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**DEVELOPMENT AND SURVIVAL OF *CULEX (CULEX) THEILERI***

**THEOBALD**

**UNDER FLUCTUATING TEMPERATURES, HUMIDITIES AND  
SALINITIES.**

by

**D.L.C. ALBERTYN**

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DEVELOPMENT AND SURVIVAL OF *CULEX (CULEX) THEILERI*  
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SALINITIES.

by

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To my family

Dalène,

Izèbeau,

and Chantélie

for love, support and

understanding

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## OPSOMMING

Aangesien byna niks bekend was aangaande die seisoenale voorkoms van onvolwasse muskiete in die westelike Oranje-Vrystaat nie, asook die invloed van wisselende temperature en baie hoë soutkonsentrasies (NaCl) op die ontwikkeling en oorlewing van die onvolwasse stadia van die muskiet *Culex theileri* nie, is hierdie aspekte gedurende hierdie ondersoek bestudeer.

Twaalf maandelikse monsternemings by 18 waterpoele het 14 muskietspesies opgelewer, waarvan *Cx. theileri* (46,15 %), *Cx. pipiens* (32,43 %) en *Cx. univittatus* (10,76 %) die volopste was. Dit is ook getoon dat al die verskillende onvolwasse stadia van die eersgenoemde twee spesies gedurende die winter, hoewel in klein getalle, teenwoordig was. Hulle was egter volop gedurende die res van die jaar. *Cx. theileri* het in 89 % van die poele voorgekom terwyl *Cx. pipiens* en *Cx. univittatus* onderskeidelik in 50 % en 56 % van die poele teenwoordig was. Laasgenoemde twee spesies het veral voorkeur getoon aan poele met meer as 30 % plantbedekking.

Die onvolwasse stadia van *Cx. theileri* en *Cx. pipiens* het drie duidelike versamelpieke getoon, met die grootste gedurende die herfs, terwyl *Cx. univittatus* twee ewe groot pieke onderskeidelik in die vroeë somer en herfs getoon het.

Hierdie versamelpieke het 'n noue verband getoon met reënvalpeike, veral in die geval van die *Aedes* spesies.

Die belangrikste beperkende faktor van muskietgetalle gedurende die winter was die lae temperature wat vlieg- en eierleggingsaktiwiteite van volwassenes verminder het en ook hoë mortaliteite onder die onvolwasse stadia tot gevolg gehad het. Gedurende die somermaande was lae reënval, beskikbaarheid van geskikte broeiplekke en digtheidsafhanklike faktore byvoorbeeld oorbevolking, kompetisie en predasie die belangrikste beperkende faktore.

Die temporale en ruimtelike verspreiding van nege spesies is ontleed. 'n Hoë temporale korrelasie ( $p > 5,5$ ) tussen *Cx. theileri*, *Cx. pipiens* en *Cx. univittatus* het voorgekom. Daarteenoor het *Aedes caballus*, *Ae. hirsutus* en *Ae. juppi* saam voorgekom. Die hoë korrelasies vir die voorkoms van die verskillende spesies binne afsonderlike groeperings dui die invloed van temperatuur en reënval op die bevolkingsgetalle van die verskillende spesies aan. *Cx. salisburyensis*, *Culiseta longiareolata* en *Anopheles squamosus* het nietemin nie saam met mekaar voorgekom nie en is ook nie met die ander spesies gekorreleer nie omdat hulle meerendeels op verskillende tye gedurende die kouer maande van die jaar voorgekom het.

Hoë ruimtelike korrelasies ( $p > 4,8$ ) tussen *Cx. theileri* en

*Cx. salisburyensis*, tussen *Ae. caballus* en *Ae. hirsutus*, en tussen *Ae. juppi* en *Cx. univittatus* het voorgekom. Hierdie groeperings weerspieël moontlike voorkeure of vereistes vir eierlegging in aanvaarbare habitats. Daarteenoor was *Cx. pipiens*, *Cs. longiareolata* en *An. squamosus* nie met mekaar of die ander muskietspesies ruimtelik gekorreleer nie omdat hulle verskillende habitatsvoorkeure gehad het.

In eksperimente met wisselende dagtemperature het die ontwikkelingstyd van 24 uur oue eerste instar larwes tot volwassenheid onderskeidelik gewissel van 50 tot 11 dae onder laboratorium toestande by gemiddelde temperature van 10°C en 24,1°C. Onder veldtoestande by gemiddelde temperature van onderskeidelik 8,2°C en 22,5°C was die gemiddelde ontwikkelingstyd 80 tot 11 dae. In veldproewe het 96 % mortaliteit onder die eksperimentele bevolking voorgekom terwyl die mortaliteit in die laboratorium 85 % by 10°C was. Die hoër mortaliteite in die veldproewe word toegeskryf aan die feit dat die wateroppervlakte gedurende die winter periodiek vir kort periodes gevries was.

Eksperimente waar die invloed van wisselende temperature en versadigingstekorte op bloedgevoede wyfies ondersoek is, het getoon dat die langste oorlewings tydperk ( $LT_{50} = 23,9$  dae) by lae temperature (10°C) en lae versadigingstekorte (0,33 kPa) voorgekom het.

Die toleransie van *Cx. theileri* ten opsigte van hoë NaCl oplossings is bepaal deur 'n kontrole groep, wat in 0,02M NaCl oplossing geteel is, te vergelyk met 'n geselekteerde groep wat in 0,12 M NaCl oplossing ( $F = 6$ ) geteel is. Die NaCl oplossings wat gebruik is, het gewissel van 0,0M (gedistilleerde water) tot 0,22M vir die eierleggingseksperimente en vanaf 0,0M tot 0,16M vir die ontwikkeling van die onvolwasse stadia.

Vir alle parameters waarvoor getoets is, het geen betekenisvolle ( $p > 0,05$ ) toename in NaCl toleransie voorgekom nie en dus geen ware seleksie vir 'n halofitiese *Cx. theileri* stam nie. Daar was egter 'n betekenisvolle ( $p < 0,05$ ) afname in eierlegging, persentasie uitbroeiing en grootte van kopkapsules, terwyl ontwikkelingstyd en persentasie mortaliteit betekenisvol toegeneem het in alle stadia by hoë NaCl molariteite, ongeag die groep.



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## 1. General introduction

*Culex theileri* Theobald is the numerically dominant mosquito in the western Orange Free State, comprising approximately 50 % of the adults trapped in New Jersey light traps from 1976 to 1978 (Hewitt *et al.*, 1982; Van der Linde *et al.*, 1982; Van der Linde, 1984). This is an area of high saturation deficits, high summer and low winter temperatures, and low rainfall (Van Pletzen & Van der Linde, 1981). It has been described by Acocks (1953) as *pan-turfveld* in which *Diplachne* pans containing water of a high salinity are common (Geldenhuis, 1982).

*Cx. theileri* is a vector of Rift valley fever (McIntosh *et al.*, 1973; Jupp *et al.*, 1980), and is thus of medical and veterinary importance. Epidemics of Rift valley fever have occurred twice in South Africa viz. during the summer of 1950 - 1951 when it was first identified (Barnard & Botha, 1977) and during 1974 - 1975 when thousands of livestock died and many people were affected some fatally (Barnard & Botha, 1977; Van Velden *et al.*, 1977; Jupp *et al.*, 1980).

The hosts of this virus, during inter-epidemic periods, are unknown. Bearing in mind that *Cx. theileri* adults are implicated in transmission of this virus, and that adults of this species occur in low numbers during winter, it is possible that they are able to transmit the virus to

successive generations. Although ovarial transmission of Rift valley fever has not been shown to occur in *Cx. theileri*, it can by no means be ruled out as a possible method of transmission.

## 2. Seasonal distribution and relative abundance of immature mosquitoes

### 2.1 Introduction

*Cx. theileri* is known to be a vector of Rift Valley fever (McIntosh, et al., 1973; Jupp et al., 1980). This served as motivation for research into the mosquito fauna of the western Orange Free State which included studies on the relative abundance of adult *Cx. theileri* and other species using New Jersey light trapping methods (Hewitt et al., 1982; Van der Linde et al., 1982; Van der Linde, 1984). These results showed that *Cx. theileri* adults were present in low numbers throughout most of the winter period and were common during the rest of the season.

Data published by Jupp indicated that *Cx. theileri* overwinters in the immature stages and are probably the major overwintering form of this species on the Transvaal highveld (Jupp 1969, 1975).

No work has been done on the seasonal abundance of immature *Cx. theileri* in the Orange Free State. The determination of the temporal distribution and seasonal abundance of immature *Cx. theileri* in the western Orange Free State will contribute to the knowledge of the ecology of this species.



Temperature and humidity in the field fluctuate widely. In spite of this the influence of these parameters on a particular species is frequently assessed at several constant levels. It is doubtful whether these results are also valid for the field situation (Peairs, 1914, 1927; Parker, 1930; Shibata, 1934, all quoted by Huffaker, 1944; Huffaker, 1944; Andrewartha & Birch, 1954; Richards, 1960; Caulton, 1976; Van As, 1980).

Recent work by Van der Linde (1984) on the biology of *Cx. theileri* which included aspects such as development and survival under constant temperatures and humidities and in a range of NaCl solutions is especially relevant here. He found that constant temperatures of 9°C and 36°C were lethal to the immature stages of this species. Jupp (1967, 1975) indicated that *Cx. theileri* larvae are able to survive up to 74 days in winter conditions in the Transvaal highveld when minimum temperatures are considerably lower than 9°C. Thus, an investigation of the effects of variable temperatures was deemed necessary.

Furthermore, Van der Linde (1984) found that a small proportion of the experimental population was able to tolerate relatively high NaCl concentrations. As salt pans are numerous in this region, the possibility of selection for NaCl tolerant local strains in the area also required investigation.

## 2.2 Materials and methods

### 2.2.1 Location and description of sampling sites

Eighteen pools were selected for sampling in the Bloemfontein district (Fig. 2.1). These sites were divided into two categories viz.:

- (i) Those with little or no vegetation in the water, the bottoms often bare rock or gravel overlaid with mud or silt (eg. Figs. 2.2 to 2.5). Sites, 1, 2, 3, 4, 5, 7, 9, 11, 12 and 13 fell in this category (Table 2.1, column A).
- (ii) Those in which more than 30 % of the water surface was covered by emergent vegetation or contained submergent vegetation within the water body (Figs. 2.6 to 2.9). Sites 4, 6, 8, 10, 14, 15, 16, 17 and 18 fell in this category (Table 2.1, column B).

The general characteristics of the sites, including conductivity and pH of the water, and the dominant vegetation associated with each site are summarised in Table 2.1, columns A and B. All sites were permanent except nos. 1, 3, 4, 12, 14, and 18 which were dry for periods of two to seven months.

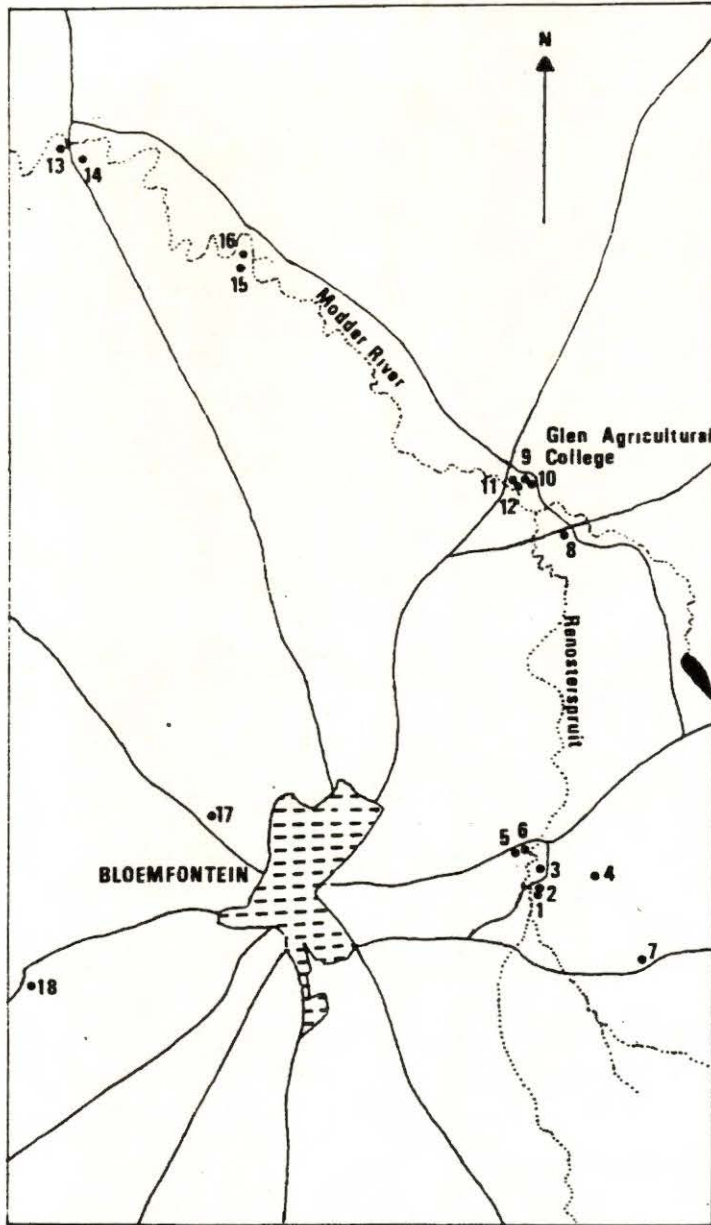


Fig. 2.1 The distribution of 18 sites sampled during May 1985 to May 1986.

Table 2.1 Dominant vegetation and physical characteristics of sites sampled during 1985-1986.

Plant species	SITES																		I	II	No of sites	% of total frequency in all sites						
	A*									B**																		
	1	2	3	5	7	9	11	12	13	1	2	3	4	5	6	8	10	14	15	16	17	18						
<i>Agrostis lacnantha</i> Nees	x	x			x																			0	3	18,8	6,8	
<i>Amaranthus</i> spp.																								0	1	6,3	2,3	
<i>Cyperus longus</i> (L.)	x	x	x										x											1	4	25,0	9,1	
<i>Gomphostigma virgata</i> (L.F.) Baill.	x	x						x																0	2	12,5	4,6	
<i>Paspalum paspaloides</i> (Mitch.) Scribn.	x	x																						6	9	56,3	20,5	
<i>Phragmites australis</i> (Car.) Trin. ex. Steud.				x											x	x	x		x	x	x			0	2	12,5	4,6	
<i>Scirpus incinus</i> (Thunb.) Steud								x																0	1	6,3	2,3	
<i>Scirpus paludicola</i> Kunth.								x					x											1	3	18,8	6,8	
<i>Typha capensis</i> (P. Rohrb.) N.E. Br.	x	x			x																			2	4	25,0	9,1	
<i>Acacia karoo</i>																x								1	1	6,3	2,3	
<i>Cynodon dactylon</i> (L.) Pers.																			x					1	1	6,3	2,3	
<i>Diplachne fusca</i> (L.) Beauv. ex. Stapf.																				x	x	x		3	3	18,8	6,8	
<i>Eleocharis palustris</i> R. Br.																					x	x		1	1	6,3	2,3	
<i>Lagarosiphon muscoides</i> Harv.																						x		1	1	6,3	2,3	
<i>Mariscus congestus</i> (Wahl.) C. Du.																x								1	1	6,3	2,3	
<i>Paspalum dilatatum</i> Poir.																x					x			2	2	12,5	4,6	
<i>Polygonum salicifolium</i> (Willd.)																						x		1	1	6,3	2,3	
<i>Rumex lanceolatus</i> (Thunb.)													x			x								3	3	18,8	6,8	
<i>Echinochloa holubii</i> (Stapf.) Stapf.																				x	x			1	1	6,3	2,3	
Species/site	5	6	1	1	3	0	0	3	0	19	3	1	5	2	-	5	4	5	-	-	-	-	25					
Conductivity (mS m <sup>-1</sup> )/site	30,3	35,6	11,2	93,5	51,4	69,0	262,7	565,2	600,6	191,1	15,8	69,7	56,0	48,6	94,2	46,8	63,7	16,5	16,0	47,0								
x pH/site	7,7	7,7	8,2	7,8	8,0	7,9	7,6	8,0	7,6	7,8	7,7	8,4	8,0	7,8	7,4	7,5	7,6	7,3	7,5	7,7								

\* Sites grouped under A: less than 30 % vegetational cover.  
 \*\* Sites grouped under B: more than 30 % vegetational cover.



Fig. 2.2      Site no. 1.    Shallow rock seepage pools  
situated below a stone and concrete weir.



Fig. 2.3      Site no. 5.    A garden rock pool permanently  
filled with water.    Well shaded in summer.



Fig. 2.4 Site no. 13. A bare rocky river bed.



Fig. 2.5 Site no. 13. A close up view to show the bare rock pools.



Fig. 2.6 Site no. 8: A marsh formed by seepage from a dam.



Fig. 2.7 Site no. 10. One of the two inlets of a dam where processed sewage water enters.



Fig. 2.8            Site no. 16.        A shallow permanent marsh filled by irrigation runoff, seepage and rain.



Fig. 2.9            Site no. 18.        A shallow marshy area terminating in a small dam and filled by seepage and rain.



## 2.2.2 Field sampling procedure

### 2.2.2.1 Immature mosquitoes

During the period May 1985 to May 1986 18 selected pools were sampled monthly. At each site the water temperature, conductivity and pH were recorded. Sampling was done with a 200 ml, long-handled dipper. Thirty samples of 200 ml each were taken at each site and divided into three groups of 10 samples each, giving three replicates per site. The larvae were counted and the instar composition determined in the laboratory. The immature stages were put individually into glass vials containing 10 ml of a rearing medium and reared to adulthood. The rearing medium consisted of a mixture of larval food\* (0,1 g/litre) suspended in NaCl solution with the conductivity equal to that found at the sampling site. Fresh solutions were provided every second day. Pupae in the samples were placed in vials containing the appropriate NaCl solution without larval food. The rearing procedure, involving the use of NaCl solutions with conductivity similar to that of water at the sampling sites, was adopted in order to minimize mortality. The female adults were identified with the aid of keys compiled by Dr. B.M. McIntosh and with reference to Edwards (1941). The female Anophelinae were identified by Dr. M. Coetzee\*\*.

\* Larval food consisted of a 1:1 mixture (m/m) of Nestum No. 1 Baby Cereal + Brewers yeast.

\*\* Dr. M. Coetzee - Entomologist, S.A.I.M.R., Johannesburg.

#### 2.2.2.2 Overwintering Adults

Two trapping methods were used for adults. In the first method, a modified vacuum cleaner (Fig. 2.10) was used to collect mosquitoes from vegetation and animal burrows. Measured areas of vegetation (10 m x 10 m), adjacent to selected pool sites were traversed systematically by sweeping the marked areas with the vacuum cleaner for 20 minutes. A similar method was employed by Magnarelli (1975) and Dr. P.G. Jupp\* (personal communication).

In the second method a trap (Fig. 2.11) was positioned over a patch of vegetation. Two ml of Citronella oil (mosquito repellent) was sprayed into the vegetation from under the edge of the trap, in an attempt to drive the mosquitoes up towards the trapping bottle at the apex. Mosquitoes were then transported to the laboratory for identification. The temperature and humidity at the sampling areas were recorded at ground level and at one metre height.

\* Dr. P.G. Jupp, Senior Researcher, Arbovirus Research Unit, Institute of Virology, Johannesburg.

