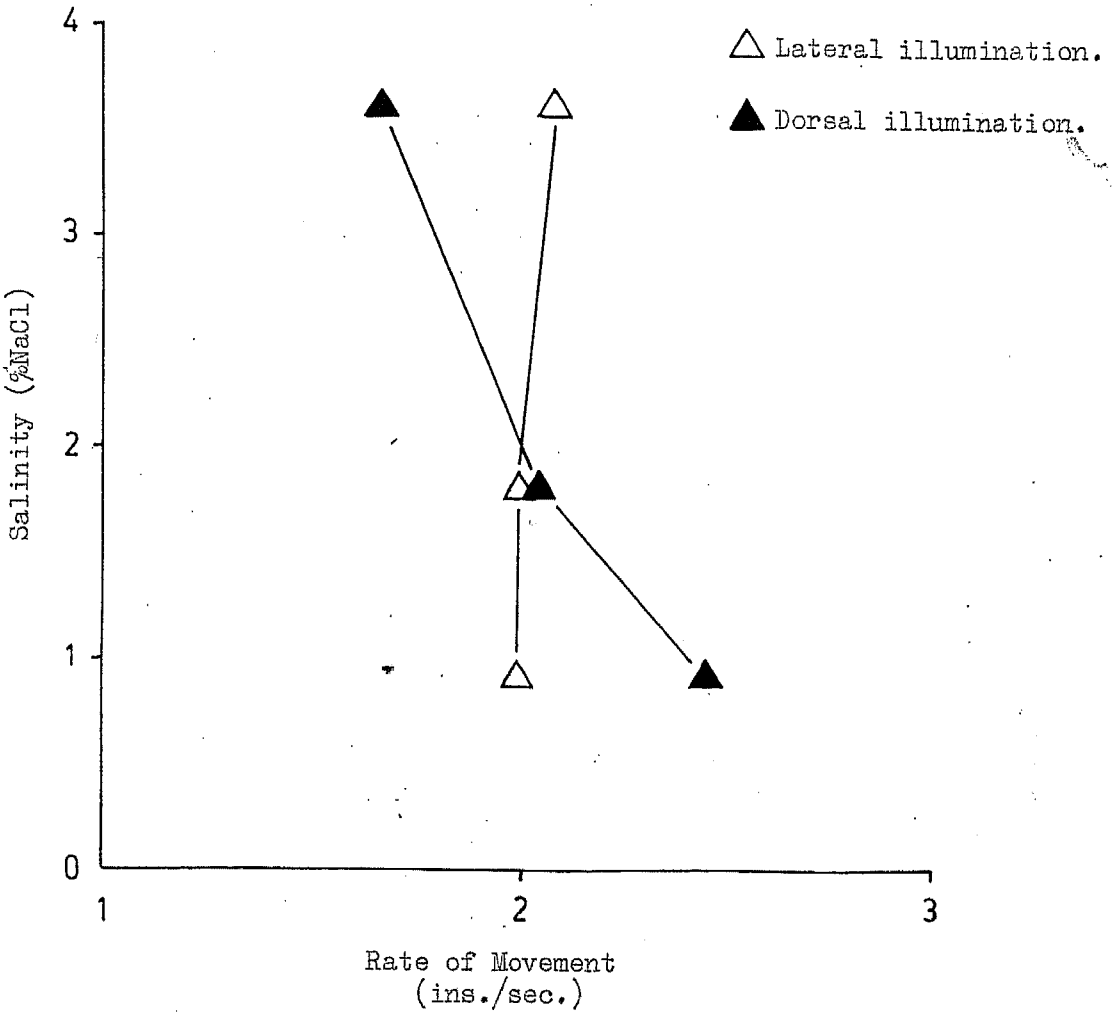


Figure 19. Influence of Direction of Illumination on the rate of Movement of Fourth Instar Larvae of A.vigilax at 30°C.

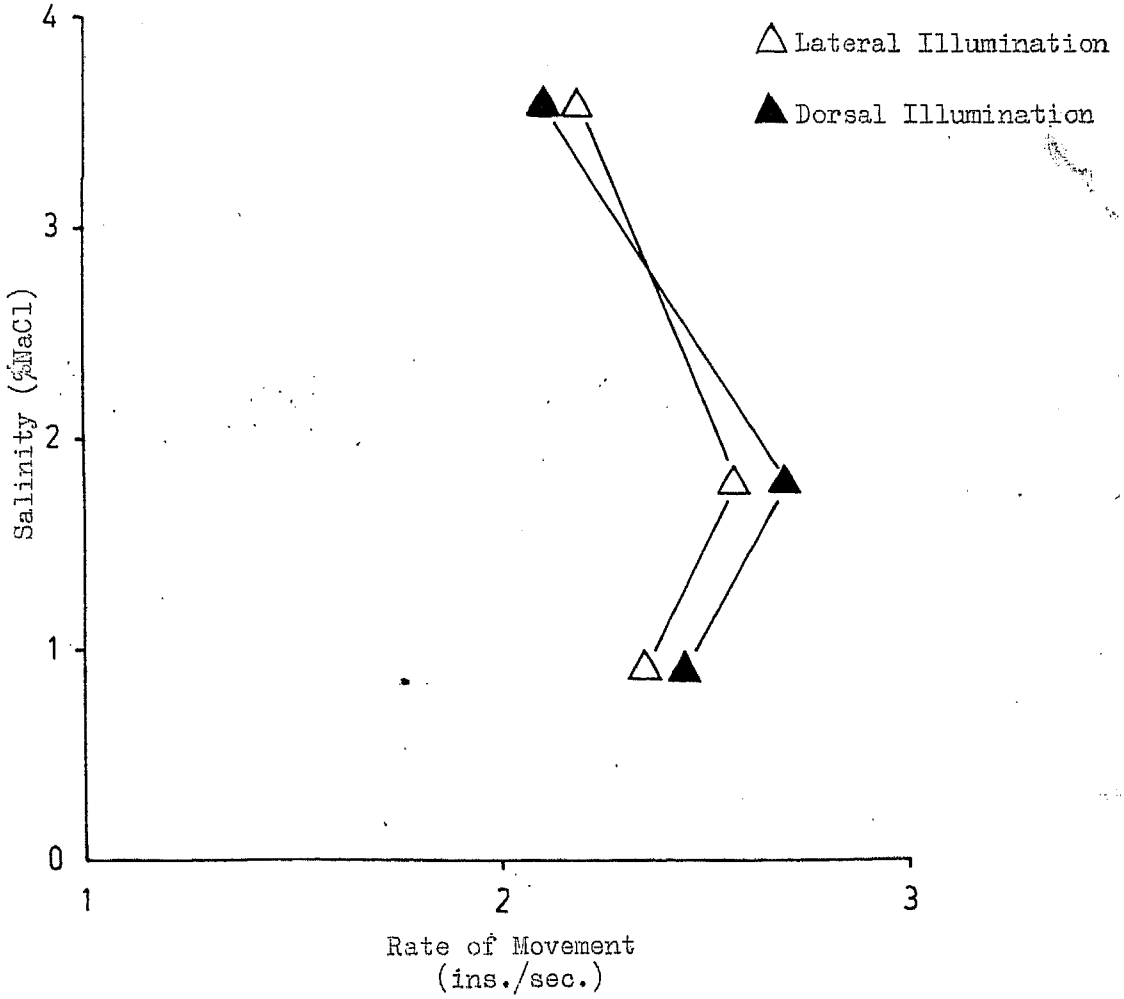


TABLE 61

Intraspecific and Interspecific Analyses of Influence of Direction of Illumination on the Rate of Movement of Fourth Instar Larvae of A.vigilax, A.australis, A.aegypti and C.fatigans at 30°C. (Checker board of t values and probabilities.)