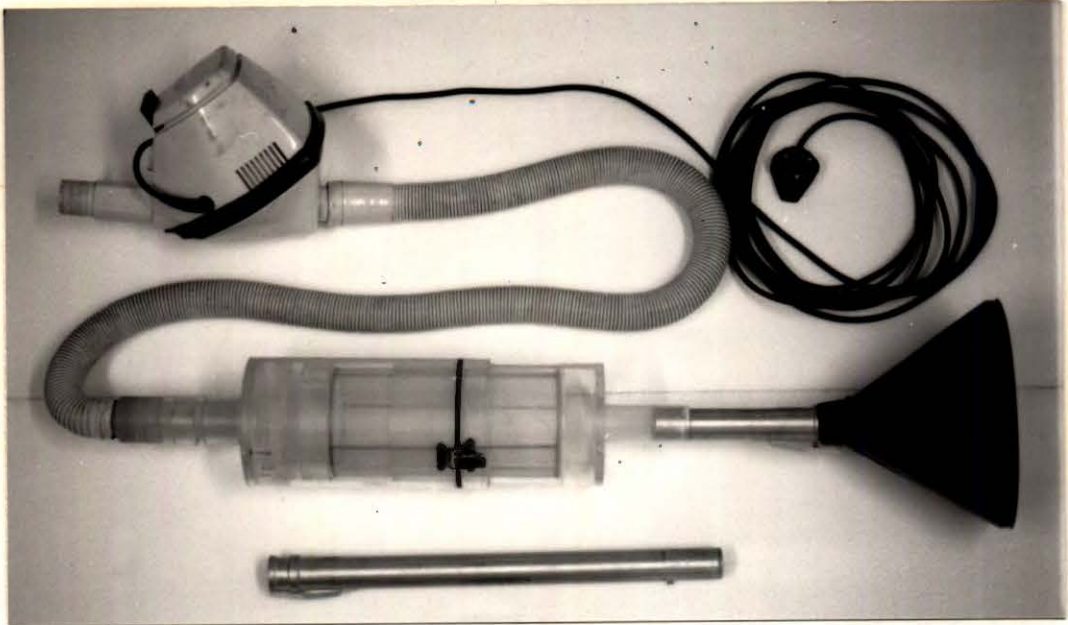


Fig. 2.10      The modified vacuum cleaner used to sample adults from vegetation during winter.



Fig. 2.11      The mosquito emergence trap used to sample adults during winter.

## 2.3 Results and discussion

### 2.3.1 Immature mosquitoes

#### 2.3.1.1 Species composition

Fourteen species were recorded during the survey period from May 1985 to May 1986 of which *Ae. vittatus* is a new record for the Orange Free State (Table 2.2). *Cx. theileri* (46,15 %) was the most abundant species followed by *Cx. pipiens* (32,43 %) and *Cx. univittatus* (10,76 %). The remaining 11 species made up the final 10,66 % of the total (Table 2.2).

#### 2.3.1.2 Habitat associations

It is evident that some sites yielded greater numbers of immature stages than others and were utilized by different species to various degrees (Table 2.3). However, the discussion here will highlight only those aspects that broadly reflect differences between sites, and the dominant species that frequent them. *Cx. theileri*, *Cx. pipiens*, *Cx. univittatus*, *Cs. longiareolata*, *An. squamosus* and some of the *Aedes* group merit individual discussion.

#### *Cx. theileri*

This species occurred at 16 of the 18 sites sampled during

Table 2.2 Species composition of female mosquitoes sampled as immatures at 18 sites during the period May 1985 - May 1986.

Species	1985 - 1986	
	Nos.	% frequency
<i>Aedes (Aedimorphus)</i>		
<i>hirsutus</i> (Theobald)	27	0,56
<i>Aedes (Neomelaniconion)</i>		
<i>unindentatus</i> (McIntosh)	10	0,21
<i>Aedes (Ochlerotatus)</i>		
<i>caballus</i> (Theobald)	93	1,94
<i>juppi</i> McIntosh	123	2,57
* <i>Aedes (Stegomyia)</i>		
<i>vittatus</i> Bigot	11	0,23
<i>Anopheles (Cellia)</i>		
<i>cinereus</i> group	8	0,17
<i>squamosus</i> group	81	1,69
<i>Culex (Culex)</i>		
<i>quinquefasciatus</i> Say	1	0,02
<i>pipiens</i> Linnaeus	1 555	32,43
<i>theileri</i> Theobald	2 213	46,15
<i>univittatus</i> Theobald	516	10,76
<i>Culex (Lutzia)</i>		
<i>tigripes</i> Grand pré & Charmoy	1	0,02
<i>Culex (Maillotia)</i>		
<i>salisburyensis</i> Theobald	68	1,42
<i>Culiseta (Allotheobaldia)</i>		
<i>longiareolata</i> Mcquart	88	8,84

\* First record in the Orange Free State .

Table 2.3 Species and number of females reared from larvae sampled at each site for the period May 1985-1986.

	Sites:																				Σ	ΣΣ
	A*										B**											
	1	2	3	5	7	9	11	12	13	Σ	4	6	8	10	14	15	16	17	18	Σ		
<i>Ae. hirsutus</i>	6		20						26	1									1	27		
<i>Ae. unidentatus</i>			10						10										0	10		
<i>Ae. caballus</i>		2	90						92	1									1	93		
<i>Ae. juppi</i>			50						50	3						70			73	123		
<i>Ae. vittatus</i>								11	11											11		
<i>An. cinereus</i>	8								8											8		
<i>An. squamosus</i>	6	7			41		17	2	73	3	2					1	2		8	81		
<i>Cx. quinquefasciatus</i>									0					1					1	1		
<i>Cx. pipiens</i>				2			3	6	11	1	1	317	1115			110			1544	1555		
<i>Cx. theileri</i>	31	5	62	47		16	1014	76	1251	41	312	75	50	2	6	438	32	6	962	2213		
<i>Cx. univittatus</i>	9	1					36		46	2	6	111	112		16	218	5		470	516		
<i>Cx. tigripes</i>									0							1			1	1		
<i>Cs. salisburyensis</i>	13	4	23			7	20		67						1				1	68		
<i>Cs. longiareolata</i>								88	88										0	88		
Tot. females/site	73	19	170	87	88	0	26	1087	183	1733	52	321	503	1278	2	23	838	39	6	3062	4795	
Total males and females/site	133	31	337	148	167	0	57	1977	400	3250	83	556	980	2337	3	36	1163	89	6	5253	8503	
Grand total larvae/site	450	150	573	192	734	0	130	2317	590	5146	86	646	1299	2347	38	1041	3172	107	129	8857	14008	

\* Sites grouped under A: less than 30 % vegetational cover.

\*\* Sites grouped under B: more than 30 % vegetational cover

1985 - 1986. It was present in slightly higher numbers (1251 - 56,53 %) in pools containing less than 30 % vegetational cover (Table 2.3, column A) than in other locations (962 - 43,47 %) (Table 2.3, column B). The mean pH of all sites was similar ranging between 7,3 - 8,4 (Table 2.1), while the mean conductivity of sites under column A was substantially higher (191,1 mS m<sup>-1</sup>) than under column B (47,5 mS m<sup>-1</sup>) (Table 2.1). Although *Cx. theileri* preferred sites 12, 6 and 16, it was obvious that this species frequented a wide range of pools containing temporary or permanent water of varying conductivities (see Tables 2.1 and 2.3). The presence or absence of vegetation in the pools did not seem to influence their suitability as larval habitats. The apparent wide larval and pupal tolerances of *Cx. theileri* may contribute to its dominance in the Orange Free State (Jupp and McIntosh, 1967).

In addition, *Cx. theileri* was found to be present throughout the year. Peak numbers were caught during November 1985 (348 - 15,73 %) and January (222 - 10,03 %), March (360 - 16,27 %), April (894 - 40,40 %) and May 1986 (203 - 9,17 %) after heavy rains in these and the preceding months (Table 2.4).

#### *Cx. pipiens*

*Cx. pipiens* was present in eight of the 18 pools sampled

during this survey and thus frequents a more limited range of habitats than *Cx. theileri* (Table 2.3). The majority of *Cx. pipiens* were collected in pools containing more than 30 % vegetational cover (Table 2.3, column B). The mean conductivity of the water in pools frequented by this species was approximately 47,5 mS m<sup>-2</sup>. Highest numbers of *Cx. pipiens* (115 - 71,70 %) (Table 2.3, Column B) were captured at site no. 10 which was enriched by sewage effluent (Fig. 2.7). The presence of this mosquito at sites rich in sewage effluent is well known (Jupp, 1967).

Peak numbers of *Cx. pipiens* were recorded in September (110 - 7,07 %), October (101 - 6,50 %) and November 1985 (293 - 18,84 %) and March (192 - 12,35 %), April (476 - 30,61 %) and May 1986 (318 - 20,45 %) (Table 2.4). Peak numbers were usually associated with high rainfall during or in the month before the larvae were captured (Table 2.4). The high numbers of *Cx. pipiens* (110 - 7,07 % and 318 - 20,45 %) recorded in September 1985 and May 1986 in the absence of rain probably reflects suitable high mean atmospheric temperatures (16,3 and 13,4°C) for oviposition of adults in permanent water bodies (eg. site no. 10) during these periods (Fig. 2.7). While the high numbers sampled in October 1985 (101 - 6,50 %) and March 1986 (192 - 12,35 %) are due to high rainfall (Table 2.4).



Table 2.4 Species composition of females reared from larvae sampled monthly during 1985-1986

Species	Months:													Tot.	% Tot.
	1985						1986								
	M 5	J 6	J 7	A 8	S 9	O 10	N 11	D 12	J 1	F 2	M 3	A 4	M 5		
<i>Ae. hirsutus</i>						27								27	0,56
<i>Ae. unidentatus</i>						10								10	0,21
<i>Ae. caballus</i>						93								93	1,94
<i>Ae. juppi</i>						53	70							123	2,57
<i>Ae. vittatus</i>												11		11	0,23
<i>An. cinereus</i>								8						8	0,17
<i>An. squamosus</i>	10	2	4					3	1	6		16	39	81	1,69
<i>Cx. quinquefas</i>														1	0,02
<i>Cx. pipiens</i>	3	19	6	4	110	101	293	11	11	11	192	476	318	1555	32,43
<i>Cx. theileri</i>	12	13	5	9	31	67	348	27	222	22	360	894	203	2213	46,15
<i>Cx. univittatus</i>	2				6		161	34	14	13	90	151	45	516	10,76
<i>Cx. tigripes</i>	1													1	0,02
<i>Cx. salisburyensis</i>		2	18	7				20				21		68	1,42
<i>Cs. longiareolata</i>	7	14	7	20	16								24	88	1,84
Σ females	35	51	40	41	163	351	872	103	248	52	642	1569	629	4795	
Σ males & females	81	93	89	73	276	398	1593	158	455	97	931	3046	1223	8503	
Tot. all larvae	385	394	333	92	291	1329	2730	245	1335	269	1489	3112	2006	14008	
Total mean monthly rainfall (mm)	0,0	31,4	0,4	0,0	1,0	83,9	60,3	83,7	35,0	34,9	136,8	77,7	0,0	545,1	
Mean monthly air temperature (°C)	16,3	9,5	8,3	12,5	16,3	20,3	21,1	21,8	23,7	22,4	20,4	17,7	13,4	233,7	

*Cx. univittatus*

This species was found in 10 of the 18 pools sampled during 1985 - 1986 (Table 2.3). As with *Cx. pipiens*, the majority of *Cx. univittatus* (470 - 91,08 %) were collected at sites in which more than 30 % of the water surface was covered by emergent or contained submergent vegetation (Table 2.3, column B). This supports records by Edwards (1941). It is noteworthy that the largest number of *Cx. univittatus* (218 - 42,25 %) were collected at site no. 16 and to a lesser extent at site nos. 8 (111 - 21,51 %) and 10 (112 - 21,71 %) (Table 2.3, column B). Site nos. 8 (Fig. 2.6) and 16 (Fig. 2.8) are marshy areas filled by irrigation seepage and also rainfall. Site no. 10 (Fig. 2.7) was a moderately sized dam fed by processed sewage water. The water in site no. 8 was usually very clear, while site nos. 10 and 16 were slightly turbid darker water. The largest numbers of *Cx. univittatus* were sampled during November 1985 (161 - 31,20 %) and April 1986 (151 - 29,26 %) after high rainfall in October/November 1985 and March/April 1986 (Table 2.4) which filled these sites, including a large expanse of grass adjacent to the marshes (site nos. 8 and 16). As dipper sampling was concentrated along the edges of the marsh, rather clear water over emergent grass was sampled at the time. Jupp (1967) also indicated that *Cx. univittatus* was collected most frequently in rainwater accumulating over grass.

*Cs. longiareolata*

*Cs. longiareolata* was only found at site no. 13 (Table 2.3, column A). This site is composed of many small bare isolated rock pools with steep sides (Figs. 2.4 and 2.5). It also had the highest mean annual conductivity of 600,6 mS m<sup>-1</sup> (Table 2.1) recorded for all sites. The preference of *Cs. longiareolata* for small steep-sided pools has been reported by Van Pletzen & Van der Linde (1981). Since *Cs. longiareolata* was successfully reared in the laboratory in distilled water (Van Pletzen & Van der Linde (1981) and was collected in high conductivities at site no. 13 it is clear that conductivity does not influence the choice of a site for oviposition in this species to any marked degree. The wide tolerance of *Cs. longiareolata* is substantiated by work done on this species in the Central Negev desert (Dimentman & Margalit, 1981). These authors considered *Cs. longiareolata* to be eurythermal, euryhaline and euryionic. They were present in various pools throughout the year in which mean water temperatures ranged from 7°C to 31°C, conductivity from 250 mS m<sup>-1</sup> to 2250 mS m<sup>-1</sup> and pH from 6 to 10.

The reason that *Cs. longiareolata* has a predilection for small pools (Gillett, 1971; Van Pletzen & Van der Linde, 1981), often devoid of vegetation is obscure. This, together with their predatory behaviour (Van der Linde\* -

\* Dr. T.C. de K. van der Linde, Senior Researcher, Department of Zoology and Entomology, University of the Orange Free State, Bloemfontein, Republic of South Africa.

personal communication\*) could give them an ecological advantage over other species. Furthermore, it is probable that these small pools, by virtue of their relative isolation, serve to reduce the chance of location by predators, whilst predators that do invade these pools have no vegetation from which to launch attacks on the larvae. In addition Van Pletzen & Van der Linde (1981) have suggested that wave-action, which may inhibit oviposition, is less pronounced in small pools than in more open locations.

It is also noteworthy that *Cs. longiareolata* was only sampled at site no. 13 (Table 2.3, column A) and only during the winter months during this survey (Table 2.4). The highest numbers recorded occurred during June (14 - 15,91 %) and August 1985 (20 - 22,73 %) and May 1986 (24 - 27,27 %) (Table 2.4). This supported work done by Van Pletzen & Van der Linde (1981). However, adults were trapped by these authors throughout the year, suggesting that *Cs. longiareolata* is a multivoltine species. The absence of larvae during the summer months at site no. 13 can possibly be attributed to the high temperatures that occur in these small, exposed rock pools during this time. However, in winter, the heat absorbed by the rocks during the day might act to offset low night-time temperature.

*An. squamosus*-group

Nine out of 18 sites surveyed were frequented by *An. squamosus*. The highest numbers (73 - 90,12 %) occurred in pools with up to 30 % vegetational cover (Table 2.3, column A) and a mean conductivity value of 191,1 mS m<sup>-1</sup> (Table 2.1). Site nos. 7 and 12 were preferred breeding places for *An. squamosus* where the highest numbers (41 - 50,62 % and 17 - 21 % respectively) were sampled during the year (Table 2.3, column A). A pH of 8 was recorded for both these sites. The mean pH range in which the species occurred in the other sites was 7,3 to 8,4 (Table 2.1, column A). Mean annual conductivity recorded for both sites 7 and 12 was 51,4 and 565,2 mS m<sup>-1</sup> but varied from 30,3 to 6006,6 mS m<sup>-1</sup> at other sites where they were caught. Thus conductivity does not seem to be a prime factor in the choice of a breeding site for this species.

Common features of these two sites were the presence of reeds (*Typha capensis* - site no. 7 and *Phragmites australis* - site no. 12), still, clear water, and the fact that both pools were isolated, located in rather deep depressions protected from wind and early morning and late afternoon sunlight. In addition, both sites had bands of either emergent grass (*Paspalum paspolodis* - site no. 7) or sedges (*Cyperus longus* and *Scirpus incinus* - site no. 12) overhanging and shading the edges (Table 2.1, column A).

Though exceptions abound (Gillet, 1971), anophelines often prefer still, shaded, rather clear cool pools for oviposition (Muirhead-Thompson, 1940; Edwards, 1941; Hopkins, 1952). It thus seems likely that the common features presented above may have been important factors in the choice of these two sites for oviposition by *An. squamosus*. Hopkins (1952) indicated that *An. squamosus* larvae are most prevalent in habitats with temperatures ranging from 15,1°C to 26°C.

The *An. squamosus* apparent preference for more temperate climatic conditions was borne out by the greater numbers collected during the colder months of May 1985 (10-12,35 %), April 1986 (16 - 19,75 %) and May 1986 (39 - 48,15 %) with a small peak (6 - 7,41 %) after rain in February 1986 (Table 2.4). Mean atmospheric temperatures during these four months were 16,3, 17,7, 13,4 and 22,4°C respectively. *An. squamosus* seemed able to oviposit at mean temperatures as low as 13,4°C, judging from the peak numbers sampled (39 - 48,15 %) in May 1986 (Table 2.4).

The months in which peak numbers of *An. squamosus* were caught in this study support findings by Hewitt *et al.*, (1982) and Van der Linde (1984) who found peak adult populations of the species during the same months.

### The *Aedes* group

The *Aedes* mosquitoes occurred mainly at sites grouped under column A (Table 2.3) and specifically site no. 3 at which very little vegetation was present within the water body. The mean conductivity for this site was  $11,2 \text{ mS m}^{-1}$  (Table 2.1). *Ae. juppi* was also collected in large numbers at site no. 16 (Fig. 2.8) (Table 2.3, column B) together with *Cx. univittatus* and *Cx. pipiens*. The site specificity, viz. site no. 3, of the *Aedes* group is probably linked to a large extent with the occurrence of a large expanse of gently sloping silt and mud around the edges of the dam that provided suitable sites for oviposition. It is well known that floodwater mosquitoes oviposit on moist soil margins of pools placing the eggs singly or in small groups in cracks and interstitial spaces between soil particles (Kennedy, 1942; Horsfall & Morris, 1952; Beckel, 1955; Gillett, 1955; Horsfall, 1963; Russo, 1978). Pools with gently sloping sides (viz. site no. 3; Table 2.3, column A) and a slow recession rate of water presents a more suitable oviposition site for a longer period than those with steep, sloping margins (Horsfall, 1963). Edwards (1941), Jupp (1967) and McIntosh *et al.*, (1973) indicated that *Ae. caballus* and *Ae. hirsutus* frequented drier regions where heavy thunderstorms provide ground pools with little or no vegetation while *Ae. juppi* is found more frequently in regions of slightly higher rainfall where water accumulates

over grassland viz. site no. 16 (Tables 2.1, 2.3 & 2.4).

### 2.3.1.3 Seasonal distribution of all immature stages

The total numbers of immature mosquitoes sampled during the survey period showed three population peaks (Fig. 2.12) closely associated with mean total monthly rainfall and mean monthly air temperatures. A smaller population peak is evident during January when less rain was recorded and is probably due to continued oviposition by adults in other permanent water bodies (Fig. 2.12).

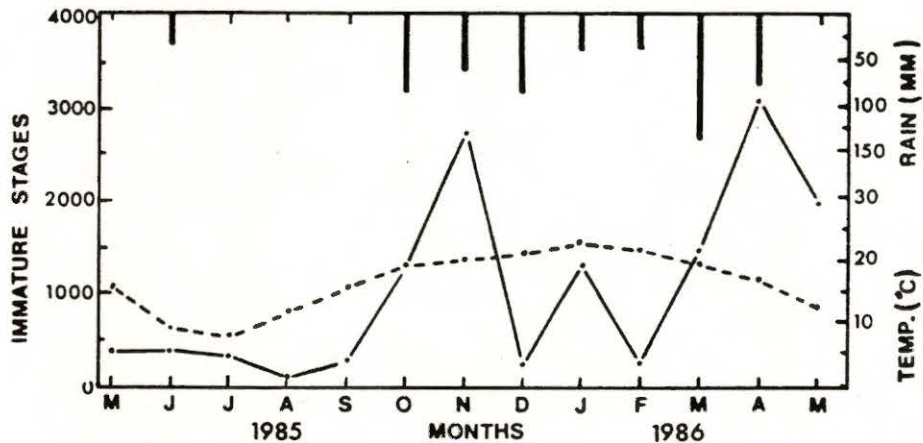


Fig. 2.12 Seasonal distribution of the total numbers of immature mosquitoes sampled from May 1985 to May 1986. Immature stages = solid line. Mean monthly atmospheric temperature = broken line. Rainfall (mm) = columns.



#### 2.3.1.4 Relative frequencies of individual instars

The relative frequencies of individual instars of all species during 1985 - 1986 indicated three distinct population peaks for 2nd, 3rd and 4th instar larvae, while four peaks were observed for instar one larvae and pupae (Fig. 2.13). During the period May to June 1985 the numbers of all instars remained relatively constant. In July, instars one and two showed slight reductions in numbers sampled, while a small increase in frequencies of the remainder occurred (Fig. 2.13). The reductions indicated for instars one and two (Fig. 2.13) were probably due to mortality, reduced oviposition by adults and delayed hatching of eggs as the mean air temperature for July was approximately 8°C. The rise in numbers of instars three, four and pupae was possibly due to progression of some instars eg. one and two, to later instars. During August, fewer instar three and four larvae and pupae were sampled. These reductions may have been due to mortality as no change occurred in instar one and two numbers during this period. On the other hand, it is also possible that temperatures were high enough during August to allow some of the fourth instar larvae and pupae to reach adulthood. The constant number of instar one and two larvae in August could be ascribed to the low mean temperature at which hatching occurred. This would have prolonged their developmental time. Mean air temperatures were between 9°C and 10,5°C

TOTAL NUMBERS OF ALL SPECIES

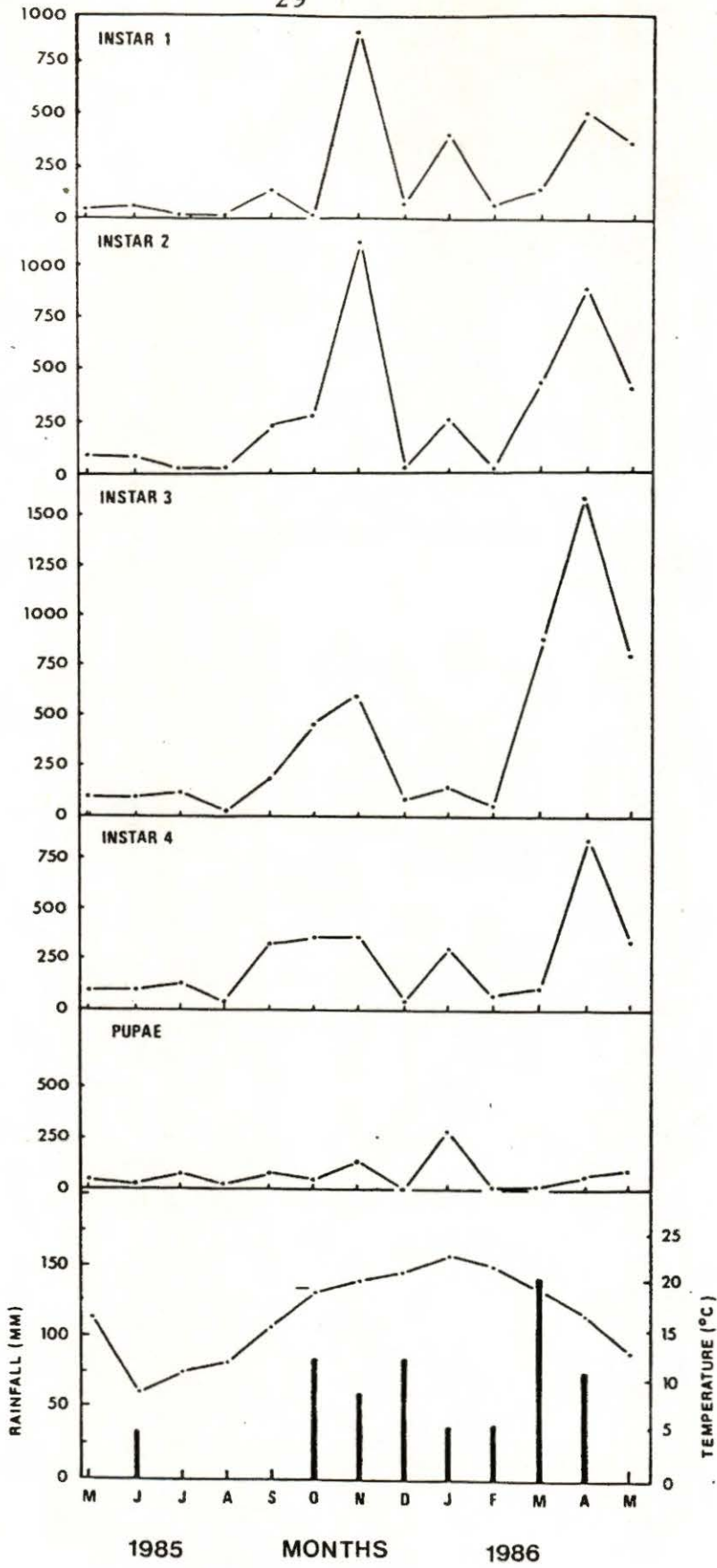


Fig. 2.13 The relative frequencies of instars sampled during May 1985 to May 1986.

during August (Fig. 2.13). Van der Linde (1984) has shown that the temperature threshold of development for immature *Cx. theileri* was between 6,3°C and 7,9°C under constant temperature regimes. However according to Van der Linde (1984) none of the larvae exposed to a constant temperature of 12°C reached adulthood. Thus, the reductions in instars three, four and the pupal stage observed during August may be due to the combined effect of mortality in some larvae while more robust individuals were able to reach adulthood under the lower variable diurnal temperatures occurring in the field. Furthermore, it is generally agreed that, compared to constant temperatures, variable field conditions result in reduced developmental thresholds (May, 1985).

During September, the numbers of all the instars increased as mean air temperatures rose from approximately 14°C to 18°C owing to an increase in the developmental rate of the immature stages (Fig. 2.13). Furthermore, the higher temperatures may have stimulated oviposition by newly emerged and possibly some overwintering adults which would replace earlier instars which had progressed to later stages. Van der Linde (1984) has indicated that under constant temperature regimes, 70 % and 80 % of the *Cx. theileri* females oviposited at temperatures as low as 15°C and 18°C respectively. In addition, he showed that 85 % of the eggs laid, hatched in spite of limited exposure to a temperature of 2°C for four days.

During October the populations of all instars increased with the exception of instar one and pupae, which showed a decline. The increase in the former instars and the reduction in the latter two were probably due to progression of earlier instars to later stages. The reduction in instar one and pupae may also be due to mortality or eclosion and reduced replacement oviposition by adults. However this seemed unlikely as temperatures were high enough in October (between 18°C and 21°C) to allow for oviposition and eclosion.

The best explanation is that the substantial rainfall (84 mm) (Fig. 2.13 and Table 2.4) in October reduced adult populations or destroyed freshly laid egg rafts which resulted in the reduction in instar one larvae in October. Van Pletzen & Van der Linde (1987) have shown that large raindrops are highly damaging to *Cx. quinquefasciatus* egg rafts, reducing egg hatch substantially.

In November, the largest numbers of first, second and third instar larvae were recorded (Fig. 2.13). This rise in population numbers is probably due again to accelerated oviposition and developmental rate as mean air temperatures were above 20°C from mid-October (Fig. 2.13). In addition, the 84 mm of rain recorded in October provided the first substantial range of springtime breeding sites for

mosquitoes (Fig. 2.13). During December 1985 and February 1986 numbers of all instars were reduced. A small peak occurred in January when less rain was recorded. It is interesting that the greatest numbers of pupae captured in the whole survey, occurred in the "drier" January 1986 period (Fig. 2.13). The reason for this is not clear. Possibly a generally lower level of oviposition occurred with adults taking refuge during the hot dry period. Alternatively, other factors such as competition between younger instars for food and space may have caused differentially higher mortalities than that which may have occurred within the pupal populations which do not compete for food (Van Pletzen, 1981; Van der Linde, 1984). White (1980), in an intensive study of field populations of *Cx. tarsalis* indicated that adult and especially larval populations were unexpectedly reduced in mid-summer periods. He suggested that four factors were largely responsible for this phenomenon viz. with respect to the larvae: fluctuations in water level of the field pool site, intraspecific density dependent factors (eg. nutrition, overcrowding, competition etc.) and predation; and with respect to the larvae and the adults: high water and air temperatures. Baily & Gieke (1969, quoted by White, 1980) and Reisen (1975) agreed that high water and air temperatures might influence the numbers of larvae and adults. A similar situation may have occurred during the "drier" mid-summer period during this study in which

immature populations were reduced (Figs. 2.12 and 2.13).

It is interesting that there were large larval populations in April, but much smaller populations in May. This could be related to the mean air temperature which dropped from 20°C to 13°C. However, the numbers of pupae increased slightly (Fig. 2.13). It is possible that these pupae give rise to adults that may take a blood meal and oviposit during early winter. Alternatively, these adults may form the nucleus of an overwintering population. The low winter temperatures, and the fact that all the immature stages of mosquitoes were present during the winter months, suggest that the first and second larval instars that were present at the onset of winter were able to continue development, albeit at a reduced rate. *Cx. theileri* and *Cx. pipiens* instars in all stages of development were collected throughout the winter months (Table 2.4). The large population peaks recorded in autumn (April 1986) represent mostly *Cx. theileri* and *Cx. pipiens* immature stages and to a lesser extent *Cx. univittatus* (Table 2.4). Jupp (1969) showed that in the Transvaal highveld, the fourth instar larvae of *Cx. fatigans* took 60 days to reach adulthood, while *Cx. pipiens* instar one larvae took 108 days, and second instar larvae of *Cx. theileri* took 74 days to reach adulthood in winter (Jupp, 1975). He also reported that *Cx. pipiens* and probably *Cx. theileri* and *Cx. univittatus* immature stages which might be present in the autumn and

early winter can survive throughout the winter and thus produce viable adults in the spring. However, during the present study, immature *Cx. univittatus* were not collected during June, July and August. It is possible that larvae of this species were not recorded because of their very low numbers during these months. On the other hand, as only approximately 60 % of the total numbers of immature stages captured during the survey, reached adulthood in the laboratory, it is possible that a number of species including *Cx. univittatus* might have died in the laboratory before reaching adulthood. They may have died of heat shock when transferred to the warmer laboratory from the colder field conditions. As no attempts were made to identify species in the larval stage during this study, larvae dying in the laboratory were ignored in subsequent analyses.

Another possibility is that *Cx. univittatus* may overwinter largely as adults accounting for the apparent lack of immature stages in mid-winter. Support for this is given by Jupp (1969, 1975). He found that adult *Cx. univittatus* populations increased in density in the spring faster than either *Cx. pipiens* or *Cx. theileri* and generally at an earlier date. Furthermore, Jupp (1969, 1975) showed that parous rates of *Cx. univittatus* were higher in autumn and spring than those of *Cx. pipiens* and higher than *Cx. theileri* in spring. Parous rates of 91 % were recorded for *Cx. univittatus* almost a month before the other two species

showed any increases (Jupp, 1975). Parousness in females is taken to indicate that oviposition has occurred in these individuals prior to capture. In Jupp's studies (1969, 1975), these parous females were from two likely sources, viz. young females that had emerged and oviposited in late autumn, hibernated during winter as low temperatures precluded oviposition and were captured in spring when temperatures were favourable for a resumption of activity. On the other hand, these females might have emerged in winter and early spring and been able to oviposit in warmer periods prior to capture. However, as no immature stages of *Cx. univittatus* were found in winter during this survey it is possible that females of this species overwinter in the parous condition (Jupp, 1975). But since the summer biology of *Cx. pipiens*, *Cx. theileri* and *Cx. univittatus* is similar, Jupp (1975) considers this unlikely. Thus, it is still uncertain whether *Cx. univittatus* overwinters in the immature or adult form.

#### 2.3.1.5 Seasonal distribution of individual species

The temporal distribution of larvae of the seven most abundant species (Table 2.4) are considered individually. They are *Cx. theileri*, *Cx. pipiens*, *Cx. univittatus*, *Cs. longiareolata*, *An. aquamosus*, *Ae. juppi* and *Ae. caballus*.



*Cx. theileri*

Three population peaks were evident. The larger peaks of summer (November) and autumn (April) (Fig. 2.14 A) being associated with rainfall peaks during October to December and March to April (Fig. 2.14 D). A smaller population peak in the drier mid-summer period (January) during 1986 probably represents continued oviposition by *Cx. theileri* in more permanent water bodies at this time. Thus, this species utilizes both temporary and permanent pools for breeding (Edwards, 1941, Jupp, 1969, 1975). The large (see also section 2.3.1.2) peaks in population numbers are associated in all probability with the greater use of widespread temporary pools (except site no. 3) during peak rainfall periods which augment the more permanent pools (except site no. 9).

*Cx. pipiens*

*Cx. pipiens* also has three obvious population peaks, viz. September and November 1985 and in April 1986 (Fig. 2.14 B). The population peaks become progressively larger from spring through summer to autumn (Fig. 2.14 B).

The sharp increase in *Cx. pipiens* larval populations in September 1985 almost two months earlier than *Cx. theileri* (compare Figs. 2.14 B and 2.14 A) is attributed to the increased ovipositional activity of the former at lower mean atmospheric temperatures and the presence of a very favourable permanent breeding site (no. 10, Fig. 2.7). This supported to some extent by Jupp (1975) who reported that parous rates for *Cx. pipiens* were higher (47 %) than *Cx. theileri* (20 %) in October in the Transvaal highveld. One would expect a similar trend in parous rates for the two species in September. Unfortunately Jupp (1975) trapped too few of both these species in his study to confirm this. *Cx. pipiens* (Fig. 2.14 B) also differs from *Cx. theileri* (Fig. 2.14 A) in that the peak in January is absent. The reason that larval populations of this species remained low during December 1985, and January and February 1986 (Fig. 2.14 B) is possibly due to adults taking refuge from the hot drier weather (Reisen *et al.*, 1984) or due to heightened predation of larvae or interspecific competition for food in permanent pools in summer (White, 1980).

#### *Cx. univittatus*

There were two large population peaks, one occurring during November 1985 and one in April 1986 (Fig. 2.14 C). The

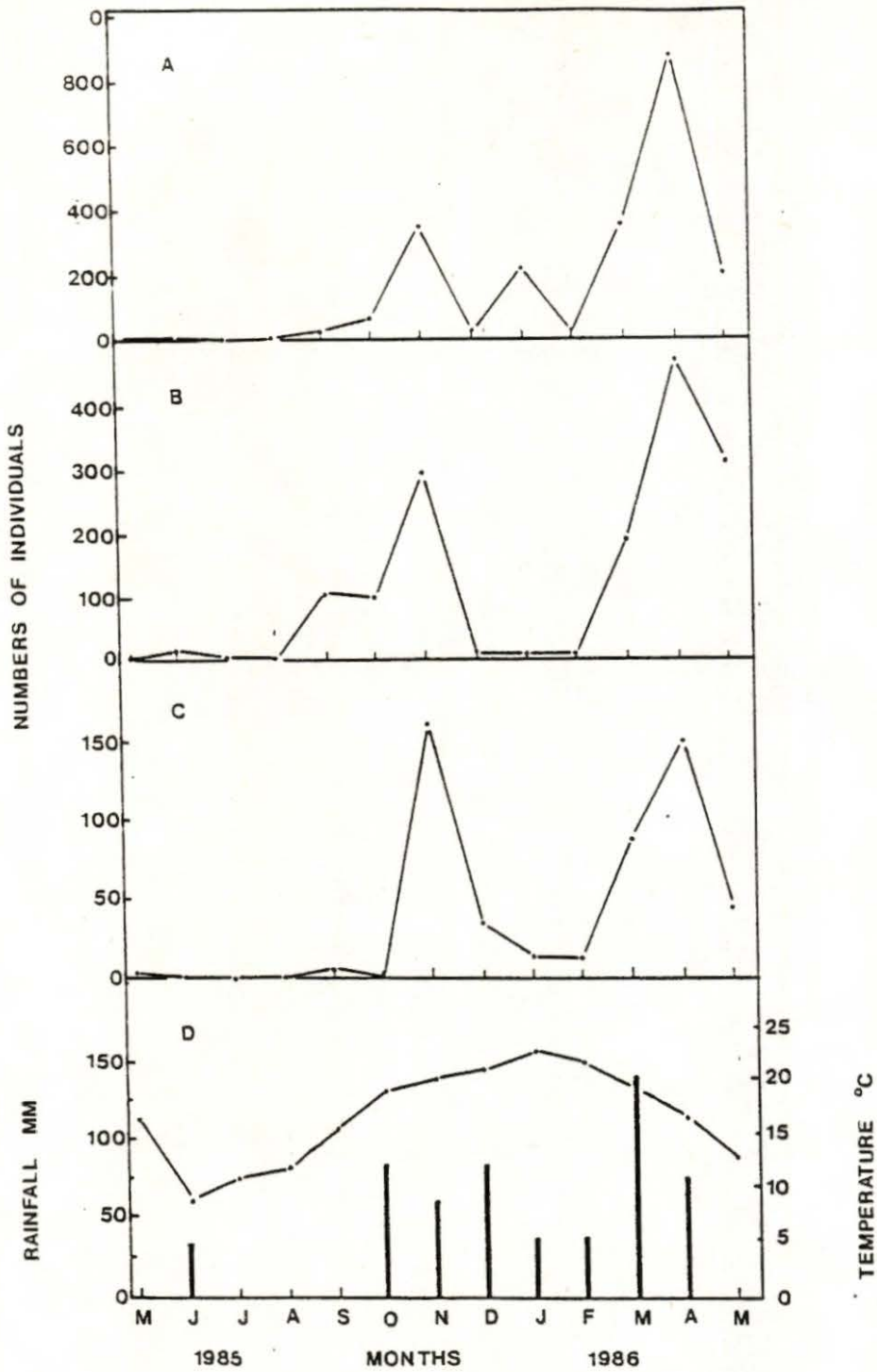


Fig. 2.14 The seasonal distribution of the immature stages of the three most abundant species sampled during 1985 to 1986, viz. A: *Cx. theileri* (46,15 %), B: *Cx. pipiens* (32,43 %) and C: *Cx. univittatus* (10,76 %), D: Precipitation (columns = monthly totals) and temperature (dots = monthly means).

peaks in immature numbers appeared to be even more closely associated with rainfall than was the case with *Cx. theileri* and *Cx. pipiens* (Fig. 2.14 A and B) (see section 2.3.1.2). However, *Cx. univittatus* differs from both *Cx. theileri* and *Cx. pipiens* in that no immature stages were collected during June, July, August and October (Fig. 2.14 C).

*Cs. longiareolata* and *An. squamosus*

As these two species occurred in greatest numbers in autumn and winter (Table 2.4) they are discussed together. *Cs. longiareolata* was found only from May to September, with a peak in August 1985 and one in May 1986 (Table 2.4). Similar results were obtained in the field by Van Pletzen & Van der Linde (1981) at a site in the Bloemfontein area. A probable explanation why larvae were absent from samples during the summer months in the present study, is that thunderstorms and the consequent flooding of the Modder River destroyed site no. 13 during October and November 1985 (see Section 2.3.1.2).

*An. squamosus* were found during April and May 1986 (Table 2.4) and were absent from August to November 1985 and March 1986. However, Bedford (1928) and Van der Linde (1984) trapped large numbers of adults during the summer months. The reasons that larvae were not present in samples during spring and summer in this survey are not clear. However,

predation may be an important factor (White, 1980) (see Section 2.3.1.2).

#### The *Aedes* group

The majority of immature *Aedes* were collected in samples taken during October, with *Ae. juppi* occurring again in November 1985 and *Ae. vittatus* only in April 1986 (Table 2.4). The presence of these floodwater mosquitoes in a very narrow time period during this survey is correlated with high rainfall during the summer (Table 2.4). It is known that the eggs of *Aedes* laid on damp soil need to be washed out by heavy rain and completely submerged for hatching to occur (Horsfall, 1956).

#### 2.3.1.6 Temporal and spatial co-abundance of species

Based on the numerical abundance for temporal and spatial co-abundance, correlation coefficient matrixes were calculated for the nine dominant species. As not all species were present at all sampling dates and sites, the following criteria were used to select the species to be analysed.

In the temporal co-abundance analyses it was arbitrarily decided to include only species that were present on at least five out of the 13 sampling dates. The exception was

the *Aedes* species where all were included, even if found only once, provided 20 or more individuals were sampled. This was done because of their obvious grouping at a very narrow period in time, ie. after heavy rains. In the latter case, the use of correlation coefficients might be in doubt, but their inclusion was necessary for a more complete picture.

The same species were subjected to spatial co-abundance analyses. From these matrixes, dendrograms were compiled using a group average sorting strategy (Lance & Williams, 1967). Only clusters at a minimum level of significance of  $p = 0,05$  were considered and are discussed below.

In as far as temporal co-abundance is concerned two groups, *Culex* and *Aedes* were correlated at above the 0,55 ( $p = 0,05$ ) level (Fig. 2.15). Group I comprised *Cx. theileri*, *Cx. pipiens* and *Cx. univittatus*, with the former two species being slightly more closely associated in time than the latter (Fig. 2.15). The fact that these three species were present throughout the year (except for *Cx. univittatus* in winter) (Fig. 2.15), their habit of laying on permanent water, and the fact that their population numbers tended to rise and fall at approximately the same times probably explains the high correlation coefficient value indicated for them.