Of Illumination			Lateral				Dorsal			
Direction	Species		A.vigilax	A.australis	A.aegypti	C.fatigans	A.vigilax	A.australis	A.aegypti	C.fatigans
Lateral	A.vigilax	t p								
	A.australis	t p	3.865 < 0.001							
	A.aegypti	t p	10.160 < 0.001	9.168 < 0.001						
	C.fatigans	t p	5.213 < 0.001	6.586 < 0.001	4.190 < 0.001					
Dorsal	A.vigilax	t p	1.132 0.3-0.2	4.717 < 0.001	8.999 < 0.001	4.183 < 0.001				
	A.australis	t p	1.085 0.3-0.2	4.183 0.001	6.016 < 0.001	2.631 0.1-0.001	0.460 0.7-0.6			
	A.aegypti	t p	-	-	-	-	-	-		
	C.fatigans	t p	8.004 < 0.001	7.993 < 0.001	2.151 0.05-2.02	2.179 0.05-0.02	6.858 <0.001	4.375 <0.001	—	

TABLE 62

The Influence of Temperature and Sequence of Trials on Stimulus Satiation to Lateral Illumination of Fourth Instar Larvae of *A. vigilax* in 1.8% NaCl.

Temperature	Sequence of 10 Trials per Larva	Replicates	Mean Rates of 10 Trials Ins./Sec.	Variance	Median	p
25	Consecutive	1	2.01	0.10	1.83	
		2	2.24	0.15	2.14	
		3	2.12	0.18	2.38	
		4	2.53	0.12	2.59	
		5	1.79	0.19	1.67	
		6	2.09	0.12	2.05	
		7	2.25	0.09	2.34	
		8	2.40	0.15	2.49	
		9	2.34	0.06	2.49	
		10	2.47	0.11	2.60	
					μ 2.26	>0.10
					σ^2 0.32	
30	Consecutive	1	1.91	0.40	1.84	
		2	1.94	0.53	1.72	
		3	2.54	0.24	2.34	
		4	1.37	0.32	1.53	
		5	3.23	0.35	3.22	
		6	2.16	0.31	2.02	
		7	2.42	0.18	2.14	
		8	2.02	0.52	2.07	
		9	2.42	0.12	2.49	
		10	2.20	0.03	2.20	
					μ 2.16	>0.10
					σ^2 0.22	
30	Non-consecutive (15 minutes between trials)	1	2.70	0.09	2.60	
		2	1.83	0.82	1.90	
		3	2.24	0.21	2.37	
		4	2.38	0.38	2.33	
		5	1.63	0.65	1.65	
		6	2.69	0.54	3.09	
		7	1.95	0.40	2.06	
		8	2.30	0.08	2.32	
		9	2.84	0.74	2.48	
		10	2.41	0.03	2.36	
					μ_2 2.32	>0.10
					σ^2 0.15	

4. DISCUSSION

Since the aquatic immature stages of species of mosquitoes are obliged to occupy certain ecological habitats which are determined by the sites of oviposition chosen by the gravid females in response to environmental stimuli, natural selection will favour the survival of those individuals whose genotypes will permit their adaptation to the environmental factors associated with those habitats. The adaptation displayed by the surviving individuals may be either of a physiological or of a behavioural form.

In this investigation the physiological survival and behavioural responses of the four selected species to certain environmental factors were studied in the laboratory, to correlate these responses with survival.

Many field workers do not consider that behavioural responses shown in the laboratory, which is an abnormal environment, are significant. However, other authors (including Muirhead-Thomson, 1940) have observed in various species responses exhibited to stimuli in the laboratory, which were comparable with those responses exhibited in the field.

If a behaviour pattern or tropism, or a physiological response is inherent, it should be exhibited in the laboratory as well as in the field.

The response to an external stimulus by an animal at the time of application of the stimulus depends on its physiological state, and the environment. In mosquito larvae, the nutritional state (Miller, 1940) and age (Omardeen, 1957) have been shown to affect behaviour, and in addition, these factors must surely affect physiology. In the laboratory studies, nutrition was assumed to be adequate, since the larvae were fed

prior to, and during the experiments with finely ground "K9" dog biscuits, and age was controlled in the experiments in which the responses of the four species were compared, by using only fourth instar larvae. Since environmental conditions also affect behaviour and physiology, care was taken to ensure that all these factors (viz. light, temperature, salinity), with the exception of those varied experimentally, were kept constant. Thus it may be assumed that all the physiological survival and behavioural responses to the selected environmental factors exhibited by the immature stages of the four species in the laboratory were valid, and comparable with those shown in the field.

However, before any physiological or behavioural responses of the immature stages of the four species could be correlated with survival, it was necessary to have some knowledge of their ecology. Therefore, the ecology of the immature stages of A.vigilax was determined by field observations (Section 2.2), whilst literature (O'Gower, 1956, 1960; Woodhill and Pasfield, 1941) (Section 1.2) provided the necessary information about the other three species.

It has been observed that the tidal salt marshes which form the breeding place of A.vigilax are subjected to great diurnal and seasonal variations in temperature (Table 1, Figures 4, 5, 6, 7 and 8), moisture content (Tables 1 and 2) and salinity (Tables 1 and 3), and analyses have shown that variations in the climatic conditions associated with these marshes were caused by associations between certain of these environmental factors (Table 9). Thus here, the survival of the immature stages would be greatly influenced by the possession of physiological and behavioural adaptations, which would enable them to overcome the adverse effects of

these climatic factors, and to escape detection by the predaceous A.alternans (Section 1.21).

Physiological adaptations have been observed in both the egg and larval stages of A.vigilax.

Results of transect soil sampling across pools (Tables 5 and 6) indicate that the eggs of this species were deposited on soil partly covered by emergent vegetation (e.g. Salicornia australis), which, by retaining shade would reduce moisture loss from the soil and eggs (Section 2.231). These findings were in agreement with those of Bradley and Travis (1942, 1943), Elmore and Fay (1958), Bodman and Gannin (1950), and Horsfall (1961) (Section 1.21).

Since the marshes may be repeatedly flooded by either rain, or sea water (Sections 2.221, 2.222), the embyros in the eggs must have the ability to hatch under a wide range of salinities. This ability has been proved by the detection of first instar larvae in the pools on the days immediately following such floodings.

The unsuccessful attempts at hatching eggs from soil samples taken from the field during late autumn and winter (Table 5), together with the observations (Tables 1 and 7, and for analyses Table 9) of a great decline in the population densities of the immature stages in the pools during these seasons of the year could indicate that a diapause mechanism operates in the egg stage. Such a mechanism would favour survival of this species under the adverse temperatures of winter.

Winter diapause has been observed in the adult female (Bullock, Murdoch, Fowler, and Brazzel, 1959; Danilevskiĭ, 1958; Wallis, 1959), the larval stages (Chapman, 1959b; Howard, Dyar and Knab, 1912; Mellanby,

1940; Mitchell, 1907) and the egg stage (Beckel, 1958; Gillett, 1955) of many species of mosquitoes from the Northern Temperate Zone. However, in those species breeding in salt marshes in the Northern Temperate Zone, diapause usually occurs in the egg stage (Khelevin, 1959; Marshall, 1938; Telford, 1957, 1958). Therefore it is reasonable to assume that such a mechanism would occur in the egg stage of A.vigilax.

The occasional occurrence of some immature stages in the pools during the winter months (Table 1 and 7) was probably due to the possession by certain individuals in the population of a physiology permitting their hatching and development under temperature conditions adverse for the remainder of the population. The rates of development of these individuals (deduced from the weekly determinations of the ratios of first: fourth instar larvae in a pool (Table 7) were much slower than those of individuals hatching during the summer months. Thus it would appear that the rate of development of this species, as in other species of mosquitoes (Lal, 1953 and others) would be directly proportional to the environmental temperature. However, the environmental factor of pH within the limits recorded in the field did not affect the occurrence of the immature stages (Table 3).

Since the larval stages of this species have been recorded in the field over a wide range of salinities from fresh water (Hamlyn-Harris, 1933) to approximately 9.0% NaCl (Table 4), they must have an efficient means of osmoregulation.

Osmoregulation in mosquito larvae, involves active absorption of walter and salts through the anal papillae, which are the only areas of the body which are freely permeable to these substances. It has been shown that in species (including A.vigilax) whose larvae occupy saline habitats,

the anal papillae are reduced in length in comparison with those of species breeding in fresh waters (Hill, 1925; Komp, 1955; Marshall, 1938; Woodhill, 1938, and others). This reduction in length is directly proportional to the environmental salinity (Gibbons, 1932; Wigglesworth, 1938).

Since the rate of absorption of chloride (and other) ions by the anal papillae is a function of their surface area, this rate would be very reduced in those species from saline habitats. This slow rate of absorption would enable these larvae to tolerate for a limited time, and probably survive, temporary lethal elevations in salinity, caused by evaporation, and terminated by rainfall.

The powers of osmoregulation of the fourth instar larvae were demonstrated by their ability to survive for several hours in solutions of NaCl greater than those normally encountered in the field, and to survive completely in fresh water (distilled water plus food) (Table 9, Figures 10, 11).

The observation that 10% NaCl was lethal in the laboratory but not in the field can be explained by considering the factor of acclimatization to salinity. In salt marshes, the larvae acclimatize to the gradual increase in salinity ($\simeq$ 2%/24 hours (Table 3)) of the water caused by evaporation, and thus they would be expected to tolerate higher salinities for a longer time than if suddenly transferred from a low to a high salinity. That acclimatization to salinity does affect survival was proved experimentally, for after acclimatization at a low salinity, the mortality rates of the fourth instar larvae in the upper lethal salinities were significantly greater than those rates observed after acclimatization in a higher non-lethal salinity (Table 9, Figure 11).

In addition, analyses of this data have shown that the mortality

rate of the fourth instar larvae in an upper lethal salinity is a function of the combined effects of previous acclimatization salinity, the upper lethal salinity encountered and its temperature.

In addition to these physiological adaptations to their environmental salinity, the larvae of this species also exhibited behaviour patterns which would have survival value. Results of bi-weekly sampling have shown that irrespective of season, the larvae were not randomly distributed in the pools, but were clumped in areas that either contained emergent vegetation (Salicornia) or were relatively deep (3" - 6½") (Table 8, Figure 9). The probability of the larvae being detected by the predaceous larvae of A.alternans would be very small in both these situations where the light intensity would be reduced and where the soil would act as a camouflage. The negative phototropism (Table 37) would cause the larvae to move to the darker regions of the pool, irrespective of whether or not they were the deepest regions. Whilst the kinesis elicited by mechanical shock and given direction by light and gravity (Tables 33 and 37) would induce them to move away from major disturbances of the water caused by the movement of the giant larvae of A.alternans.

The ineffectiveness of many combinations of normally encountered temperatures and salinities at appreciably altering the comparatively fast rates of movement of the fourth instar larvae under dorsal and lateral illumination (Tables 57 and 59. Figures 16,17, 18 and 19), could have survival value by ensuring that under normal conditions these larvae would always be able to move away from, and escape any predator. However, the rates of movement of the first, second and third instar larvae would be too slow for their escape, from such a situation, whilst pupae might survive by fast vertical movement. (Table 57).

Although stimulus satiation to light shock has been observed in several species of mosquitoes (Mellanby, 1958), it was apparently absent from the behaviour pattern of A.vigilax (Table 62). This absence of stimulus satiation could have survival value for A.vigilax by always permitting their response to sudden changes in light intensity such as would occur as a predator approached.

The aspects of the ecology of A.vigilax which have not been investigated here are the influence of the biotic and edaphic factors on the distribution and abundance of its immature stages, and the oviposition behaviour of the gravid female.

A biotic factor which could influence survival of the immature stages would be that of competition for resources (e.g. food, shade and camouflage from predators, and water surface area for respiration). However, this factor would only be important under conditions of crowding, caused by great evaporation of the water in the pools forming the larval habitats.

Since this investigation was concerned with the study of the ecology of the immature stages only, of A.vigilax, the oviposition behaviour of the gravid female was not determined. However, since it has been shown (O'Gower, 1955, 1957a, 1957b, 1958b; Woodhill, 1941b; and others) that the oviposition site (which eventually becomes the site of the larval habitat) chosen by the gravid female is determined by its behavioural responses to certain environmental stimuli, it is essential that the oviposition behaviour should be determined as soon as this species has been colonized in the laboratory.

A far greater diurnal and seasonal variation in salinity is associated with the marine rock pools forming the breeding place of

A.australis than is encountered in the larval habitat of A.vigilax. Therefore survival of the immature stages of A.australis in their ecological habitat would be greatly influenced by their ability to osmoregulate.

In the larvae of this species the anal papillae, which are the sites of osmoregulation, are vestigial (Woodhill, 1936). Therefore, since the rate of absorption of the chloride (and other) ions through these papillae is a function of their surface area, this rate would be greatly reduced. Thus it would be valid to assume that the duration of a lethal salinity necessary to cause mortality of these larvae would be great in comparison with that needed by other species possessing larger gills.

This assumption has been proved correct, for the fourth instar larvae of A.australis in addition to surviving in fresh water (distilled water + food), survived the upper lethal salinities for a significantly greater duration than did the fourth instar larvae of A.vigilax, A.aegypti and C.fatigans which all possess significantly longer gills (Table 10, Figures 13 and 14). In addition, it was shown that the mortality rate of the fourth instar larvae of A.australis was directly proportional to the upper lethal salinity (Figure 11).

The apparent discrepancies between these results, which showed that solutions of 10% and 20% NaCl were lethal to the fourth instar larvae, and those observations of O'Gower (1960) of the occurrence of the immature stages in rock pools containing water of salinity approximately 24%, are resolved when the factor of acclimatization to salinity is considered. In rock pools evaporation would cause a gradual increase in the salinity of the water. Gradual acclimatization to increasing salinity would enable the larvae to tolerate higher salinities for a greater duration than if suddenly

transferred from a low to a high salinity. It has been proved that acclimatization to salinity does affect survival, for after acclimatization at a low salinity, the mortality rates of the larvae in the upper lethal salinities were significantly greater than those rates recorded in the solutions after acclimatization in a higher non-lethal salinity (Table 9, Figure 11).

An analysis of these results showed that the mortality rate of the fourth instar larvae of this species in an upper lethal salinity was a function of the combined effects of previous acclimatization salinity, the upper lethal salinity encountered, and its temperature.

The significantly greater duration required by the 30% NaCl solution at 25°C to cause mortality of A.vigilax in comparison with that required by A.australis could be accounted for by the fact that since this salinity was greater than that encountered by either species in its ecological habitat, the mode of interaction between the abovementioned factors could be slightly different. Thus these results would not contradict the field observations of O'Gower (1960).

As well as having to withstand great variations in salinity, the immature stages of A.australis in the rock pools are subjected to occasional flushings by large waves at high tide, and by heavy rainfall. The kinesis exhibited by the fourth instar larvae in response to mechanical shock, and given direction by light and gravity (Tables 39 and 43), in addition to the negative phototropic responses of the fourth instar larvae (Table 21), would prevent their being washed away on these occasions.

For, in response to the mechanical shock caused by the water movement the larvae would move down to the deeper sheltered areas of the pool. In addition, their fast rate of lateral movement (Table 60) would be

of an advantage when moving away from the splash of an oncoming wave, which would spread laterally across the pool.

Although the immature stages of A.aegypti usually inhabit fresh water of negligible salinity, they occasionally have been recorded from brackish water by various authors (reviewed by Christophers, 1960). However, Macfie (1914, 1921) found that although a solution of 0.5% NaCl did not affect larvae of this species, diluted sea water of salinity 1.6% was lethal within 24 hours, whilst undiluted seawater caused mortality within 2-4 hours. These results were confirmed by Woodhill (1942), and Nakata (1957), who showed that complete development could only occur in water of salinity 1% or less, although pupae could tolerate a salinity of 7% (Woodhill, 1942).