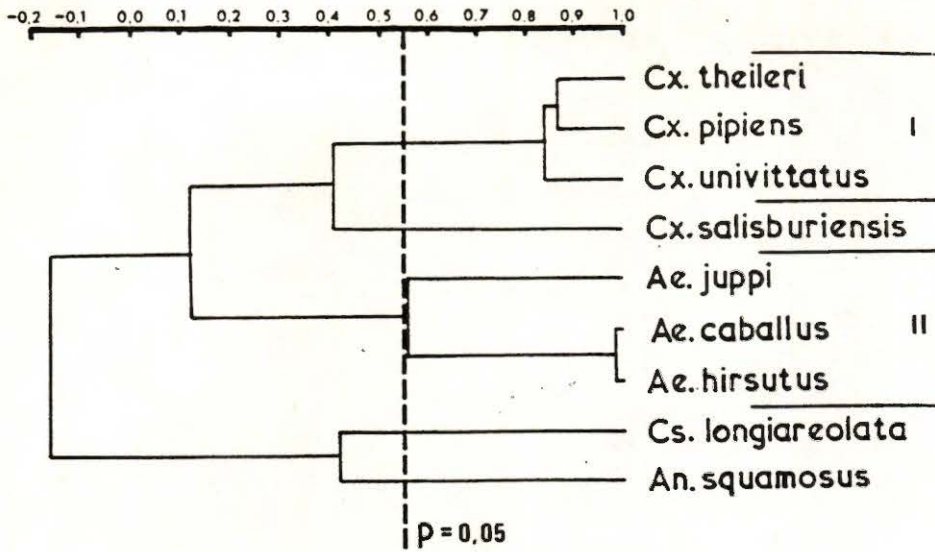


Fig. 2.15 A dendrogram showing the temporal co-abundance of species during May 1985 to May 1986.

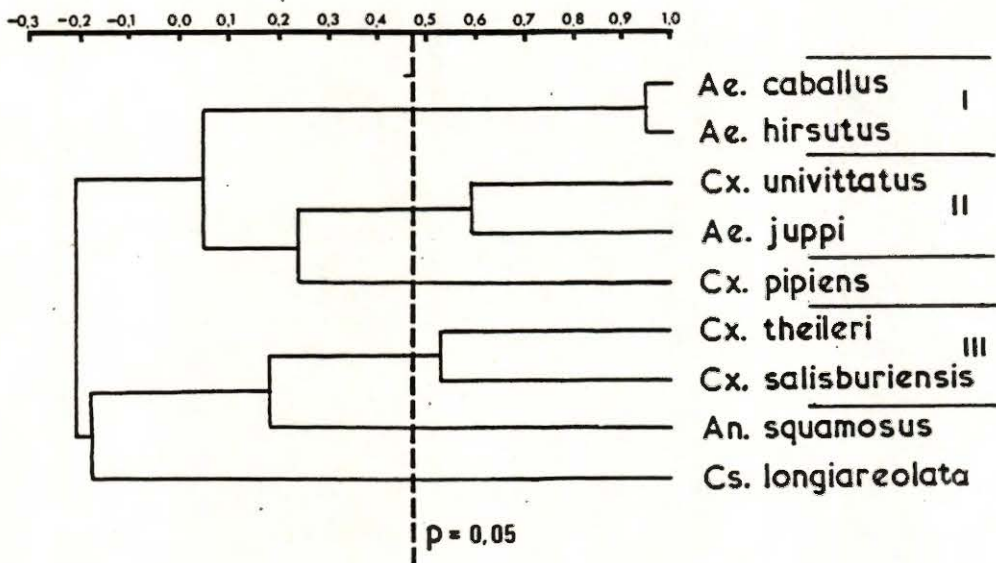


Fig. 2.16 A dendrogram indicating the spatial co-abundance of species from May 1985 to May 1986.

Group II was made up of *Ae. caballus*, *Ae. juppi* and *Ae. hirsutus*, which are all floodwater mosquitoes (Fig. 2.15). The high correlation between these three species, especially *Ae. caballus* and *Ae. hirsutus* is probably due to the fact that they were only found during October and November when sufficient rain had fallen. Total submergence of the eggs is a prerequisite for the hatching of the eggs in these species which oviposit in moist soil in areas where water accumulates after heavy rain (Horsfall, 1956). Thus, their high degree of co-abundance is not unexpected.

*Cx. salisburyensis*, *Cs. longiareolata* and *An. squamosus* are not associated in time with each other or with species in groups I and II (Fig. 2.15). These three species attain their highest numbers at different times in the cooler autumn, winter and spring months (Table 2.4) compared to groups I and II which are "summer" mosquitoes. Although these three species all require permanent water in which to oviposit, the different times at which their numbers rise and fall probably explains their low correlation in time.

In the spatial co-abundance analysis, three groups were associated at a correlation coefficient above 0,48 ( $p = 0,05$ ) (Fig. 2.16). Group I comprised *Ae. caballus* and *Ae. hirsutus*. This grouping is not surprising since they are both floodwater mosquitoes. However, the absence of *Ae. juppi* from this group is unexpected. The reason may be that



*Ae. juppi* was found in highest numbers at another site (site no. 16; (Table 2.3) in which the other *Aedes* species were absent. Although this phenomenon has been reported by Dr. T. van der Linde (personal communication), some support for the dissociation of *Ae. juppi* from *Ae. caballus* is given in work by Edwards (1941), Jupp (1967) and McIntosh *et al.* (1973). Edwards (1941) noted that *Ae. caballus* and *Ae. hirsutus* generally breed in temporary rain or floodwater pools, which are usually open with little vegetation and Jupp (1967) reported that *Ae. caballus* was generally found in rainwater overlying bare soil. McIntosh *et al.*, (1973) however, reported that *Ae. juppi* larvae occur in temporary rainpools in grassland. The vegetational cover of site no. 3 and 16 differ considerably (Table 2.1). Site no. 3 has virtually no vegetational cover while no. 16 has abundant cover. This confirms the observations of Jupp (1967) and McIntosh *et al.* (1973) mentioned above.

The co-abundance of *Ae. juppi* and *Cx. univittatus* in space might seem somewhat unexpected (group II, Fig. 2.16). *Ae. juppi* oviposits in soil near the water, while *Cx. univittatus* oviposits on the water surface. A possible explanation for their co-abundance is that *Cx. univittatus*

prefers clear rain water accumulating over emergent vegetation, low in dissolved organic matter and suspended solids (Jupp, 1967). Furthermore, as noted above under group I, *Ae. juppi* larvae often occur in temporary grassland rainpools (McIntosh *et al.*, 1973).

Group III consists of *Cx. theileri* and *Cx. salisburyensis*. The resemblance in their oviposition needs probably explain their spatial co-abundance. However, *Cx. salisburyensis* has usually been collected in permanent pools, open with little vegetation (Table 2.4) or in the shaded edges and backwaters of streams (Edwards, 1941), while *Cx. theileri* occurs in a wide range of habitats (Table 2.4) (Jupp & McIntosh, 1967).

The spatial separation of *Cx. pipiens*, *An. squamosus* and *Cs. longiareolata* (Fig. 2.16) from each other and from species in groups I, II and III can be attributed to oviposition preferences. *Cx. pipiens* prefers sewage effluent (Jupp, 1967), while *An. squamosus* frequents still, deep often shady pools (Hopkins, 1952) and *Cs. longiareolata* is usually found in small, steep-sided pools often devoid of vegetation (Gillet, 1971; Van Pletzen & Van der Linde, 1981). The association between the latter two species reported by Van Pletzen & Van der Linde (1981) is not supported in this study.

It is noteworthy that species closely associated in time

(Fig. 2.15) are often not closely associated in the habitats occupied by the larvae (Fig. 2.16). For instance, *Cx. theileri* and *Cx. pipiens* are closely associated temporally (Fig. 2.15) but are spatially dissociated (Fig. 2.16). The reason for this may be that these two species probably compete directly for available food sources in situations where they co-occur. On the other hand it is certain that *Cx. pipiens* prefers water of different quality such as sewage water (Jupp, 1967). A similar situation probably exists between *Cx. univittatus* and *Ae. juppi* (Figs. 2.15 and 2.16). Spatial or temporal dissociations of mosquito species would act to minimize interspecific competition for resources. *Ae. caballus* and *Ae. hirsutus* were found to be co-abundant in space and time (Figs. 2.15 and 2.16). This would suggest that these two species are direct competitors for resources. However, competitive exclusion has apparently not occurred as both these species were present in the region in previous surveys of adults in the western Orange Free State (Van der Linde *et al.*, 1982). It is probable that larval *Ae. caballus* and *Ae. hirsutus* differ in their micro-habitat requirements, in feeding behaviour, or utilization of different resources in the food web. Resource partitioning is known to occur in other species: Van Pletzen has shown in 1981 that *Cs. longiareolata* larvae spend most of their time browsing on the substratum. *Cx. univittatus* larvae on the other hand are mainly filter-feeders (Mostert, 1981) although some browsing does occur.

The various instars of *Cx. univittatus* also differ in the time spent on a variety of activities; eg. instar four larvae browse on the substratum more than instar one larvae (Mostert, 1981). These examples indicate that behavioural and morphological differences between species and between instars within spatially co-abundant species are likely to be reflected in partitioning of the micro-habitat in these species and can lead to coexistence. An in-depth study of the behavioural activities of immature stages, such as was done with *Cs. longiareolata* (Van Pletzen, 1981) of co-abundant species of *Aedes* could undoubtedly throw more light on resource partitioning in aquatic environments.

### 2.3.2 Overwintering adults

The trapping of adult mosquitoes around the pool sites during the winter months was unsuccessful. On only two occasions were adult mosquitoes found viz. at site no. 13 during May and August 1985. The only species captured with the emergence trap (Fig. 2.11), was *Cx. theileri*. In both instances, the mosquitoes were caught in fairly dense stands of volunteer oats surrounding a concrete sewage reservoir. Paucity of adults during winter and the fact that in cool conditions, adult mosquitoes are loathe to fly, even when disturbed by agitation with a suction apparatus (Fig. 2.10) or repellants (eg. Citronella oil) probably explains the difficulty in finding overwintering survivors in the field.

Furthermore, adults rarely remain around the pool sites from which they may have emerged in winter (Van der Linde - personal communication).

An emergence trap (Fig. 2.11) left in position for one or two days in areas to be sampled would possibly provide more acceptable results as it would probably trap any mosquitoes present should they become active at favourable temperatures. However, the use of the suction apparatus (Fig. 2.10) proved completely unsuccessful under winter conditions in the Bloemfontein area. It could be that the areas traversed (10 m x 10 m) were too small, or that the time allotted (20 minutes/100 m<sup>2</sup>) was insufficient, or that the suction rate at the mouth of the funnel (Fig. 2.10) was inadequate to capture mosquitoes in vegetation. However, the most likely reason is that adults are scarce and inactive in winter and probably do not remain in the immediate vicinity of pool sites.

## 2.4 Conclusion

This study provided concrete evidence that *Cx. theileri* and *Cx. pipiens* overwinter in the western Orange Free State in the field as larvae. [The fact that larval populations are drastically reduced in the cold, dry winter months,] the lowest numbers caught representing approximately four percent of the peak autumn populations, underscores the role of low temperature and low rainfall in limiting mosquito numbers in the field. [While low temperatures in winter reduce the rate of development of immature stages and oviposition by adults as well as increasing mortality, reduced rainfall during the summer period is probably a major factor in limiting mosquito populations,] [High rainfall is usually followed by peak populations a month later,] The indicated time lag of approximately a month between high rainfall and larval population peaks is probably not a true reflection of generation time for the majority of the species represented here. Evidence from laboratory studies is that generation times in summer for *Cx. theileri* (Van der Linde, 1984), *Cx. univittatus* (Mostert, 1981) and *Cs. longiareolata* (Van Pletzen, 1981) would probably be between 18 and 28 days in the field. Furthermore, weekly or fortnightly samples and weather records would indicate time lags between rainfall and increase in immature populations more accurately, and possibly that generation turnover occurs more frequently

than indicated in this survey. However, as White (1980) has indicated, peak populations seem to occur from spring through to summer as rising temperatures caused initially distinct successive cohorts to develop more and more rapidly, so that cohorts merged by the time they emerged, or were sampled, giving the appearance of one large cohort or population peak. Population reductions that occur later in summer and autumn are probably related to intrinsic density-dependent factors viz. overcrowding, competition, predation, etc. together with extrinsic factors such as low rainfall, seasonal reduction in temperature, etc. (White, 1980). However, this explanation needs to be confirmed by intensive field and laboratory studies.

(In this study, temperature and rainfall were certainly the most important factors influencing immature mosquito numbers.) However, the fact that larger populations did not always occur after favourable rainfall implies that other factors may be involved. The lack of response in *Cx. pipiens* and *Cx. univittatus* populations and the relatively small peak in *Cx. theileri* numbers during January and February (Table 2.4) after 84 mm of rain was recorded in December are indications of this phenomenon. Apart from the possible influence that the high mid-summer temperatures may have had in reducing survival of both larvae and adults and fecundity (White, 1980), predation may have played a significant part in preventing or limiting population growth

(White, 1980). Predators would tend to have increased greatly in numbers by mid-summer, especially in more permanent water bodies and this may have accounted for the low numbers recorded in mid-summer. As virtually nothing is known about the influence of predators on the population dynamics of mosquitoes in the western Orange Free State, an intensive study in this respect would certainly contribute to the ecology of the insect fauna in the region.

The temporal and spatial co-abundance of species reflects to a large extent the overriding importance of temperature and rainfall in the former and ovipositional requirements in the latter. The influence of climatic conditions on seasonal abundance of mosquitoes is fairly well researched. However, the factors that influence spatial co-abundance, especially at the micro-habitat level in the field, has received little attention. This is probably due to the practical difficulty of measuring multiple variables and the complexity of events and their interactions with respect to choice of a site by a female to assure the survival of her progeny. However, intensive studies such as those carried out by White (1980) can probably be adapted to answer some questions on the co-existence of various species at selected sites and possibly the mechanisms by which competition is avoided. An example is the temporal and spatial co-abundance of *Ae. caballus* and *Ae. hirsutus* in this study. However, partitioning of the micro-habitat needs to be considered and studied in order to



solve this problem.

Finally, the problem of identifying the winter resting sites of adult mosquitoes that emerge in late autumn or early winter needs to be addressed. The fact that the use of a suction apparatus or a modified emergence trap as considered here, proved unsuccessful, indicates either that other trapping methods must be considered or that adults generally do not survive long during winter periods. It certainly seems that they do not remain in the vicinity of the pool sites surveyed. Furthermore, the necessity of obtaining information on overwintering adults is based on the fact that in the case of *Cx. theileri* the transmission of Rift valley fever has been demonstrated by such adults (McIntosh, et al., 1973; Jupp, et al., 1980).

### 3.0 The influence of fluctuating temperatures and humidities on the development and survival of *Culex theileri*.

#### 3.1 Introduction

There is evidence that the established procedure of using constant temperature and humidity regimes in the laboratory to some extent precludes precise assessment of developmental potential and survival of animals under field conditions (Huffaker, 1944; Richards, 1960). Some recent studies have indicated that the developmental rate and eventual body mass of animals bred under variable temperature regimes similar to those in the field were higher than those recorded under constant temperatures (Wilkinson & Daugherty, 1970; Odum, 1971; Caulton, 1976; Van As, 1980). A reduction in the developmental thresholds is also known to occur in variable temperature regimes (Headlee, 1940; 1941; Huffaker, 1944; Andrewartha & Birch, 1954; Messenger & Flitters, 1959; Christophers, 1960; Richards, 1960).

Work on the mosquito fauna on the South African highveld has shown that *Cx. theileri* occurs throughout most of the winter in the immature stages (Jupp, 1969, 1975) and as adults (Hewitt, et al., 1982; Van der Linde et al., 1982; Van der Linde, 1984). The lower lethal limit for immature stages of *Cx. theileri* at constant temperatures is 12°C (Van der

Linde, 1984). The  $LT_{50}$  of blood fed females at 6°C and a saturation deficit (sd) of 0,40 kPa was 14,4 days (Van der Linde, 1984). The fact that the weekly mean temperatures on the Transvaal highveld during winter are often lower than this (i.e. 2°C to 9°C) (Jupp, 1969, 1975) implies that the threshold of development in *Cx. theileri* is probably lower in the field than under constant temperatures in the laboratory. The discrepancy between laboratory and field results was investigated in this study.

## 3.2 Materials and methods

### 3.2.1 The influence of variable temperatures on the development of *Cx. theileri* larvae

#### 3.2.1.1 Laboratory trials

Egg rafts laid by *Cx. theileri* females from a laboratory colony over a six hour period, were placed in the different incubators simulating various fluctuating temperature regimes (Table 3.1). These temperatures fell within the range observed in the Orange Free State during past seasons (Hewitt *et al.*, 1982; Van der Linde *et al.*, 1982). The larvae that hatched were transferred to plastic containers containing 0,02 M NaCl rearing solution and kept in their respective temperature regimes for 24 hours before developmental trials were started.

One hundred and twenty instar one larvae were selected at random from each regime and placed individually in Apex vials (no. 8) containing 10 ml of 0,02 M NaCl rearing solution. The vials were placed in racks in water baths running at the desired temperatures. The latter were recorded continuously with thermographs.

Fresh rearing solution was provided every second day for first and second instar larvae and daily for third and

fourth instars. Pupae were left unchanged until eclosion. Observations were made daily. Exuviae from the fourth instar larvae were removed and the head capsules measured to assess the influence of variable temperatures on the growth rate of the larvae.

#### 3.2.1.2 Trials under natural climatic conditions

Temperature conditions in natural pools were simulated by placing a portable plastic pool ("Porta-pool": 3,0 m in diameter by 1,0 m in height) filled with tap water to a depth of 45 cm in the field (Fig. 3.1). The temperatures recorded with thermographs are given in Table 3.2. The experimental procedure was the same as described for laboratory trials, with the exception that the egg rafts were hatched in containers floating in the pool. The larvae were transferred to Apex vials which were supported in floating polystyrene racks (Fig. 3.1). Observations were made daily. Exuviae from the fourth instar larvae were removed and treated as in 3.2.1.1

The experiments were conducted consecutively, except during the winter period when two experiments were conducted concurrently due to the extended life cycle, making consecutive experiments impossible.

Table 3.1 The various fluctuating temperature regimes used in laboratory trials.

Regime	Temperatures (°C)		
	$\bar{x}$	$\bar{x}$ max	$\bar{x}$ min
1	10,0	14,3	5,3
2	11,6	15,8	7,4
3	12,8	18,1	7,0
4	15,3	19,2	7,0
5	22,5	29,5	14,1
6	24,1	29,7	18,5

Table 3.2 Temperatures recorded during various times in field trials.

Regime	Temperatures (°C)		
	$\bar{x}$	$\bar{x}$ max	$\bar{x}$ min
1	8,2	10,8	5,4
2	8,2	10,8	5,4
3	12,9	16,9	7,9
4	17,2	21,4	12,9
5	20,6	26,0	17,9
6	20,9	24,0	16,6
7	22,4	26,0	17,2
8	22,6	27,1	19,0
9	22,8	26,4	18,6

3.2.2 The influence of variable temperatures and saturation deficits on female *Cx. theileri*

Four mean saturation deficits (0,32, 1,10, 0,53 and 1,44 kPa) were established in desiccators (Table 3.3). The methods of Buxton & Mellanby (1934) and Solomon (1951) were used. The chemicals used are given in Table 3.3. The temperature and saturation deficit chosen fell within the range measured in the field (Hewitt *et al.*, 1982; Van der Linde *et al.*, 1982; Van der Linde, 1984). Individuals from an established laboratory colony, which was replenished regularly with new field collected females were used. Only blood-fed females were used as these were known to live longer than males or unfed females (Van der Linde, 1984). Within two hours of a blood meal, the individual females were lightly anaesthetized with CO<sub>2</sub> and their masses determined.