C.fatigans is another species of mosquito whose immature stages, although usually found in fresh water, have also been recorded from brackish water (Hamlyn-Harris, 1929). In addition Woodhill (1942) showed that complete development was possible in distilled water or water of salinity not exceeding 1%, and that pupae were not affected by a salinity of 7%.

The larvae of these species thus have a very poor osmoregulatory ability. This is caused by their possession (in common with larvae of other species inhabiting fresh water) of long anal papillae, which present a comparatively large surface area for the absorption of chloride ions (Koch, 1938) and water (Wigglesworth, 1933b).

In their ecological habitats, because the level of chloride ions in the water is very low, or negligible, these large anal papillae are not disadvantageous, and the larvae are able to maintain their haemolymph hypertonic to the environment by means of non-chloride solutes (Wigglesworth, 1938). However, in hypertonic media of above 1% salinity death soon occurs. Wigglesworth (1938) believes that the cause of death is the

raising of the chloride content and total osmotic pressure of the haemolymph to the level of the external media, by the great absorption of the chloride ions through the anal papillae. However, the shrunken appearance of these larvae in hypertonic solutions would indicate that their death is caused by loss of water to the surrounding media, i.e. by desiccation.

However, since it has been shown (Woodhill, 1941b) that the gravid females of A.aegypti and C.fatigans usually discriminate against saline habitats as sites of oviposition, the chances that immature stages of these species would be exposed to high salinities would be slight. Therefore, further determination of the mortality rates of the immature stages in lethal salinities would be pointless, unless done as a basis for interspecific comparison of physiological adaptations.

It was for the comparison of physiological adaptations of the two species breeding in different fresh water habitats, viz. A.aegypti and C.fatigans, with those of the two species from different saline habitats, A.vigilax and A.australis, that further experiments were performed.

These investigations on the influence of lethal saline solutions on the mortality rates of the fourth instar larvae of these species gave results comparable with those of Woodhill (1942). In both species, a solution of 5% NaCl was lethal within two hours (Table 12), whilst an increase in salinity caused a decrease in mortality rate (Figure 12).

Analyses of these results showed that in each species, the mortality rate of the fourth instar larvae in a saline environment was a function of the combined effects of the temperature and salinity of the environment.

The behavioural responses of the fourth instar larvae of A.aegypti may also be correlated with survival in their ecological habitat. Throughout certain areas of its distribution this species breeds in protected situations such as water stored underground in tanks and wells, and fire-buckets in warehouses (O'Gower, 1956), where environmental factors would not appreciably limit survival. However, in other areas, under mild weather conditions, the breeding places are unprotected bodies of water, e.g. tins and bottles (O'Gower, 1956). In these latter situations, the environmental factors of rainfall, temperature fluctuations, and evaporation of the water in the containers could have a great influence on the survival of its immature stages.

The negative phototropic responses of the fourth instar larvae (Table 16) would tend to keep them away from the surface of the water in the containers, which, during the summer months could be heated by the sun to a lethal temperature.

In the laboratory, light and gravity were shown to give a directional component to the kinesis exhibited by the larvae in response to mechanical shock. (Tables 45 and 49). This behaviour would ensure that as soon as mechanical shock, in the form of rain splashing into, and causing overflow of the water in the bottles and tins, was received by the larvae, they would move down and away from the light, and thus reduce their chances of being washed away. However, larval mortality would still occur when extreme conditions would cause complete evaporation of the water in the containers.

The observed slow rates of movement of the fourth instar larvae under dorsal and lateral illumination (Table 60) may also be correlated with their ecology. Since the larvae of this species probably lack a specific predator, swift movement would be of no selective advantage to them.

A correlation between the responses of the fourth instar larvae of C.fatigans to the selected environmental factors of light, mechanical shock and gravity, and survival in their ecological habitat was also observed.

The breeding places of this species are open bodies of water polluted with organic matter. Here, the water is dark in colour, usually deep, has a low oxygen content, and possibly has large diurnal and seasonal fluctuations in temperature (Woodhill and Pasfield, 1941). In these situations, temperature variations, rainfall, and extremes of evaporation could affect survival of the immature stages. However, the presence in the pools of organic matter, which would retain moisture for a limited period of time and thus maintain an aquatic environment for the immature stages, would tend to reduce the probability of mortality from dessication.

The negative phototropic responses of the fourth instar larvae to light, irrespective of its direction, (Table 16) would cause the larvae to remain adjacent to, and in the shade of organic matter, and thus probably survive limited durations of extreme evaporation. The kineses elicited by mechanical shock and given a directional component by light and gravity (Tables 51 and 55) would have survival value for them in their ecological habitat. For, in the event of the flushing of their breeding place by heavy rainfall, the mechanical shock resulting from the water movement would cause them to move donwards, and thus prevent them from being washed away.

The comparatively slow rate of movement shown by the fourth instar larvae (Table 60) may also be correlated with their ecological habitat, for, since they lack a specific predator, a fast rate of movement probably would not have any selective survival value.

In addition to the previously discussed physiological and behavioural responses which would obviously have selective value, the fourth

instar larvae exhibited other behavioural patterns in the form of tropisms and kineses, which did not appear to have survival value. However, these behaviour patterns might be advantageous when exhibited in conjunction with other behavioural or physiological responses, or might be linked genetically with other responses which would favour survival of these four species in their ecological habitats. If either of these two postulated associations between responses were to occur, natural selection would ensure that these behavioural responses would be maintained in the populations of the four species (Sinnott, Dunn and Dobzhansky, 1958).

It has been shown that tropisms and kineses exhibited by an animal in response to external stimuli at the time of application of the stimuli, are dependent on the physiological state of the animal, and other environmental factors, in contradistinction to appetitive behaviour such as mating or oviposition, where, although the initial stimulus or drive is due to the physiological state of the animal, this behaviour is dependent for its release on certain environmental stimuli, and will always be released in the same way by those environmental stimuli (Tinbergen, 1958). Therefore, under different environmental and physiological conditions, the larvae might exhibit other tropism or kineses which might favour survival under those conditions.

Previous experiments (unpublished data) have shown that temperature would affect the behavioural responses of the fourth instar larvae of these four species, for, at an upper sub-lethal temperature ($35^{\circ}C$), the larvae remained at the surface of the water in the container, and gave very poor responses to light shock and mechanical shock. This behaviour would favour survival under these conditions, for water at the surface would be cooler

than that below the surface, due to evaporation. Mellanby (1958) observed a similar effect of temperature on the behaviour of larvae of A.aegypti, whose "alarm reactions" (diving movements) to dorsal light shock and mechanical shock were eliminated at very low temperatures ($< 11^{\circ}C$), and were very reduced at an upper sub-lethal temperature ($35^{\circ}C$). In addition, Miller (1940) observed that below $15^{\circ}C$, the larvae of C.pipiens were negatively phototropic, but above this temperature they were positively phototropic; and Omardeen (1957) showed that age of larvae influenced their responses to light.

Further investigations still need to be done to determine the effects on the tropisms and kineses to the other environmental factors, such as increase in salinity (or desiccation), of the larvae of these, and many other species of mosquitoes.

The responses of mosquito larvae to the environmental factors of light shock, mechanical shock, and gravity have been observed by several authors: the most recent observations being those of Folger (1946) and Mellanby (1958). However, because the experiments involved combinations of shock stimuli with gradient stimuli, and as these authors (Folger, 1946; Mellanby, 1958) failed to realize the importance of influence of pattern of application of these stimuli on the responses of the larvae, little useful information was obtained. Although some of the conclusions drawn by these authors agreed with results of my experiments, these conclusions were invalidly based on incorrect experimental procedure.

In my investigations, the effects of pattern (viz. sequential and simultaneous application) of selected combinations of light shock and mechanical shock stimuli on the behaviour of fourth instar larvae of the four species were fully analysed, and thus a far greater knowledge of their behaviour was obtained (Tables 17-56).

The fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans all gave similar negatively phototropic responses to the sequential illumination shocks irrespective of their direction (Tables 17, 21, 25 and 29), and would always give a diving response to mechanical shock in the presence of dorsal illumination. However, when mechanical shock was given in sequence with illumination shocks, the larvae all exhibited agitated kineses, which were given direction by lateral light and gravity. Negative phototropism has been observed in the larvae of many other species of mosquitoes by Folger (1946), Mellanby (1958), and others, whilst positive phototropism has been seen in larvae of C.pipiens at temperatures above 15°C (Miller, 1940).

The observations of Mellanby (1958) and Thomas (1957) of a directional response (in the form of a diving action) to mechanical shock in the presence of dorsal light by larvae of A.aegypti and C.fatigans respectively, are in agreement with my results. Whilst, in addition, this response has also been observed in several other species including Culex (Culex) molestus Forskål, and Anopheles maculipennis Meig. var. atroparvus Theil. (Mellanby, 1958).

In addition, Folger (1946), Mellanby (1958), and others maintain that the larvae of many species of mosquitoes exhibit negative geotropic responses, because they tend to remain at the surface of the water when at rest. However, although similar behaviour has been observed in the fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans, I do not consider that this behaviour is due to a negative geotropic response, but believe it to be a means of conservation of energy. For, to leave the surface of the water gaseous exchange of respiration, resting or surface feeding, the larvae must expend energy to overcome the force of surface tension. Therefore, by remaining at the surface when not actively feeding

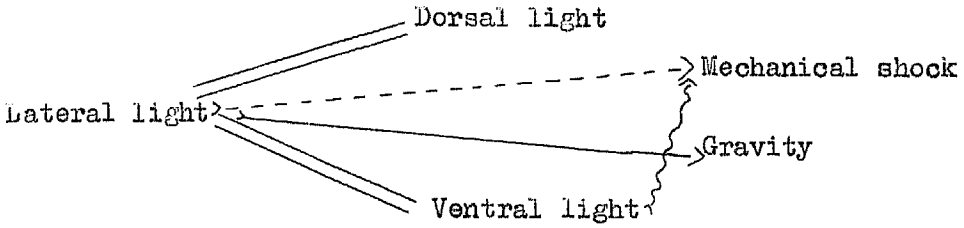
at the bottom the larvae would conserve energy that could be used more advantageously in other behavioural or physiological responses.

To fully understand the influences of light shock and mechanical shock stimuli on the behavioural responses of the fourth instar larvae of A.vigilax, A.australis, A.aegypti and C.fatigans, it was necessary to study and analyse fully the differences in their responses to simultaneous and sequential applications of certain pairs of these shock stimuli.

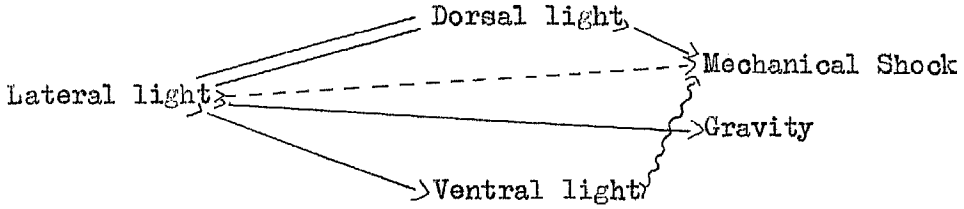
In many instances, the influence of one member of a pair of shock stimuli on the behaviour of the larvae was dominant over that of the other, when both were given simultaneously, and the responses of the larvae shown under these conditions were significantly different from those exhibited when those stimuli were given sequentially (Tables 17, 21, 25, 29, 33, 39, 45 and 51). The fact that this dominance in stimuli was not realized by previous authors was due to the designs of their experiments.

The interpretations of the behavioural responses of the four species are best represented in the following diagrams, where ——— indicates lack of dominance of stimuli, >———> indicates dominant to subordinate stimuli, >– – – – – –> indicates stimulus gives direction to kinesis, >~~~> indicates prevents diving action.

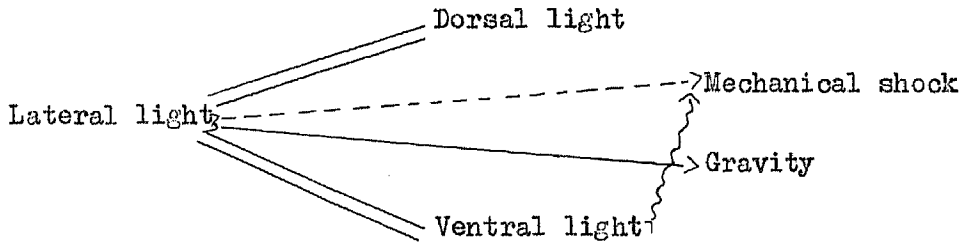
A.vigilax



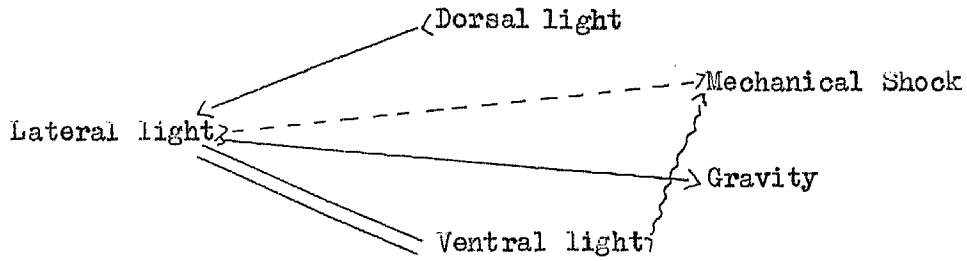
A.australis



A.aegypti



C.fatigans



From the diagrams it is obvious that in the four species the influence of dorsal light shock on the direction of movement of the larvae was never subordinate to the influences of lateral or ventral light shocks. Whilst lateral light shock gave direction to the kineses elicited by mechanical shock, causing the larvae to respond positively to gravity. In this latter response, the lateral light shock stimulus dominated the gravity stimulus (Tables 37, 43, 49 and 55). Mellanby (1958) thought that he had obtained similar responses by the larvae of A.aegypti to lateral light, mechanical shock and gravity. However, as he had applied a mechanical shock stimulus to the larvae under a constant gradient of illumination instead of as a shock both sequentially and simultaneously with a light shock, the results he obtained are not comparable with these results. Folger (1946) obtained in larvae of Culex sp. a similar positive response to gravity (or "reversal" in response, since he considered that the larvae were normally negatively geotropic), caused however, by decrease in light shock and mechanical shock. In addition, he maintained that mechanical shock given in darkness would still cause the "reversal" in geotropic response in these larvae, and that in the presence of constant illumination, mechanical shock would cause the usually positively phototropic larvae of Culex sp. to respond negatively to light. However, the results of my investigations showed that the larvae of the four species studied would respond negatively to light shock in the absence of mechanical shock. (Tables 16, 17, 21, 25 and 29).

On the application of a mechanical shock following a ventral light shock the fourth instar larvae of the four species exhibited a kinesis which lacked a directional component. This response proved that the diving action shown by these larvae in response to mechanical shock was

was actually composed of two components: a kinetic stimulus caused by the mechanical shock, and a directional one due to light shock, which caused their positive response to gravity.

Folger (1946) and Mellanby (1958) both thought that they had obtained responses similar to these in the species they had investigated, but again, since the mechanical shocks were given to the larvae under a constant gradient of illumination, the conclusions were incorrectly drawn from the experiments, and so again they are not comparable with my results.

The responses of the fourth instar larvae of A.vigilax and A.aegypti to light shock and mechanical shock were identical, whilst the responses of the other two species to these shocks differed but slightly from those of the abovementioned species. The selective survival value of certain of these responses of the four species to light shock, mechanical shock and gravity have previously been discussed, and will be mentioned again in the final summary of this discussion.

In summarising this discussion: It is obvious that the fourth instar larvae of the four species showed physiological and behavioural responses which may be correlated with survival in their ecological habitats. As would be expected with the two species that breed in fresh water, the osmoregulatory abilities of A.aegypti and C.fatigans were very limited, and although not significantly different from one another (Figure 12, Table 13b), were vastly inferior to those of the other two species (Figures 13 and 14, Table 14). On the other hand, the two species A.vigilax and A.australis, which breed in saline habitats, were observed to acclimatize to, and tolerate for a considerable time, great changes in salinity. This was due to an efficient osmoregulatory mechanism aided by the reduction in size of the anal papillae. However, although in its

larval habitat A.australis is subjected to greater changes in salinity than is A.vigilax, in its larval habitat, the osmoregulatory ability of the former was shown to be greater than that of the latter only under certain environmental conditions of temperature and salinity (Table 11b). Inter-specific differences in rates of acclimatization to salinity could account for the observation, under experimental conditions, of only slight differences in osmoregulatory ability between A.vigilax and A.australis.

Although the fourth instar larvae of the four species exhibited negative phototropic responses (Table 16), and although when given mechanical shocks they all responded with kineses given directional components by light and gravity (Tables 33, 37, 39, 43, 45, 49, 51, and 55), it was possible to distinguish differences in behavioural responses between the fourth instar larvae of the four species by the comparison of their rates of vertical and lateral movement. Many of the behavioural responses of the fourth instar larvae of the four species to light shock, mechanical shock and gravity could be correlated with survival in their respective ecological habitats.

In addition, by giving mechanical shocks and light shocks in sequence and simultaneously, a far greater knowledge was obtained of the responses of the fourth instar larvae of the four species to these shocks and to gravity than had previously been acquired of these and other species of mosquitoes by previous authors.

The influence on the distribution and abundance of the immature stages of A.vigilax of the other environmental conditions, viz. biotic and edaphic, were not studied. Since this investigation was primarily concerned with the study of the ecology of the immature stages of A.vigilax the oviposition behaviour of the gravid female was not determined.

The correlations between the physiological and behavioural responses of the fourth instar larvae of A.vigilax, A.australis, A.aegypti, and C.fatigans, and the environmental factors associated with their respective ecological habitats are best seen in the following tabulation, where + or - indicate presence or absence of a factor, and +, ++, +++ etc. indicate a semi-quantitative estimate of a factor.

Species	Salinity variation in ecological habitat	Osmoregulatory ability	Incidence of predator in ecological habitat	Temperature variation in ecological habitat	Effect of water in ecological habitat		Rate of Movement		Behavioural adaptations to environmental factors in ecological habitats		
					Flushing by rainfall	Flushing by waves	Vertical	Lateral	Negative geotropism	Kinetic response to mechanical shock	Geotropic response after light shock and mechanical shock
A.vigilax	+++	+++	+	+++	-	-	+++	+++	+	+	+
A.australis	++++	++++	-	++++	+	+	+++	++++	+	+	+
A.aegypti	+	+	-	++ or +++	+	-	+	+	+	+	+
C.fatigans	+	+	-	+++	+	-	++	++	+	+	+

It may be seen from this tabulation that a close correlation exists between the physiological and behavioural responses of these four species, and the environmental factors associated with their ecological habitats.

In conclusion: These investigations have shown that the survival of the immature stages of A.vigilax, A.australis, A.aegypti and C.fatigans in their respective ecological habitats, which they are obliged to occupy because of the oviposition sites chosen by the gravid females in response to certain environmental stimuli (O'Gower, 1955, 1957a, 1957b, 1958b; Woodhill, 1941b, and others) may be correlated with their physiological and behavioural responses to the environmental factors associated with these habitats. For example, the physiological and behavioural responses of the fourth instar larvae of A.vigilax to the environmental conditions of salinity, temperature, light shock, mechanical shock and gravity have been shown to affect their survival in their ecological habitat.

Natural selection would ensure that the genes or genotypes governing favourable physiological and behavioural responses to environmental factors would be maintained in the population (Sinnott, Dunn and Dobzhansky, 1958).

5. SUMMARY

The ecological study of a species necessitates the study of the physical, biotic and edaphic factors of the environment associated with its habitat, its physiology, and its behaviour, since its survival in a habitat is dependent on the interaction of these factors.

In the ecological investigation of the immature stages of the salt marsh mosquito Aedes vigilax Skuse, the environmental factors of topography, temperature, depth of water, pH, rainfall, tides and water table were studied, and their influence on the distribution and abundance of the immature stages were determined by statistical analyses. However, biotic and edaphic factors were not studied.

Data from these investigations indicated that the factors of temperature, salinity, light shock, mechanical shock and gravity had limiting effects on the survival of the immature stages. Therefore, these factors were chosen for further study in the laboratory.

The physiological survival of the fourth instar larvae to the interaction of temperature and salinity was determined.

The influence of light shock, mechanical shock and gravity on the behavioural patterns of the fourth instar larvae were determined by giving these shocks in sequence and simultaneously.

The physiological and behavioural responses of A.vigilax to these environmental factors could be correlated with survival in its ecological habitat.

It was found that in comparison, the physiological and behavioural responses to these factors of the fourth instar larvae of Aedes australis Erichson, Aedes aegypti Linnaeus, and Culex fatigans Wiedmann, species from different larval habitats, also correlated with survival in their respective habitats.

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Plate 1. Aerial photograph of area studied, showing larval habitat of *A.vigilax*, and position of water table pipes in relation to Woolooware Bay.


WEENEY BAY
N
I
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II
X
WOOLOOWARE BAY

Plates 2 and 3. Area studied flooded and dry.

2

3

Plates 4 and 5. Mangroves in proximity to area studied. (Photographs taken facing north from roadway shown in plates 2 and 3.)

Plate 6. Pool D flooded by spring tide.

Plate 7. Allocation of random quadrats in pool C using foot wide transect ladder.

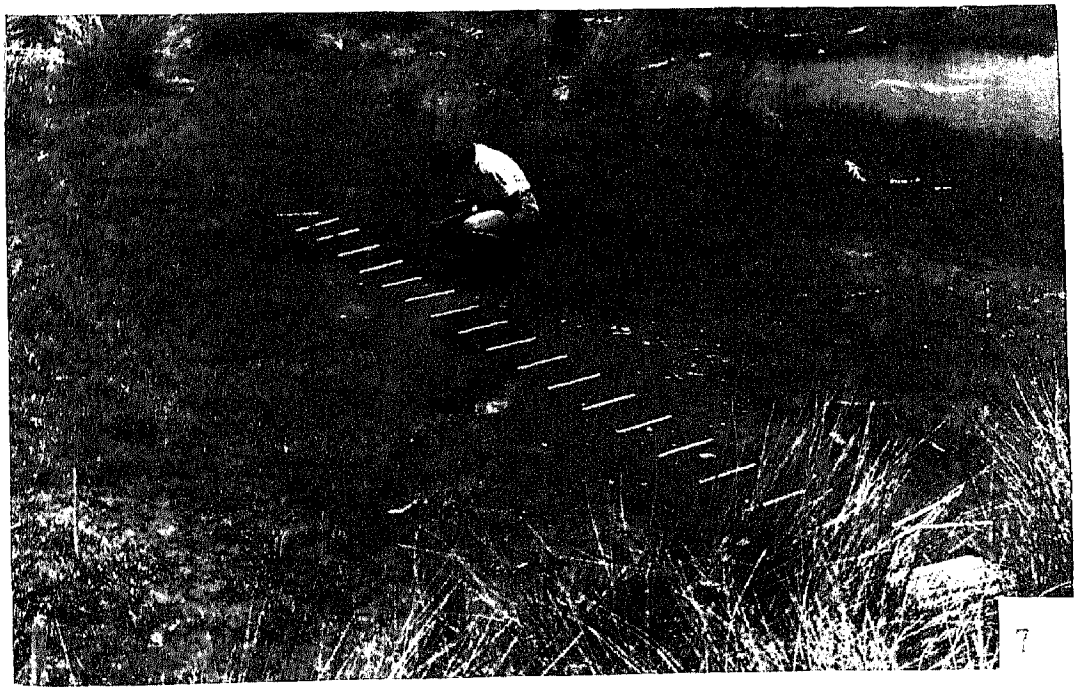

Plate 8. Pool C, dry, showing position of random quadrats.