They were placed into Apex vials (no. 8) which were then sealed with perforated plastic stoppers and placed into desiccators. The latter were placed in water baths and the temperature fluctuations were simulated by transferring the water baths and desiccators from one temperature regime to the other every 12 hours. Temperature changes were recorded continuously. The relative humidities were tested at the beginning and end of the experiment. Observations were made daily and the desiccators were opened only for brief periods

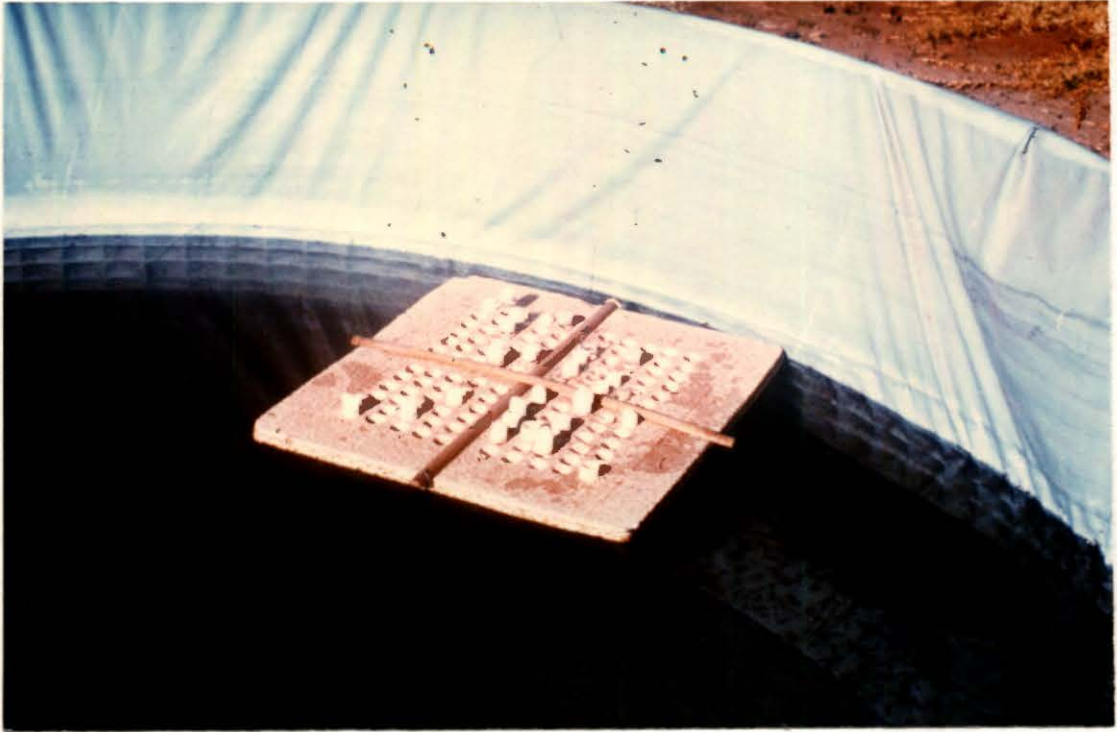


Fig. 3.1 A floating polystyrene rack in the "Porta-pool" supporting Apex vials containing the larvae.

Table 3.3 The various treatments to which bloodfed Cx. theileri females were exposed.

Temperature (°C)			$\bar{x}$ s.d.	$\bar{x}$ R.H.	Chemicals in g/100ml water	Chemicals
$\bar{x}$	$\bar{x}$ max	$\bar{x}$ min	(kPa)	(%)		
10,0	15,3	4,6	0,32	77	Super sat. soln.	NaCl
10,0	15,3	4,6	1,10	10	Super sat. soln.	ZnCl
20,0	26,5	13,5	0,53	74	65 g	KOH
20,0	26,5	13,5	1,44	38	95 g	KOH



to remove the dead mosquitoes. The dead females were weighed immediately and the percentage mass lost was calculated.

### 3.3 Results and discussion

#### 3.3.1 Developmental time under laboratory and field conditions

##### 3.3.1.1 Laboratory trials

The mean developmental time of the various immature stages of *Cx. theileri* under the different temperature regimes is given in Table A 3.1. The analysis of variance showed that there were significant differences ( $p < 0,05$ ) between the developmental times of the different stages ( $F = 1441,30$ ), between the various regimes ( $F = 520,80$ ) and between the sexes ( $F = 6,63$ ) (Table A 3.3).

Tukey's test ( $Q_{0,05} = 1,3051$ ) indicated that the developmental time of instar one larvae was significantly shorter than all the other stages, except instar two. Instar four took the longest to develop. In addition, the developmental times of individuals in the various regimes were shown by Tukey's test ( $Q_{0,05} = 2,2827$ ) to be significantly different from each other except regimes five ( $22,5^{\circ}\text{C}$ ) and six ( $24,1^{\circ}\text{C}$ ) which were the same. Finally, males developed significantly faster ( $p < 0,05$ ) than females ( $F = 6,63$ ) (Table A 3.3) at all temperatures.

### 3.3.1.2 Field trials

The mean developmental time of the different immature stages of *Cx. theileri* under natural climatic conditions is given in Table A 3.2. The analysis of variance showed that there were significant differences ( $p < 0,05$ ) in developmental time between the various immature stages ( $F = 2638,38$ ), between the different temperature regimes ( $F = 677,40$ ) and between the sexes ( $F = 85,57$ ) (Table A 3.4). Tukey's test ( $Q_{0,05} = 2,8656$ ) also showed that in regimes one and two ( $8,2^{\circ}\text{C}$ ) the individuals took significantly longer to develop than in all the other regimes with temperatures ranging from  $12,9^{\circ}\text{C}$  to  $22,8^{\circ}\text{C}$  (Fig. A 3.1). Individuals in regime three ( $12,9^{\circ}\text{C}$ ) had a significantly longer developmental time than those in regimes four ( $17,2^{\circ}\text{C}$ ) to nine ( $22,8^{\circ}\text{C}$ ). In addition individuals in regime four ( $17,2^{\circ}\text{C}$ ) took significantly longer ( $p < 0,05$ ) to develop than those in regimes eight ( $22,6^{\circ}\text{C}$ ) and nine ( $22,8^{\circ}\text{C}$ ) (Fig. A 3.1). Finally, males developed significantly ( $p < 0,05$ ) faster than females ( $F = 85,57$ ) at all temperatures (Table A 3.4).

These findings on the influence of temperature on mosquito development have been reported by numerous other workers (Bar-Zeev, 1958; Brust, 1967; Trpis & Horsfall, 1969; Parker, 1979; McDonald *et al.*, 1980; Van der Linde, 1984). However, some small differences in developmental pattern were apparent (Fig. 3.2 and 3.3). Firstly, in field trials

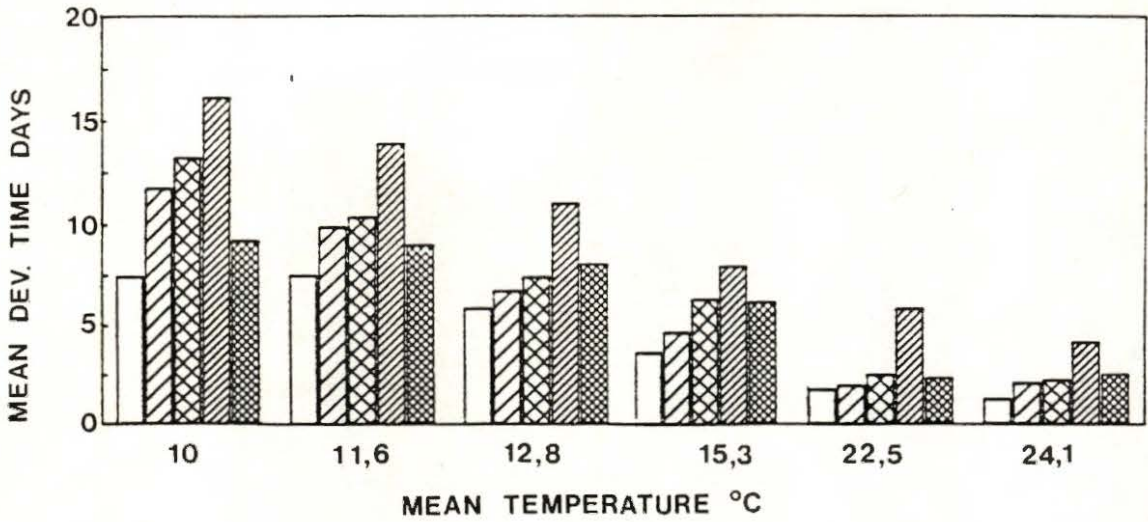







Fig. 3.2 The influence of fluctuating temperatures under laboratory conditions on the developmental times of immature *Cx. theileri* (instars 1 =  , 2 =  , 3 =  , 4 =  , pupa =  .

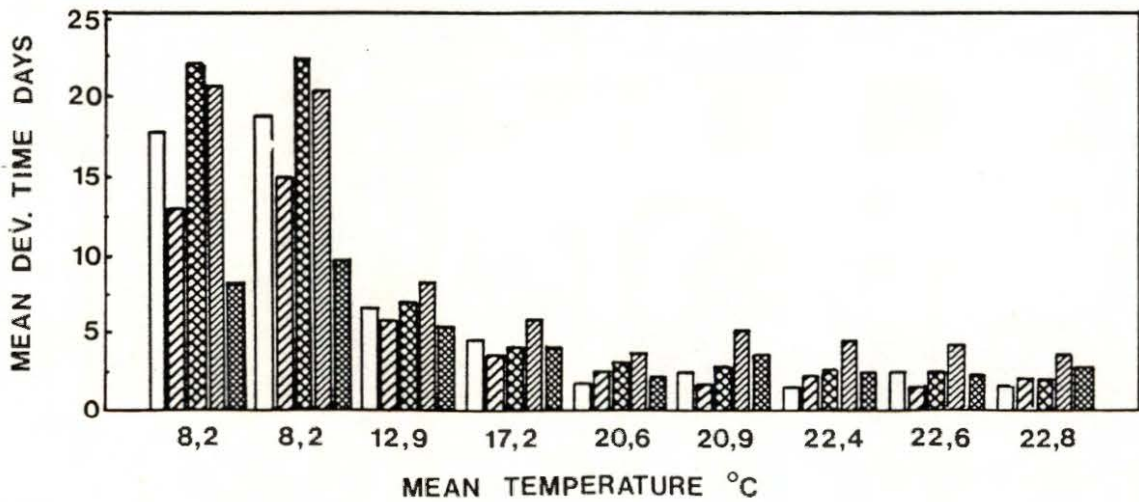







Fig. 3.3 The influence of temperature under natural conditions on the developmental times of immature *Cx. theileri*: (instars 1 =  , 2 =  , 3 =  , 4 =  , and pupae =  ).

the instar one larvae took longer to complete development than the instar two larvae (Fig. 3.3) which was not the case in the laboratory trials (Fig. 3.2). This can probably be ascribed to the fact that the average temperature in the field trials was lower than in the laboratory trials. Secondly, in the field trials the developmental time of the instar four larvae was shorter than the instar three larvae at a mean temperature of  $8,2^{\circ}\text{C}$  (Fig. 3.3). This did not occur in laboratory trials (Fig. 3.2). The differences could be attributed to the uncontrolled sub-zero temperatures that occurred in the field compared to the controlled above-zero temperatures that were maintained in the laboratory. Although mean temperatures were the same, the instar three larvae were exposed to daily sub-zero temperatures on 17 out of 21 days (81 %) while instar four had sub-zero temperatures on only 10 out of 19 days (53 %) during their respective developmental periods in the field. In addition considering the length of time individuals may spend in a particular stage (up to 20 days) the reduced exposure of instar four larvae to sub-zero temperatures probably had a profound effect by increasing developmental rates compared to instar three in winter. In spite of the greater diurnal climatic variability experienced by individuals in the field trials (Table A 3.2), these results corroborate laboratory data (Table A 3.1).

In general the influence of fluctuating temperatures on the

development of immature *Cx. theileri* in the field and in the laboratory were very similar. In this respect it is noteworthy that developmental thresholds in both experimental groups for both sexes is 0°C (Figs. 3.4 and 3.5). The relationship between the reciprocal of developmental time and temperature is best described by curvi-linear regression equations (Figs. 3.4 and 3.5). Because of the moderately high mean temperatures (24,1°C: laboratory; 22,8°C: field) and the low mean temperatures (10°C: laboratory; 8,2°C: field) obtained in this study (Tables A 3.1 and A 3.2) these equations probably approximate the lower accelerative portion of a "sigmoid" or "logistic" curve (Davidson, 1944). This curve is generally considered by many authors to be optimal in describing the interaction between developmental time and mean temperature in many poikilothermic organisms (eg. Davidson, 1944; Headlee, 1941; Huffaker, 1944; Messenger & Flitters, 1959; Richards, 1960; Bursell, 1970; Hagstrum & Hagstrum, 1970; Wilkinson & Daugherty, 1970; Tanigoshi *et al.*, 1976; and Tanigoshi, 1978 quoted by Allsopp, 1986).

However, this is only so if mean temperatures used in the trials include the upper and lower extremes in the range of temperatures occurring in the habitats of the specific species under study (Allsopp, 1986). Furthermore, though fluctuating temperatures are generally considered to provide a more accurate basis for prediction of developmental rates

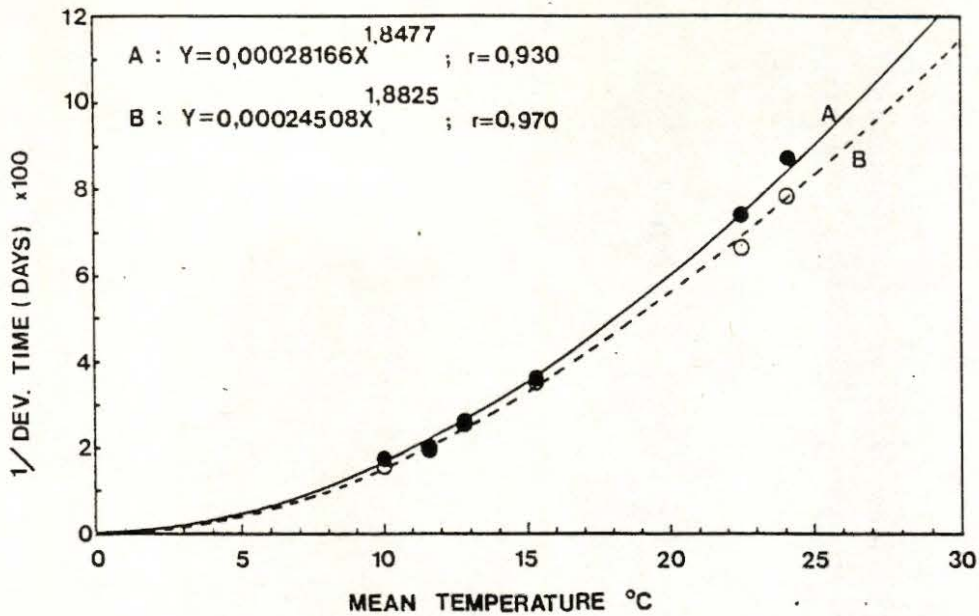


Fig. 3.4 The relationship between the reciprocal of developmental time of immature *Cx. theileri* and temperature in the laboratory (Observed points: line A = ———, closed circles; line B = - - - - -, open circles)

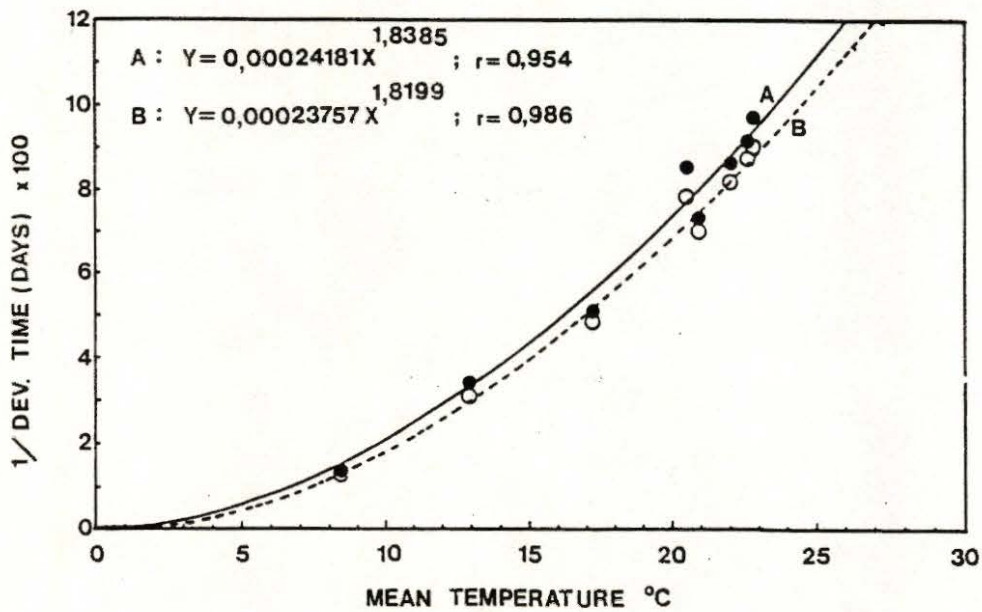


Fig. 3.5 The relationship between the reciprocal of developmental time of immature *Cx. theileri* and temperature in the field (Observed points: line A = ———, closed circles; line B = - - - - -, open circles)

in the field, Messenger & Flitters (1959) indicated that the developmental rates of the eggs of the fruit fly, *Bradysia impatiens*, were the same under constant and fluctuating temperature regimes. This was only true during the mid-range of temperatures when developmental time and temperature were directly proportional. At the upper and lower extremes of temperature, significant deviations from linearity occurred. This was more pronounced under fluctuating temperatures than under constant temperature regimes (Messenger & Flitters, 1959).

The results obtained in the present study support work done by Van der Linde (1984) who used constant temperatures. However, developmental thresholds of *Cx. theileri* under variable temperature regimes are lower and developmental times are slightly shorter at equivalent mean temperatures than under constant temperature regimes as indicated by Van der Linde (1984). Variable temperatures have been reported to reduce developmental thresholds and increase developmental rates (Huffaker, 1944; Hagstrum & Hagstrum, 1970; Allsopp, 1986). It is known that in the Orange Free State water temperatures of below freezing point do occur during winter for short periods early in the mornings. In addition, instar four larvae of *Cx. theileri* have been observed with their siphons frozen in the top mm of ice covering pools in the field. If removed and placed in the warmth of the insectary (+ 27°C), the larvae have continued



development and reached adulthood without any obvious debilitation by the experience (Dr. T.C. de K. van der Linde - personal communication) indicating that this species tolerates lower temperatures in the field than laboratory studies tend to indicate.

Finally, results in this study in trials done under field conditions (section 3.3.1.2) support Van der Linde's observation in that third and fourth instar *Cx. theileri* larvae were seen apparently frozen into the top 1-2 mm of ice in Apex vials. A small percentage (19 %) of those that were frozen in winter trials reached adulthood in spite of this. Thus, *Cx. theileri* larvae are clearly able to tolerate exposure to freezing temperatures for short periods. It is thus probable that the threshold of development of *Cx. theileri* in the field could be closer to 0°C. Richards (1960) and Bursell (1970) have shown, that thresholds of development are influenced by many factors such as the intensity and duration of exposure to lethal or sublethal temperatures, the thermal history of the individuals being tested, as well as the species. Of particular importance to survival of organisms are the temperatures or general environmental conditions to which the experimental populations were subjected prior to the initiation of the trials (Richards, 1960).

The reasons why variable temperature environments often

result in lowered developmental thresholds, increased developmental rates or even larger size (mass) individuals than in constant temperature regimes is far from clear. However, May (1985) has reviewed some of the more recent literature from a physiological vantage point and indicated that most insects prevent cold-death by physiological and biochemical resistance and behavioural selection of thermally sheltered micro-habitats. For instance, stone flies (*Zapada cinctipes*) respond immediately to cold by moving to warmer micro-habitats (Tozer, 1979, quoted by May, 1985). Baust (1981, quoted by May, 1985) has shown that glycerol and other "polyols" accumulate in large quantities in the haemolymph after cold exposure in some insects. Indeed, Steele (1981, quoted by May, 1985) has indicated that "polyols" may stabilize enzymes during freezing. However, this occurs at the expense of glycogen. This probably means that, though the effect of freezing body tissues is nullified or reduced by the "polyols", less energy in the easily catabolised form of glycogen is available. Therefore, only a shorter period of sub-zero temperatures can probably be tolerated than would normally occur. May (1985) suggests that the diel temperature changes that occur and to a lesser extent the procession of the seasons (i.e. spring rise and autumn decline of photoperiod and thermoperiod) serves to "acclimatize" insects and is probably of greater importance in affecting the development of cold-tolerance in these

animals than is generally accepted.