Plate 9. Egg transect sampling across Pool B.

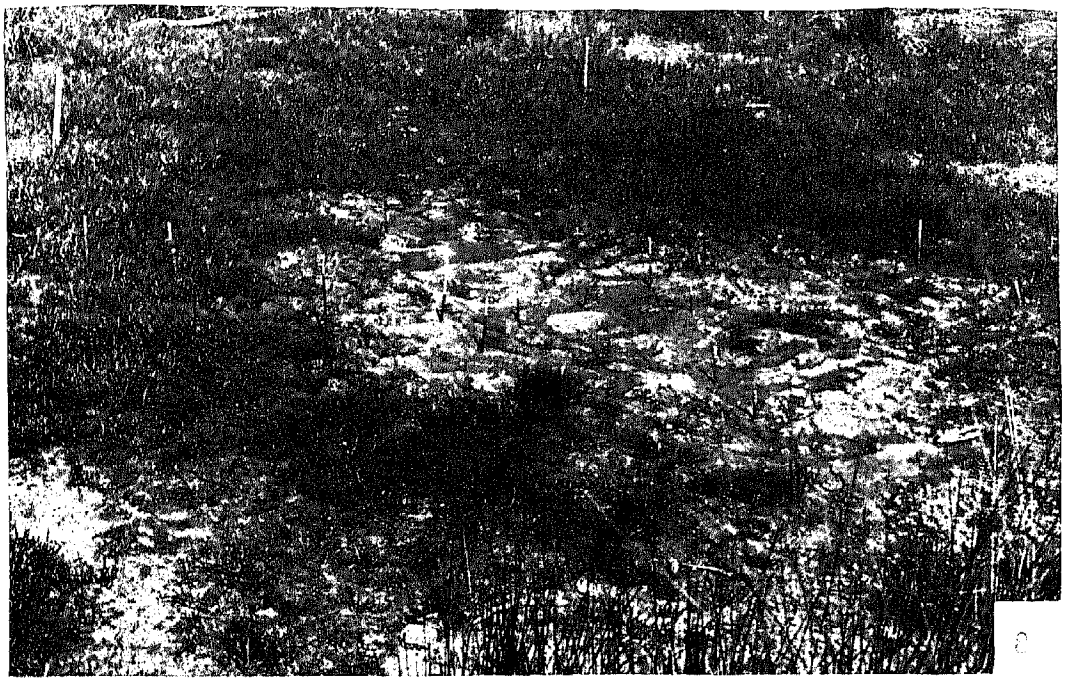

Plate 10. Measurement of depth of water table.

Plate 11. Position of water table pipe I (Pool Area), in relation to area studied. (Dry pool C may be seen slightly in front of, and to the left of, the pipe).

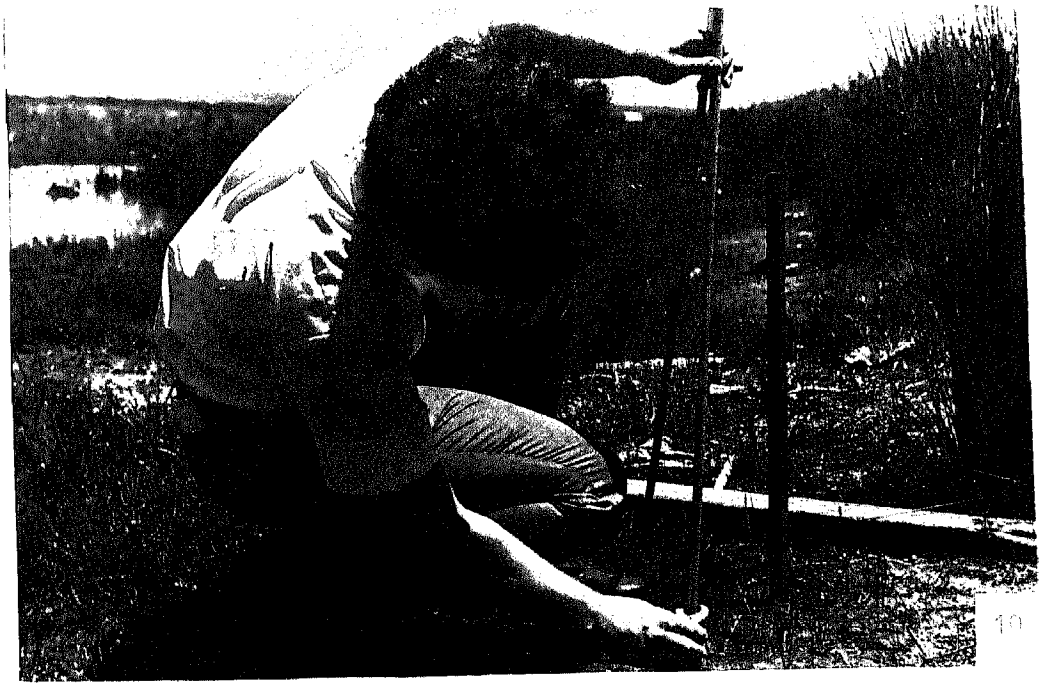

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Plate 12. A bare soil sample from egg transect sampling across pool B.

Plate 13. Flooded *Salicornia* soil sample, from egg transect sampling across pool B.

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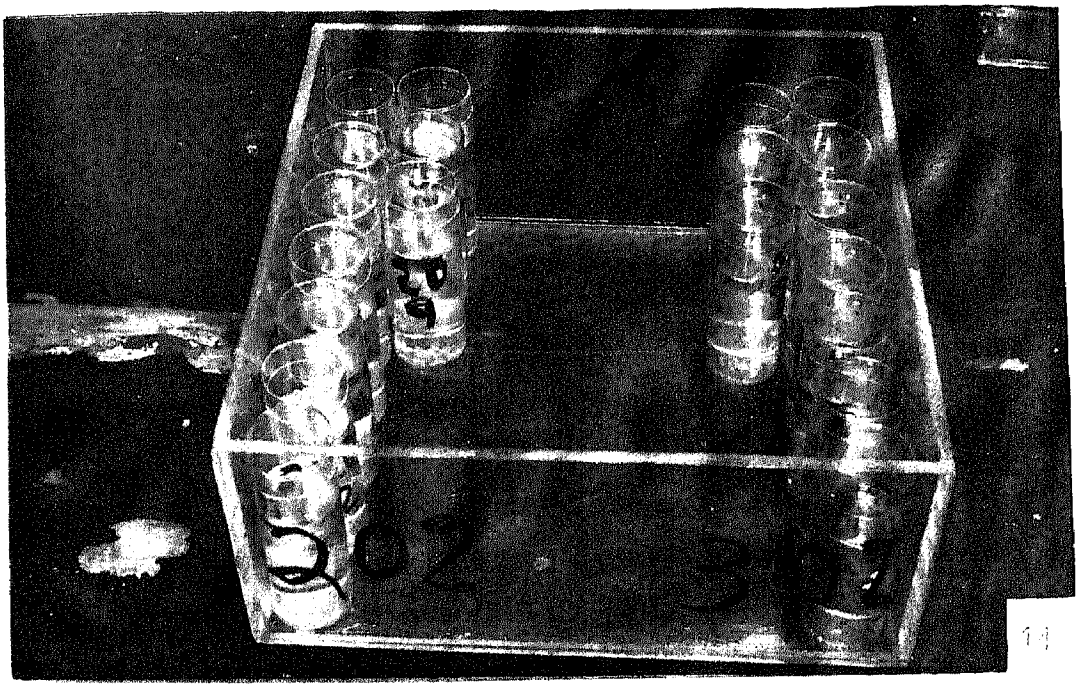
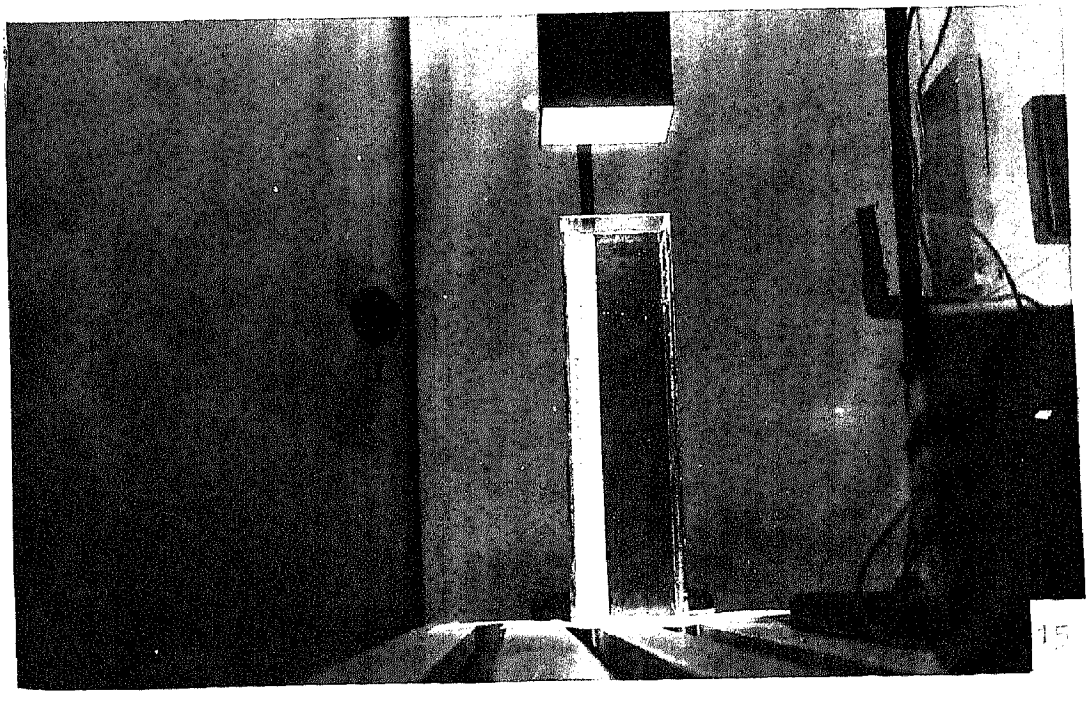
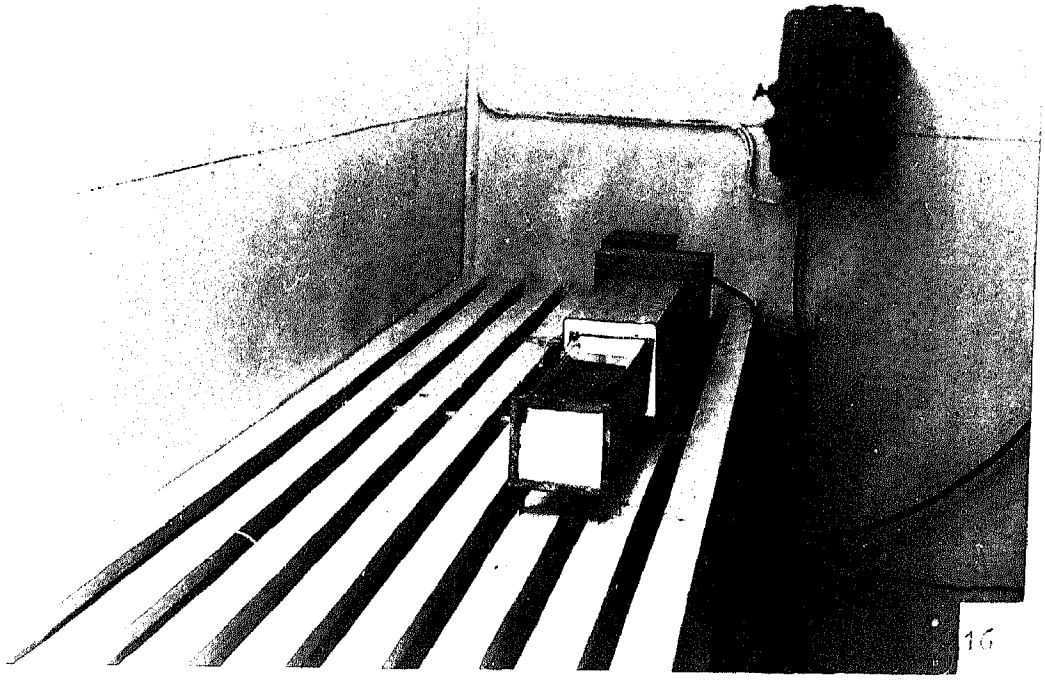
Plate 14. Replicated containers in which survival to salinity was determined.

Plate 15. Apparatus for the determination of rate of movement under dorsal illumination.





Plates 16 and 17. Apparatus for the determination of rate of movement under lateral illumination.



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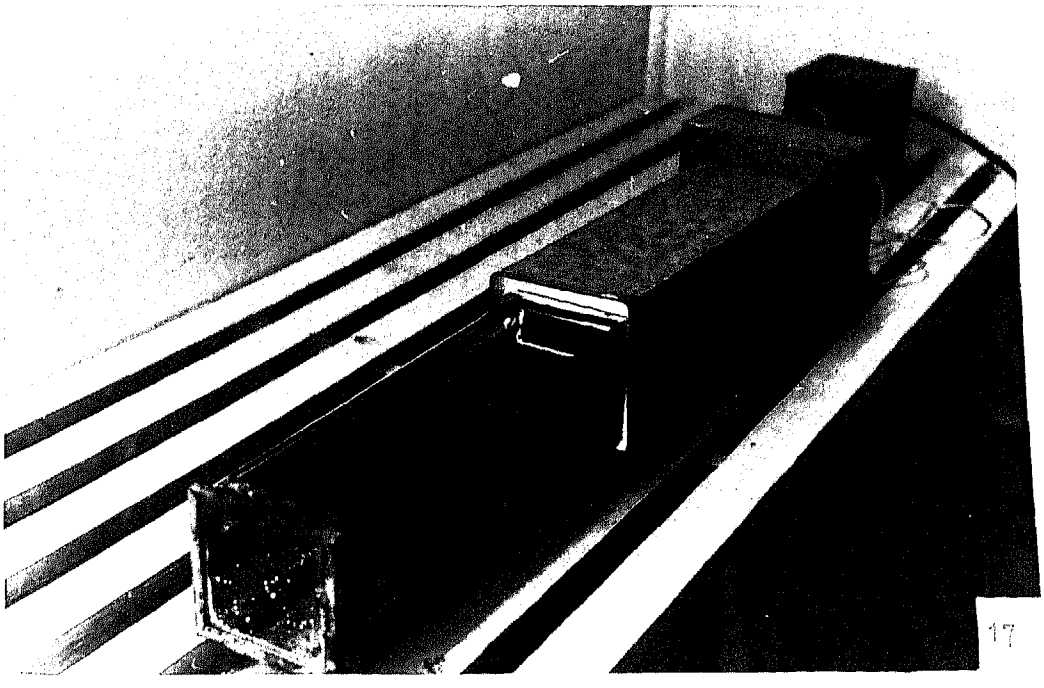

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lates 18 and 19. Apparatus for the determination of taxes and kineses to light, gravity and mechanical shock.