3.3.2 The influence of variable temperature on the percentage mortality under laboratory and field conditions.

#### 3.3.2.1 Laboratory trials

The average percentage mortality of the individuals entering each immature stage is given in Table A 3.5. An analysis of variance showed significant differences ( $p < 0,05$ ) between the different regimes ( $F = 36,69$ ) and between various stages ( $F = 6,97$ ) (Table A 3.6). Tukey's test ( $Q_{0,05} = 8,3894$ ) showed that regimes one ( $10^{\circ}\text{C}$ ) and two ( $11,6^{\circ}\text{C}$ ) had significantly higher mortalities in individuals entering each stage than the other regimes. Tukey's test ( $Q_{0,05} = 8,3894$ ) also showed significant differences between instars two and four, irrespective of the mean temperatures (Table A 3.6). Instar two had the lowest percentage mortality and instar four the highest mortality at the different variable temperatures.

#### 3.3.2.2 Field trials

The influence of natural diurnal temperature fluctuations on the percentage mortality is given in Table A 3.7. With analysis of variance significant differences ( $p < 0,05$ ) were

shown in the percentage mortality between regimes ( $F = 27,03$ ), between stages ( $F = 10,50$ ) and between some of the regime and stage interactions ( $R \times S$ ) ( $F = 3,84$ ) (Table A 3.8). In the first place, Tukey's test ( $Q_{0,05} = 15,3964$ ) indicated that regimes one and two (mean temperatures =  $8,2^{\circ}\text{C}$ ) had a significantly higher percentage mortality than all other regimes. Secondly, Tukey's test ( $Q_{0,05} = 15,3964$ ) showed that significantly higher mortalities occurred in instars one and four than in all the other stages irrespective of the mean temperature.

Similar results were obtained in both the laboratory (Fig. 3.6) and the field trials (Fig. 3.7) in which low mean temperatures increased the percentages of all instars that failed to complete each stage. However, instar one larvae had much higher percentage mortalities at a mean temperature of  $8,2^{\circ}\text{C}$  in the field trials than the other stages (Fig. 3.7), compared to mean temperatures of  $10^{\circ}\text{C}$  and  $11,6^{\circ}\text{C}$  in the laboratory (Fig. 3.6). In addition, of the 120 larvae used in each of the various temperature regimes 15,8 % and 18,3 % survived in the laboratory at mean temperatures of  $10^{\circ}\text{C}$  and  $11,6^{\circ}\text{C}$  while only 1,7 % and 5,8 % survived in the field at a mean temperature of  $8,2^{\circ}\text{C}$  (Table 3.4). The reason for these discrepancies can be attributed to the lower mean temperatures recorded in the field ( $8,2^{\circ}\text{C}$ ) and also the sub-zero temperatures to which field populations were subjected in more than 66 % of the winter days. Laboratory

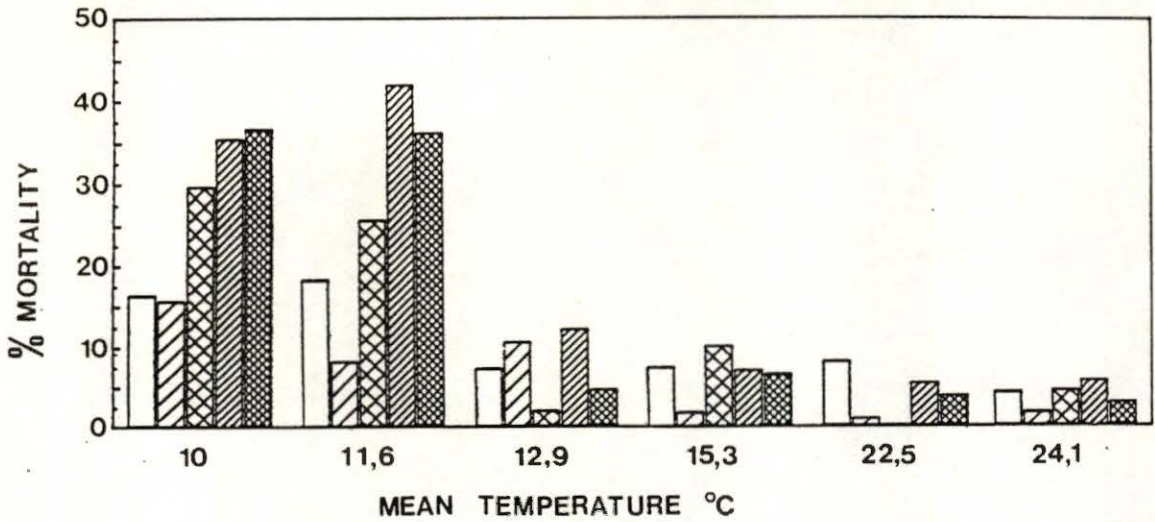







Fig. 3.6 The influence of fluctuating temperatures on the percentage mortality of immature *Cx. theileri* in the laboratory. Instars: 1 =  ; 2 =  ; 3 =  ; 4 =  ; pupae =  .

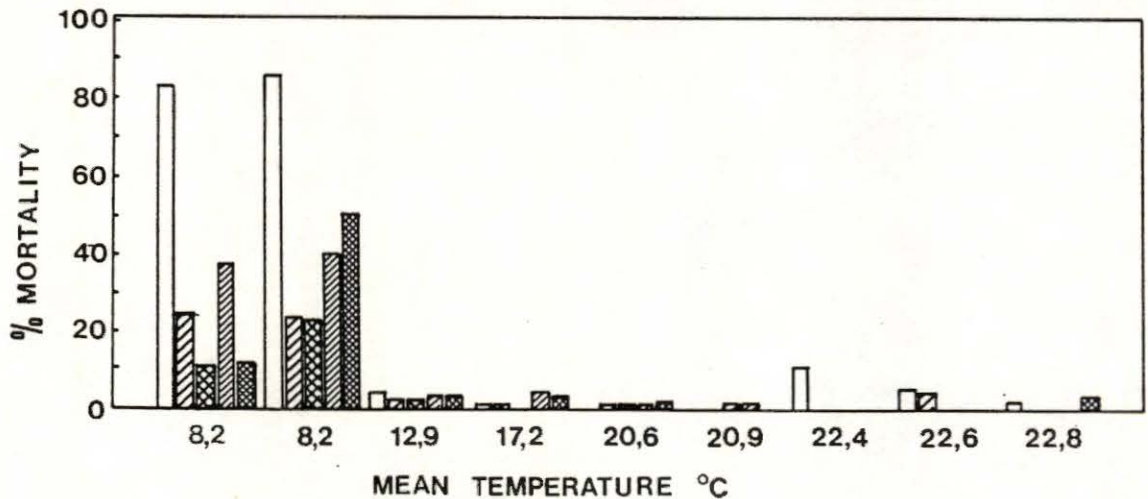







Fig. 3.7 The influence of various fluctuating temperatures on the percentage mortality of immature *Cx. theileri* in the field. Instars: 1 =  ; 2 =  ; 3 =  ; 4 =  ; pupae =  .

experimental populations were never exposed to sub-zero temperatures. Thus, a lower percentage of survivors could be expected in field trials during winter. However, the laboratory data corroborates to a large extent field data and is consistent with work done by various authors viz. Andrewartha & Birch (1954), Trpis & Horsfall (1969), Trpis & Shemanchuk (1970), Wilkinson & Daugherty (1970) and Van der Linde (1984).

Table 3.4 The percentages of the original numbers of instar one *Cx. theileri* larvae that survived to adulthood in the various mean fluctuating temperatures.

Laboratory trials				Field trials			
Temperature °C:			% Survival	Temperature °C:			% Survival
$\bar{x}$	$\bar{x}$ max	$\bar{x}$ min		$\bar{x}$	$\bar{x}$ max	$\bar{x}$ min	
10	14,3	5,3	15,8	8,2	10,8	5,4	1,7*
11,6	15,8	7,4	18,3	8,2	10,8	5,4	5,8
12,8	18,1	7,0	67,5	12,9	16,9	7,9	86,7
15,3	19,2	7,0	69,2	17,2	21,4	12,9	91,7
22,5	29,6	14,1	81,7	20,6	26,0	17,9	95,8
24,1	29,7	18,5	82,5	20,9	24,0	16,6	95,8
				22,4	26,0	17,2	89,1
				22,6	27,1	19,0	91,7
				22,8	26,4	18,6	95,8

\* only female data: no males survived in this trial.  
 $\bar{x}$  = mean temperatures during the whole developmental period.

Another important point is that variable temperature regimes seem to be more conducive to survival at low mean temperatures than constant temperature regimes. For instance, a constant temperature of 12°C was lethal to immature *Cx. theileri* (Van der Linde, 1984). However, for the same species under variable mean temperatures in the laboratory (10°C) and in the field (8,2°C) the percentage survivorship was 15,8 %, and 1,7 % to 5,8 % respectively (Table 3.4). Exactly why variable temperatures enhance survival or how development is affected remains largely unresolved (Headlee, 1940, 1941; Davidson, 1944; Huffaker, 1944; Richards, 1960; Bursell, 1970; Wilkinson & Daugherty 1970; May, 1985). Bursell (1970) has suggested that the exposure of particular species to temperatures close to the lethal limits of the species, but still within the viable range, has profound effects on aspects such as life expectance, growth rate, metabolic activity, mobility, behaviour, etc. Regarding mobility and metabolic activity it is possible that low constant temperatures may prevent individuals from obtaining enough food to replenish food reserves (Chapman, 1982). However, it is probable that they are able to replenish these reserves under a fluctuating temperature regime, provided that the intensity and duration of the temperatures to which they are exposed are above their activity threshold. It is this factor that will determine the survival or otherwise of the individuals of a particular species.

Work by Richards (1960) in which *Oncopeltus* larvae were subjected to sub-threshold temperatures indicated that the problem was far more complicated. The mortality in *Oncopeltus* was not associated with exhaustion of energy reserves. Histopathological studies showed adequate energy reserves in these larvae. Richards (1960) proposed that some biochemical "factor" eg. enzymatic, hormonal, etc., responsible for mobilizing reserve nutrients are rendered inactive by low temperatures to such an extent that it is irreversible and death ensues. This happens even if the specimen is returned to optimum conditions. Furthermore this "factor" works by being produced in response to cold, or possibly by destroying or neutralising an inhibitor, and acts within a very narrow temperature band (i.e. one to two °C). In addition, Richards (1960) suggested that photoperiodic and thermoperiodic cues modify the temperature band in which it acts. He also said that variable temperatures may allow the activation of various processes mediated by eg. enzymes of different thermal optima, resulting in the production of necessary metabolic products essential for growth, moulting and eventual emergence of some individuals. The possible answer to this phenomenon probably lies in discovering why larvae survive at low variable mean temperatures equal to constant temperatures in which all individuals die. Intensive studies of the physiological influences of low sub-lethal



temperatures on insects will be needed before this problem can be solved (Richards, 1960; Bursell, 1970).

3.3.3 The influence of variable temperatures on the head capsule widths of fourth instar larvae reared under laboratory and field conditions.

#### 3.3.3.1 Laboratory trials

There was a tendency towards an increase in the head capsule width with reduction in mean temperature for instar four larvae in the various regimes (Table A 3.9). An analysis of variance showed that significant differences ( $p < 0,05$ ) in head capsule width between treatments ( $F = 3,5$ ) occurred (Table A 3.10). Tukey's test ( $Q_{0,05} = 0,0358$ ) showed that only the head capsule widths of larvae in regime six (mean temperature =  $24,1^{\circ}\text{C}$ ) were significantly smaller than those in regime one (mean temperature =  $11^{\circ}\text{C}$ ). The other treatments did not differ significantly from each other.

#### 3.3.3.2 Field trials

The mean head capsule widths of fourth instar larvae exposed to fluctuating temperatures are given in Table A 3.11. An analysis of variance showed that no significant differences ( $p > 0,05$ ) in the head capsule widths occurred in the various regimes ( $F = 0,14$ ) (Table A 3.12).

In view of the laboratory trials above (section 3.3.3.1) in which the head capsule widths of instar four larvae reared at low temperatures (10°C) were significantly larger than those reared at higher temperatures (24,1°C) larger head capsules were expected in the field trials. However, the relatively small number of larvae (3,6 %) that reached adulthood in the winter (regimes one and two combined) (Table A 3.11) may be the reason for these results compared to the laboratory trials in which 15,8 % reached adulthood at a mean temperature of 10°C (Table 3.4).

In the laboratory a difference in mean temperature of approximately 14°C was necessary during rearing of *Cx. theileri* larvae for a significant difference in head capsule width to occur (Table A 3.1). The ultimate size of immature insects in the field is known to be influenced by temperature (Wallace & Merritt, 1980). The overwintering generation of multivoltine species attain higher individual masses than those of summer generations (Wallace & Merritt, 1980). The phenomenon of larger individuals associated with rearing at low temperature has also been reported in fruit flies, *Bradysia impatiens* (Wilkinson & Daugherty, 1970), in mayflies, *Isonychia bicolor* (Sweeney, 1978, quoted by Wallace & Merritt, 1980) and simuliids (*Simulium* spp.) (Ladle et al., 1977, quoted by Wallace & Merritt, 1980). Laboratory reared *Culex tarsalis* (Hagstrum & Workman, 1971) display similar tendencies.

Why low temperatures result in larger individuals could possibly be due to the fact that the longer period of development of insects at low temperatures allows for the intake of larger amounts of food and the accumulation of greater energy reserves than would otherwise occur at higher temperatures.

#### 3.3.4 Survival of *Cx. theileri* females under fluctuating temperatures and saturation deficits.

The  $LT_{50}$ -value of the various groups of mosquitoes exposed to each temperature-saturation deficit regime were calculated by the method of Andrewartha & Birch (1954). The relationship between  $LT_{50}$ -values and the treatment parameters was determined with multiple linear regression analysis.

The regression coefficients and the calculated  $LT_{50}$ -values for each of the different treatments are given in Table A 3.13. The analysis of variance indicated significant differences ( $p < 0,05$ ) in the mean survival times between treatments ( $F = 296,16$ ) (Table A 3.14). This indicated that fluctuating temperatures and saturation deficits affected the longevity of female *Cx. theileri*. Tukey's test  $Q_{0,05} = 1,7461$  showed that mosquitoes in all the treatments differed significantly from each other in longevity. The  $LT_{50}$ -values of the various experimental populations are given in Table 3.5.

Table 3.5  $LT_{50}$ -values for blood-fed *Cx. theileri* females exposed to various regimes of fluctuating temperatures and saturation deficits.

Regime	Temperature ( $^{\circ}\text{C}$ )			sd(kPa)	$LT_{50}$ -values (days)
	$\bar{x}$	$\bar{x}$ max	$\bar{x}$ min		
A	20,0	26,5	13,5	1,44	4,8
B	10,0	15,3	4,6	1,10	10,1
C	20,0	26,5	13,5	0,53	18,4
D	10,0	15,3	4,6	0,32	22,8

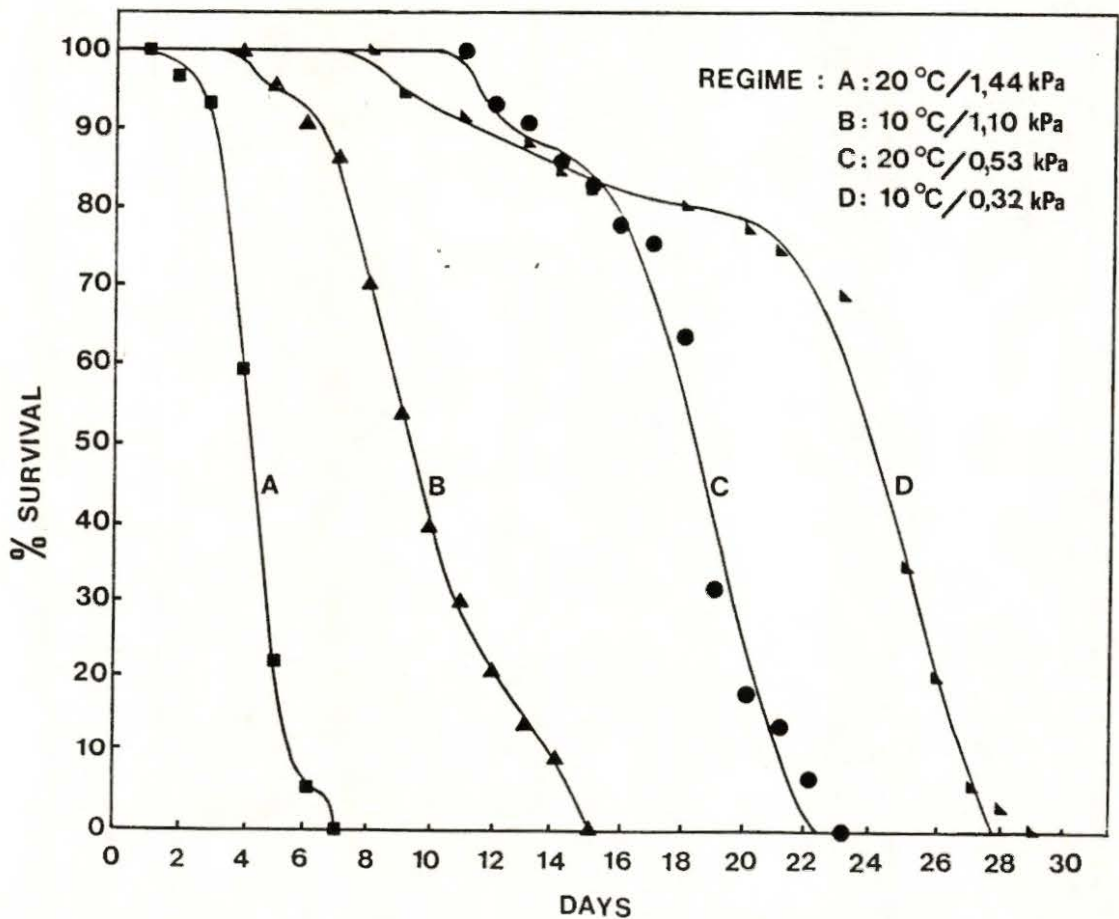


Fig. 3.8 The percentage survival of bloodfed *Cx. theileri* exposed to different fluctuating temperatures and saturation deficits.

It is noteworthy that the mosquitoes survived the longest in the low saturation deficits viz. 0,32 kPa and 0,53 kPa (Table 3.5) irrespective of the influence of temperature. Saturation deficit appears to be of overriding importance in the longevity of *Cx. theileri* females in this trial. A graphic presentation of the significant polynomial relationships between percentage survival and longevity is given in Fig. 3.8. It can be seen that in all the graphs, after an initial period of one (graph A) to 11 days (graph C) in which no mortality occurred, a slow reduction in the percentage that survived is followed by a period in which a rapid reduction in percentage survival is evident (Fig. 3.8).

However, the period before the "linear" phase is considerably longer at lower saturation deficits viz. 0,32 kPa and 0,53 kPa (graphs D and C) than at high saturation deficits viz. 1,10 kPa and 1,44 kPa (graphs B and A) (Fig. 3.8).

The probable reason for this was that water loss is reduced in environments with low saturation deficits and low temperatures (Bursell, 1970). Although low temperature is known to play an important role in survival, the results obtained here suggest that at moderate temperatures (eg. 20°C) and under variable temperature regimes, saturation deficit has the greater influence on mosquito survival and longevity.

Similar results have been obtained by Van der Linde (1984) using constant temperatures and saturation deficits. He found that the  $LT_{50}$ -value for blood-fed females was 14,4 and 13,7 days at constant temperatures of 6°C and 15°C and a saturation deficit of 0,40 kPa. This is considerably shorter than for a mean temperature/saturation deficit of 10°C/0,32 kPa found in this study in which the  $LT_{50}$ -value was 22,8 days (Table 3.5). This supports the view by a number of authors eg. Huffaker (1944); Andrewartha & Birch (1954); Richards (1960); Caulton (1976); Van As (1980). The results of this study indicate that the majority of *Cx. theileri* females would be unable to survive a winter of approximately 80 days in the Orange Free State since mean four-weekly temperatures and saturation deficits in this area ranged between 7,4°C and 11,4°C and 0,32 kPa and 0,64 kPa respectively during winter (Hewitt, et al., 1982; Van der Linde et al., 1982; Van der Linde, 1984). However, this view is based on the simple assumption that females cannot choose or find suitable winter resting sites, and that they may feed only once during the winter period when conditions are favourable and hosts available. In nature, it is probable that females will feed on available green plants during winter for carbohydrates and also actively seek suitable winter habitats in which to take refuge. Van der Linde (1984) has indicated that 50 % of female *Cx. theileri* which fed on sugar water had a  $LT_{50}$ -value of 17 days at a constant temperature of 6°C and on a saturation

deficit of 0,4 kPa. Thus, the possibility of females surviving for fairly long periods in winter cannot be ruled out. In fact, the results of New Jersey light trap catches for the period 1976 to 1978 indicated that *Cx. theileri* adults were absent for two weeks and 14 weeks during the winters of 1976 and 1977 respectively. The mean weekly atmospheric temperatures ranged between 7,4°C and 11,4°C and mean weekly minimum temperatures were between -3°C and 2°C during these periods in winter (Hewitt *et al.*, 1982; Van der Linde *et al.*, 1982). It is possible that adult *Cx. theileri* were absent from the light trap catches because it was too cold for them to fly. On the other hand, their absence may mean that these adults had died as a result of the low minimum temperatures experienced during this period. It is unknown whether *Cx. theileri* hibernates in winter, though Jupp (1975) has indicated that approximately 70 % of this species that were trapped were nulliparous and had developed a moderately sized fat body by the end of May. Fat bodies were still present in individuals trapped at the end of October (Jupp, 1975). The presence of a fat body is often associated with the accumulation of energy reserves (Ross *et al.*, 1982) for use during periods of inclement weather and food scarcity.

Adults emerging from pupae in the late autumn probably survive the early part of winter and may oviposit during favourable periods. However, the evidence from the

New Jersey light trap catches in the region and the results of the current trials make it improbable that adult *Cx. theileri* survive the whole winter period. The reappearance of adults in New Jersey light traps in the late winter are probably those emerging from overwintering immature stages. Nulliparity and the presence of a fat body in adults trapped in late winter does not necessarily imply that these adults overwintered. Adults emerging from pupae in favourable periods during winter are probably just as likely to feed on the available green plants containing sugars and perhaps build up a small to moderate fat body prior to capture. It is also possible that females may prefer plant sugars to a blood meal during winter because the former contains more readily available carbohydrates that can be used for survival and flight.

#### 3.3.4.1 Percentage mass loss

The average percentage mass loss is given in Table A 3.15. Analysis of variance of mass loss of the females showed significant differences ( $p < 0,05$ ) between the different treatments ( $F = 14,41$ ) (Table A 3.16). Tukey's test ( $Q_{0,05} = 4,9802$ ) showed that significant differences in percentage mass loss occurred between regime A ( $20^{\circ}\text{C}/1,44$  kPa) and all other regimes. A tendency to increased mass loss with increasing saturation deficit is not indicated here. However, the highest reduction in mass



(76 %) did occur at the highest mean temperature (20°C) and saturation deficit (1,44 kPa) (Table 3.6). Why significant mass loss did not occur between the other regimes is not clear. Except for the low temperature (10°C)/ high saturation deficit (1,10 kPa) (regime B, Table A 3.15), all the ingested blood in individuals exposed to the other regimes had apparently been completely digested.

Table 3.6 The percentage mass loss of blood-fed mosquitoes in the various temperature and saturation deficit regimes.

Regime	$\bar{x}$ sd (kPa)	Mean percentage mass loss at:	
		$\bar{x}$ = 10°C	$\bar{x}$ = 20°C
A	1,44		76,7
B	1,10	65,1	
C	0,53		70,4
D	0,32	69,0	

But, females in regime B which had died, usually still contained dark-coloured distended abdomens reminiscent of recently blood-fed females. In addition, in some of these females the posterior one to two thirds of the abdomen was still dark-coloured, while the remaining anterior portion was light-coloured - reminiscent of half gravid females. It

is highly probable that these individuals contained ova and undigested blood. Furthermore, it seems likely that the rate of digestion at mean fluctuating temperatures of 10°C (regime B: Table A 3.15) was reduced to such an extent that many individuals perished before the digestive process could be completed. This phenomenon was not seen in individuals exposed to regime D (10°C, saturation deficit = 0,32 kPa) (Table A 3.15). A possible explanation is that the temperature/saturation deficit regime in B was such that the females died from dehydration before digestion could be completed. Thus, the implications are that the rate of digestion at a mean temperature of 10°C proceeds at a much slower rate than loss of moisture from the mosquito in a very dry environment (Saturation deficit = 1,10 kPa).

It is also possible that at high mean temperatures and high saturation deficits, the differential between rate of moisture loss and rate of digestion may be reduced. Digestion can thus be completed. Finally, as moisture loss in regime D would be reduced by the low saturation deficit (i.e. 0,32 kPa) digestion could have been completed in spite of the low mean fluctuating temperature (i.e. 10°C). Whether this is correct awaits further research. Though Lewis (1933) has shown that mosquitoes are unable to shut their spiracles and thus reduce moisture loss during respiration, more recent studies indicate that this is not

true for all species. Krafsur (1971) has shown that in *Ae. aegypti* and *Ae. triseriatus*, both the duration and the size of the spiracular opening is influenced by relative humidity (R.H.). Low relative humidity results in reduction of the spiracular openings for shorter periods, while high relative humidity has the opposite effect. Furthermore, depleted water reserves enhance the effect of spiracular closure (Bursell, 1957, quoted by Krafsur, 1971). Whether this occurs in *Cx. theileri* is unknown. Though not directly comparable, it is noteworthy that Van der Linde (1984) also found with *Cx. theileri* that, under various combinations of constant temperatures and saturation deficits, the mass loss, temperature, and saturation deficit are less clearly related in blood-fed females. In some combinations of temperature and saturation deficit, mass reduction is less than expected. Van der Linde (1984) showed that at 15°C, mass loss at 0,4 kPa was 66 % compared to 60 % at 1,2 kPa. While at higher temperatures i.e. 24°C the trend was reversed with the highest mass loss (76 %) occurring in a saturation deficit of 1,2 kPa and the lowest (69 %) in a saturation deficit of 0,4 kPa. A similar trend is evident in the present study (Table 3.6). At 10°C mass loss at 0,32 kPa was 60 % compared to 65 % at a saturation deficit of 1,10 kPa. At 20°C the trend was also reversed. Mass loss at a saturation deficit of 0,53 kPa is 70 % compared to 77 % at a saturation deficit of 1,44 kPa (Table 3.6). Thus it would seem that at low temperatures and high saturation deficits mass loss is increased.

### 3.4 Conclusion

*Cx. theileri* is certain to overwinter in the immature stages in the field. The potential number of generations of *Cx. theileri* could exceed eight per annum in the Bloemfontein region. It is quite clear that *Cx. theileri* is a multivoltine species and at least five generations per year can probably be expected. The survival of young adults throughout the winter although possible seems improbable. Variable temperature regimes in the laboratory studies give more precise estimates of the developmental potential and survival of field populations than constant temperature regimes. However, the exposure of experimental populations to actual climatic conditions in the field seems to be even more precise, as the latter resulted in a higher rate of development and higher percentage survivorship of immature stages than laboratory simulation of field conditions. Thus, the design of experiments to accommodate the use of natural environmental conditions is to be recommended.

As the effects of low temperature and to a lesser extent high temperature phenomena together with the influence of saturation deficits are of importance in the survival of *Cx. theileri* in the Orange Free State, the associated physiological and biochemical changes merit attention.

4. The influence of sodium chloride solutions on the development and survival of *Culex theileri* in the laboratory.

#### 4.1 Introduction

It is well known that mosquitoes are selective in their choice of oviposition sites (Buxton & Hopkins, 1927; Wallis 1954a, b; Hudson, 1956; Nakamura, 1978; Gillespie & Belton, 1980; Maire, 1982; Pappas & Pappas, 1983; Van der Linde 1984). A wide variety of factors such as vegetational cover, water temperature, pH, salinity, wind speed, water movement, scototaxis, oviposition pattern and diurnal oviposition rhythm influence the choice of a site. However, where no choice of oviposition site is given eg. in the laboratory, females may oviposit in the aqueous solution available (Fielding, 1919; Van der Linde, 1984). Salinity is a common deterrent with the "rejection concentration" varying from species to species and perhaps within a species (Wallis, 1954a, b). In the absence of suitable oviposition sites in nature, selection of salt tolerant strains of the local species could occur. Parker (1979) has shown that *Ae. dorsalis* could become tolerant to NaCl solutions (25 000 ppm NaCl  $\approx$  0,14M) within four generations in the laboratory. However, in *An. albimanus*, no selection for salt tolerance could be achieved (Bailey, et al., 1981).

Van der Linde (1984) showed that in general, *Cx. theileri* had a wide range of tolerance to NaCl with a small proportion of a laboratory population tolerant to very high NaCl concentrations. It is not known whether this represented the upper limit of NaCl tolerance in *Cx. theileri*, or whether an even higher tolerance to NaCl solutions could be genetically selected for. This question served as motivation for this study.

## 4.2 Materials and methods

Two groups of mosquitoes from the laboratory were compared for tolerance to NaCl solutions of various molarities. The control group came from an established laboratory colony which had been augmented from time to time with field collected individuals. The immature stages were reared continuously in 0,02M NaCl solutions. The "selected" group originated from the control group but the immature stages had been reared for six successive generations in 0,12M NaCl before the experiment was initiated. The 0,12M NaCl solution was chosen because Van der Linde (1984) indicated that approximately 25 % of the immature stages reached adulthood at this concentration. Both groups were otherwise treated in the same manner. The laboratory rearing of *Cx. theileri* was done as described by Van der Linde (1984).

### 4.2.1 Oviposition in black petri-dishes

Four days after the *Cx. theileri* females had taken a blood meal, twelve black petri-dishes were placed in the mosquito cages of the control group and the selected group respectively. Each petri-dish was filled with a NaCl solution of one of the following molarities: 0,0M (distilled water), 0,02M, 0,04M, 0,06M, 0,08M, 0,10M, 0,12M, 0,14M, 0,16M, 0,18M, 0,20M and 0,22M. Observations were made daily and the total number of egg rafts per petri-dish

were recorded over a period of 10 days. The NaCl solutions were replaced daily with fresh NaCl solutions. The positions of the petri-dishes were also changed at random to prevent possible positional effects. The eggs were allowed to hatch in the NaCl solutions on which they were laid. The number of eggs per egg raft were counted and the percentage that hatched were calculated.

#### 4.2.2 Oviposition in vials

Females of both the control group and the selected group were confined individually to glass vials (75 x 25 mm) each containing 10 ml of NaCl solution. The same series of NaCl solutions mentioned in 4.2.1 was used. Observations were made daily and the number of egg rafts per solution were recorded. All eggs were allowed to hatch in the vials. The number of eggs per egg raft were counted and the percentage of eggs that hatched were calculated.

#### 4.2.3 The development of the immature stages

In both the control group and the selected group all the larvae which had hatched within a six hour period were transferred to rearing pans containing the various NaCl solutions. Here they were fed and kept at 27°C for 24 hours before they were used in experiments to determine the effect of the various NaCl solutions on the development of immature



*Cx. theileri.*

Twenty-four hour old larvae from both groups were randomly selected and placed individually in glass vials containing 10 ml of rearing medium (Section 3.2.1). The vials were sealed with plastic stoppers to prevent evaporation of the water.

Since the larvae that had hatched in NaCl solutions of 0,18M and higher died within 24 hours, these solutions could not be used in the development trials. Thus, NaCl solutions up to 0,16M were used.

### 4.3 Results and discussion

#### 4.3.1 Oviposition on NaCl solutions of various molarities

Kendall's test (Yeomans, 1970) indicated that the ovipositional response of consecutive populations of mosquitoes used in the various NaCl trials were similar. There were no significant differences ( $p > 0,05$ ) within the control group for petri-dishes ( $X^2 = 0,65$ ;  $df = 3$ ) or for oviposition vials ( $X^2 = 0,13$ ;  $df = 2$ ) (Table A 4.1), since they oviposited in a consistent fashion. Thus, the different consecutive mosquito populations were regarded as replicates within each trial series and subsequently analysed by standard statistical methods.

##### 4.3.1.1 Oviposition in black petri-dishes

A significant reduction ( $p < 0,05$ ) in the percentage of egg rafts laid with increase in NaCl molarity for the control group ( $F = 72,4$ ;  $r = -0,91$ ) and the selected group ( $F = 18,4$ ;  $r = -0,75$ ) is shown with polynomial regression analysis (Fig. 4.1). Since the 95 percentage confidence limits for both control and selected group overlap these groups do not differ significantly from one another.

##### 4.3.1.2 Oviposition in vials

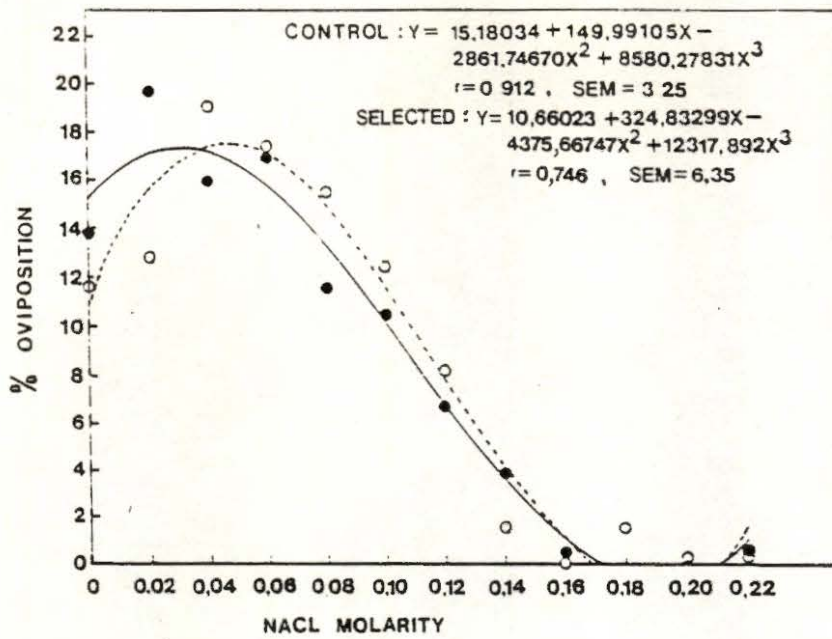


Fig. 4.1 The percentage oviposition in petri-dishes containing various NaCl solutions. Control group: regression = solid line; mean % oviposition = closed circles. Selected group: regression = broken line; mean % oviposition = open circles.

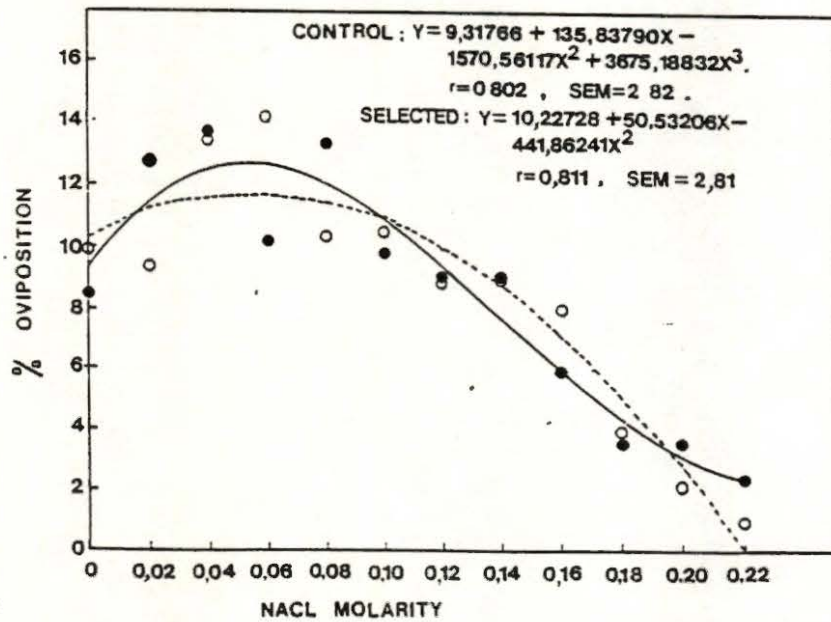


Fig. 4.2 The percentage oviposition in vials containing various NaCl solutions. Control group: regression = solid line; mean % oviposition = solid circles. Selected group: regression = broken line; mean % oviposition = open circles.

The results of the regression analysis indicated that a significant reduction ( $p < 0,05$ ) in the percentage oviposition occurred with an increase in NaCl molarity in the control group ( $F = 19,3$ ;  $r = -0,80$ ) and the selected group ( $F = 31,6$ ;  $r = -0,81$ ) (Fig. 4.2). In addition at the 95 percent confidence level, the upper and lower limits of the variables for the control group and the selected group overlap (Table A 4.3). The two groups therefore do not differ significantly from each other.

The ovipositional behaviour of the control group and the selected group did not differ within each experimental situation, indicating that no selection for high NaCl molarities had occurred. Based on these results it appears that locally selected strains of adult *Cx. theileri* tolerant to salt solutions of high concentrations are unlikely to develop in the field. Although ovipositional behaviour as such was not tested, Bailey *et al.*, (1981) also found no evidence that salt tolerant strains of *An. albimanus* could be selected for. The tendency to avoid laying on unsuitable water (eg. water containing high NaCl concentrations) (Figs. 4.1 and 4.2) support findings by Wallis (1954a), Hudson (1956), Russell (1979) and Van der Linde (1984). The one obvious difference between oviposition in petri-dishes (Fig. 4.1) and vials (Fig. 4.2) was that the eggs were laid on higher NaCl molarities in the vials than in the petri-

dishes. Similar results were obtained by Van der Linde (1984). The reason for this is that the probability of locating water in the confined space of an oviposition vial is greatly enhanced compared to finding water in black petri-dishes in cages (Van der Linde, 1984).

The ecological implication of the oviposition data are that *Cx. theileri* would exercise choice in selection of suitable (eg. low salinity) oviposition sites. However, where such sites are not available and choice cannot be exercised, a proportion of the population could still utilize the unfavourable (eg. high salinity) sites. However, females in the field are unlikely to oviposit in pools in which the salinity exceeded 0,18M NaCl equivalent. The reason for this is that no larvae that hatched in NaCl molarities of 0,18M survived (section 4.2.3).

#### 4.3.2 The number of eggs per raft laid in various NaCl solutions

##### 4.3.2.1 Black petri-dishes

The average number of eggs/raft oviposited on the various NaCl solutions are given in Table A 4.4. With an analysis of variance no significant differences ( $p > 0,05$ ) in the number of eggs/raft laid in the various NaCl molarities ( $F = 1,61$ ) were shown for either the control or the selected

groups (Table A 4.5). However, the selected group as a whole laid significantly more ( $p < 0,05$ ) eggs/raft than the control group ( $F = 24,07$ ) (Table A 4.5).

#### 4.3.2.2 Oviposition vials

The average number of eggs/raft oviposited on the various NaCl solutions are given in Table A 4.4. An analysis of variance showed that the selected group as a whole produced significantly more ( $p < 0,05$ ) eggs/raft than the control group ( $F = 4,35$ ) (Table A 4.6). In addition, there were significant differences ( $p < 0,05$ ) in the number of eggs/raft between some of the NaCl molarities in the control group and the selected group ( $F = 2,08$ ) (Table A 4.6).

It is noteworthy that in both the free choice (petri-dishes) (section 4.3.2.1) and the no choice situation (vials) (section 4.3.2.2) the selected group as a whole had a significantly higher ( $p < 0,05$ ) fecundity than the control group, irrespective of the NaCl molarity of the various solutions. This is contrary to the findings of some authors (eg. Bailey *et al.*, 1981; Parker, 1982) since they have indicated that high NaCl salinity and stress in the immature stages resulted in reduced adult size at emergence and decreased fecundity of the adults. A probable explanation is that the selected group in general are better able to survive the high NaCl molarities as a result of continuous

rearing in 0,12M NaCl solutions than the control group which was reared continuously in 0,02M NaCl. Therefore, some selection for tolerance of high NaCl molarities may have occurred in the selected group of mosquitoes. This is partly supported by Reisen *et al.* (1984) who indicated that stress in the immature stages tends to eliminate the physiologically less well adapted individuals without significantly affecting the fecundity, fertility and longevity of the survivors. He also suggested that in the field, only those species with the widest range of ovipositional preferences or physiological tolerance survive since salinity associated with silting and organic pollution rise during the dry season as water evaporates from pools. Thus, the increased fecundity of the selected group of females in this study can probably be explained in terms of a higher physiological tolerance of NaCl solutions in successive instars and generations of both immature stages and adults.

It is obvious that NaCl molarity had no effect on the numbers of eggs/raft in petri-dishes ( $F = 1,61$ ) while significant differences ( $p < 0,05$ ) occurred in oviposition vials ( $F = 2,08$ ) (Table A 4.5 and A 4.6 respectively). The reason is that too few egg rafts were laid at high NaCl molarities in petri-dishes compared to oviposition vials (Table A 4.4) to be amenable to the analysis of variance.

With Tukey's test ( $Q_{0,05} = 30,5842$ ) significantly more eggs/raft were laid in vials containing 0,0M, 0,08M, 0,10M and 0,14M than in 0,22M NaCl solutions. This is in contrast with the findings of Wallis (1954a) and Van der Linde (1984). However, Wallis (1954a) did report that the number of eggs/raft differed in culicine females but not in relation to the NaCl salinity of the test solutions. It is possible that the numbers of eggs/raft laid by various females depends on the size of the individual (Parker 1979, 1982; Bailey et al., 1981) or on the amount of blood imbibed (Clements, 1963). The higher incidence of incomplete, disarranged egg rafts that occurred on the higher NaCl molarities in oviposition vials probably implies that in a no choice situation, females that may have been weakened over the 10 day period allowed for oviposition may have been incapable of ovipositing their full complement of eggs. On the other hand, the high NaCl molarity may have been irritating to sensitive tissues at the tip of the abdomen or on the tarsi (Hudson, 1956) when laying occurred, thus inhibiting oviposition. Finally, the possibility that some resorption of ova may have commenced before weakened females oviposited, cannot be excluded. However, evidence of this is lacking in *Cx. theileri*.

Individual fecundity is unaffected by the molarity of the NaCl solution but, since the numbers of females laying in the various solutions differs, population fecundity is



affected. Thus, in the field fewer eggs will be laid as salinities increase.

#### 4.3.3 The percentage of eggs hatched

The average numbers of eggs/raft that hatched in both the control and the selected group are represented in Table A 4.7.

In Fig. 4.3 the effect of high NaCl molarities and the percentages of eggs that hatched in the various concentrations, is illustrated. With the analysis of variance significant differences ( $p < 0,05$ ) in the hatching percentages of eggs ( $F = 45,5$ ) were shown (Table A 4.8). Tukey's test ( $Q_{0,05} = 22,7249$ ) showed significant differences in the hatching percentage of the eggs that hatched on 0,0M to 0,14M solutions compared to 0,16M to 0,22M NaCl solutions. The two groups (the control and the selected group respectively) did not differ significantly ( $F = 0,07$ ) (Table A 4.8). The detrimental effect of the high NaCl molarities on the percentages of eggs hatched supports work by a number of authors (eg. Wallis 1954a, b; Hudson 1956; Van der Linde, 1984). The steep decline in the hatching percentage of eggs from 0,16M to 0,18M NaCl (Fig. 4.3) represented a limit in the ability of the majority of *Cx. theileri* eggs to absorb water. It is known that developing eggs absorb water (Chapman, 1982) and that water

is essential for the hatching of the eggs (Harwood & Horsfall, 1959).

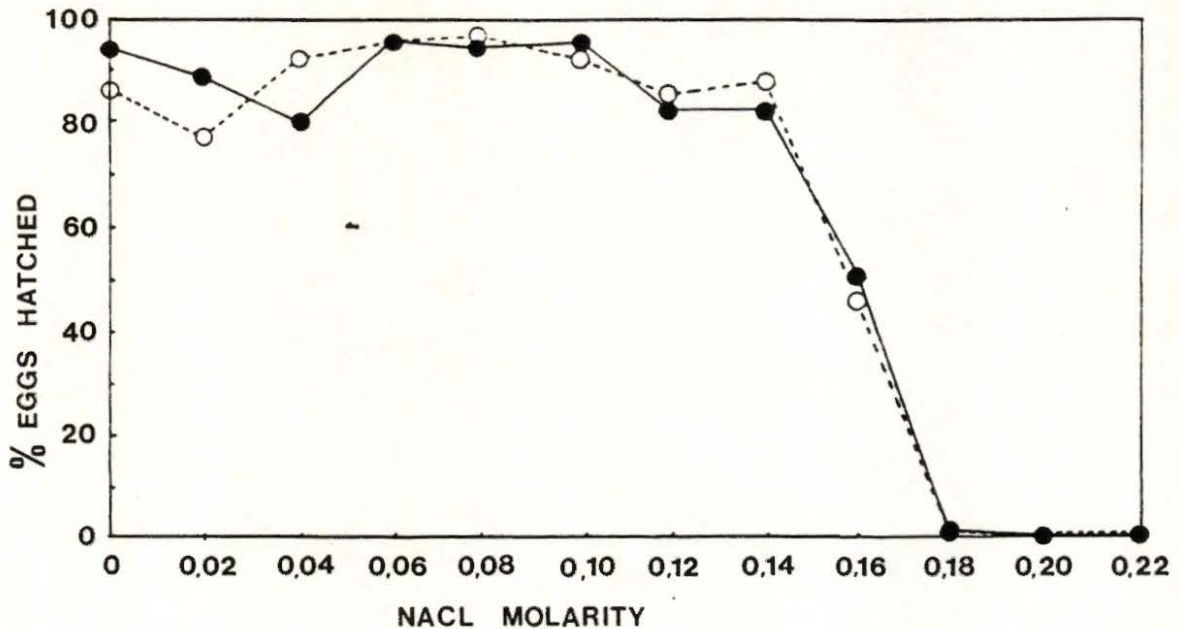


Fig. 4.3 The mean percentages of eggs that hatched on the various NaCl solutions.

The fact that the control group and the selected group did not differ significantly from each other in the influence of NaCl solutions on the percentages of eggs that hatched, suggested that no selection for the tolerance of high NaCl salinities occurred, despite the attempted selection for the one group of mosquitoes for this purpose (i.e. the selected group). The limiting factor here is the inability of eggs to absorb water from NaCl solutions of 0,20M and higher (Downs, 1951, quoted by Clements, 1963).

From an ecological viewpoint, data presented in the present

study implies that in the field, *Cx. theileri* populations will be limited to pools ranging from fresh water to salinities of 0,16M NaCl equivalent. Furthermore, local strains of *Cx. theileri* that are tolerant of high salinities are unlikely to develop in nature.

#### 4.3.4 The influence of NaCl solutions on the development and survival of the immature stages of *Cx. theileri*

##### 4.3.4.1 Developmental time

The influence of salinity on developmental time is represented in Figs. 4.4 and 4.5 and Table A 4.9.

The analysis of variance showed that there were significant differences ( $p < 0,05$ ) in the developmental times of the larvae reared in the different NaCl solutions ( $F = 15,39$ ) (Table A 4.10). There were also significant differences ( $p < 0,05$ ) between the control and the selected groups ( $F = 26,71$ ) and also between the different sexes within a certain group ( $F = 290,75$ ) (Table A 4.10).

With Tukey's test it was shown that the control group male larvae reared in 0,02M NaCl solution developed significantly faster ( $p < 0,05$ ) than those reared in 0,0M (distilled water), 0,04M, 0,14M and 0,16M (Fig. A 4.1.1). The

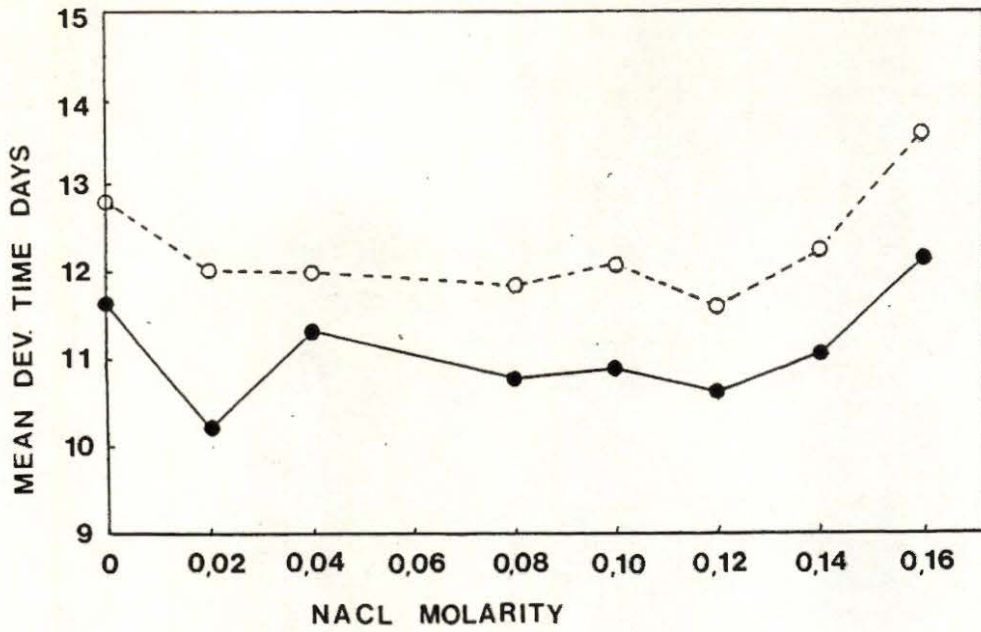


Fig. 4.4 The influence of various NaCl solutions on the developmental time of immature *Cx. theileri* (Control group). Males = ●—●, Females = ○---○.

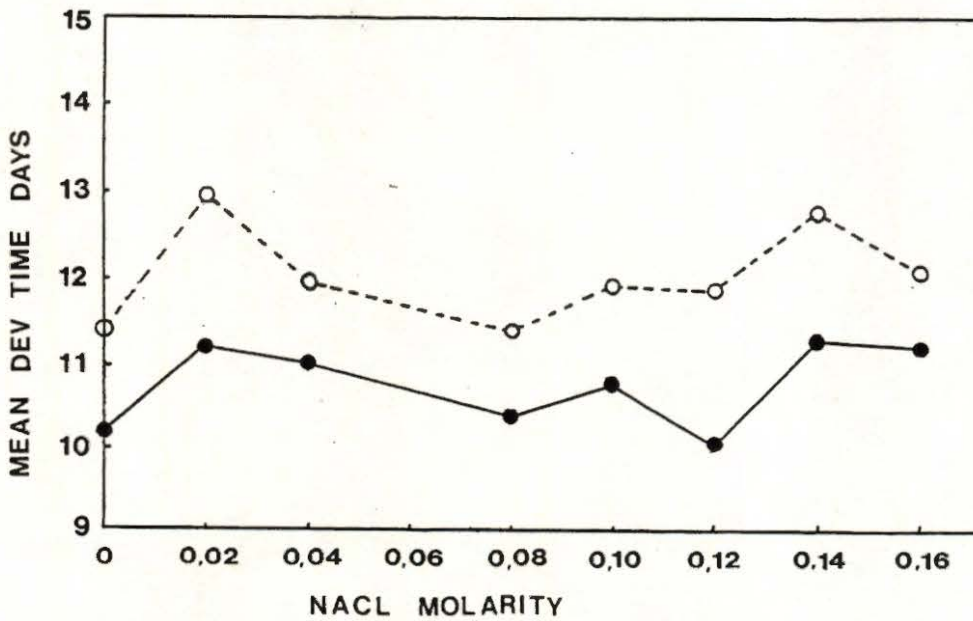


Fig. 4.5 The influence of various NaCl solutions on the developmental time of immature *Cx. theileri* (selected group). Males = ●—●, Females = ○---○.

developmental time of larvae reared in 0,08M, 0,10M and 0,12M was significantly shorter ( $p < 0,05$ ) than those reared in 0,00M and 0,16M NaCl solution, while larvae reared in 0,04M and 0,14M solutions were only faster than those in 0,16M (Fig. A 4.1.1).

Tukey's test indicated that in the control group, the female larvae reared in 0,16M NaCl were significantly slower ( $p < 0,05$ ) than those reared in all other solutions, while those reared in 0,02M, 0,04M, 0,08M and 0,12M only developed significantly faster ( $p < 0,05$ ) than the larvae in 0,0M (distilled water) (Fig. A 4.1.2).

Except for the increased developmental time of males at 0,02M development in the various NaCl solutions followed a similar pattern in males and females in the control group (Fig. 4.4). Both a lack of or an excess of NaCl in solution increases the developmental time of the immature *Cx. theileri* in the control group (Van der Linde, 1984).

In the selected group, Tukey's test showed that the developmental time of the male larvae reared in 0,12M NaCl solution was significantly faster ( $p < 0,05$ ) than all others except those reared in 0,0M (distilled water) and 0,08M (Fig. A 4.1.3). Larvae reared in 0,0M (distilled water) were also significantly faster ( $p < 0,05$ ) in development than those reared in 0,02M, 0,04M, 0,14M and 0,16M NaCl

solutions. Male larvae reared in 0,08M were only faster than those in 0,02M, 0,14M and 0,16M NaCl (Fig. A 4.1.3).

With Tukey's test it was shown that the selected group female larvae developed significantly faster ( $p < 0,05$ ) in 0,0M (distilled water) than larvae reared in all other solutions except 0,08M NaCl (Fig. A 4.1.4). Larvae reared in 0,04M to 0,12M NaCl solutions were also significantly faster ( $p < 0,05$ ) in their development than those reared in 0,02M and 0,14M, while those reared in 0,16M were only faster than those in the 0,02M (Fig. A 4.1.4).

The developmental pattern effected by the NaCl solutions was very similar for males and females in the selected group (Fig. 4.5). However, the short developmental time for both sexes of the selected group shown at 0,0M and the levelling off of the graphs at 0,14M to 0,16M NaCl is unexpected and not clear (Fig. 4.5). However, the levelling off of the graphs at 0,14M and 0,16M NaCl is similar to results obtained by Van der Linde (1984).

In comparing the two groups, the selected group larvae developed significantly faster ( $p < 0,05$ ) than those for the control group ( $F = 26,71$ ) (Table A 4.10). This can probably be ascribed to the fact that the selected group was reared continuously for six generations in a 0,12M NaCl solution while the control group was reared in a 0,02M NaCl solution.

Thus, the former were probably better adapted to high NaCl molarities than the latter group. Thus, there appears to be some selection for increased tolerance to high NaCl molarities.

The linear regression correlation between developmental time and increasing NaCl molarities for both sexes of the control group was non-significant (Males:  $F = 1,9$ ;  $r = 0,09$  and females:  $F = 0,97$ ;  $r = 0,06$ ) ( $p > 0,05$ ). On the other hand, the correlations for males ( $F = 5,3$ ;  $r = 0,15$ ) and females ( $F = 8,1$ ;  $r = 0,18$ ) of the selected group were both significant ( $p < 0,05$ ). The reason for the difference in developmental time between the two groups is not readily explained. In the control group developmental times increased at both ends of the NaCl spectrum (Fig. 4.4) while in the selected group the developmental times were shortest in distilled water (except 0,02M) and longest in 0,14M and 0,16M NaCl (Fig. 4.5).

The developmental time to adulthood of male larvae was significantly shorter ( $p < 0,05$ ) than the females ( $F = 290,75$ ) (Table A 4.10). This is a well known fact and needs no elaboration.

The above-mentioned results point to the fact that if the salinity of the natural habitat is very low or very high it will take longer for *Cx. theileri* larvae to develop to

adulthood. However, *Cx. theileri* is capable of utilizing habitats in the range of 0,0M to as high as 0,16M NaCl.

#### 4.3.5 The mortality of the immatures stages

Though the percentage mortality varies for the individual instars, increased mortalities tend to occur at both ends of the spectrum of NaCl molarities tested (Figs. 4.6 and 4.7) (Table A 4.11). This is less obvious in the control group at low NaCl molarities. The lowest mortalities occurred in 0,02M (control group) and 0,12M (selected group). This is not surprising since the two laboratory colonies from which the control group and the selected group were taken, were reared continuously over a period of time in the respective NaCl concentrations. It is assumed that some selection must have taken place.

An analysis of variance of the percentage mortality of the immature stages of *Cx. theileri* indicated significant differences ( $p < 0,05$ ) between instars ( $F = 12,85$ ), between NaCl molarities ( $F = 9,27$ ), for interactions, between instars and groups ( $F = 13,58$ ), between instars and NaCl molarity ( $F = 5,73$ ) and between NaCl molarity and groups ( $F = 15,96$ ) (Table A 4.12).

Tukey's test ( $Q_{0,05} = 2,5856$ ) indicated that significantly fewer instar two and three larvae died than instar one and



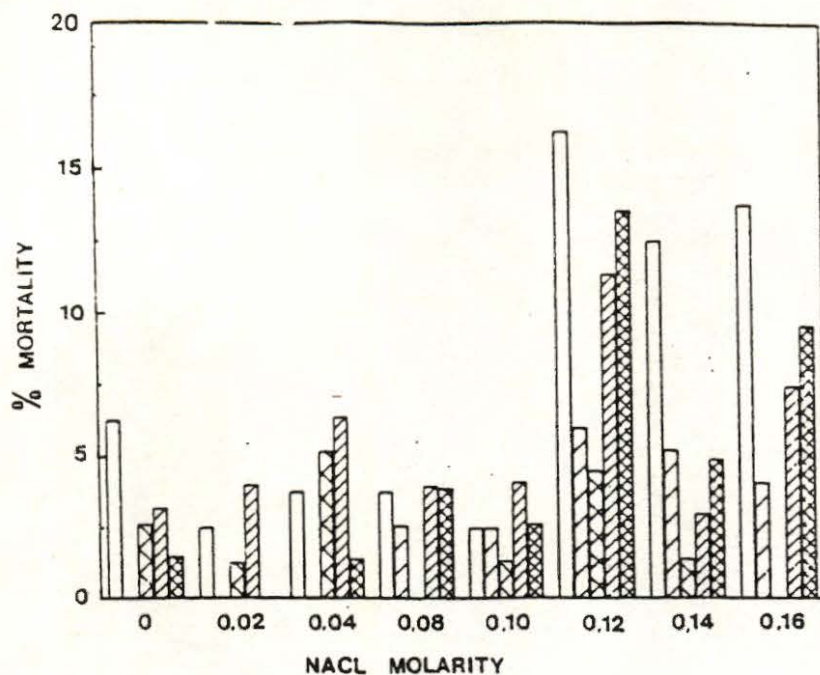







Fig. 4.6 The influence of NaCl solutions of different molarities on the percentage mortality of immature *Cx. theileri* (control group). Instar 1 =  ; Instar 2 =  , 3 =  , 4 =  and pupae =  .

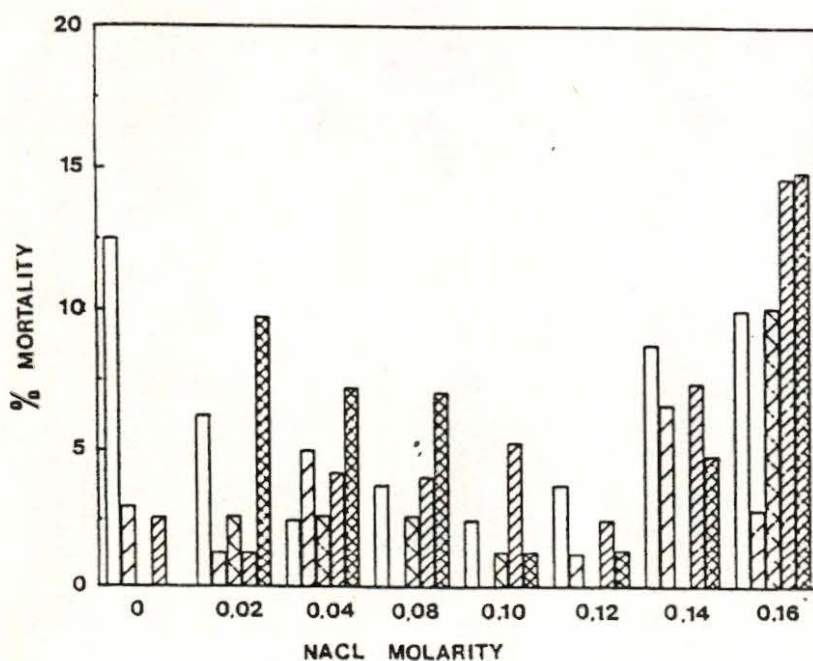







Fig. 4.7 The influence of NaCl solutions of different molarities on the percentage mortality of immature *Cx. theileri* (selected group). Instar 1 =  , 2 =  , 3 =  , 4 =  , pupae =  .

four or pupae ( $F = 23,85$ ) irrespective of groups or NaCl molarities (Table A 4.12). The lowest mortalities occurred in instar three followed by instar two, while the highest mortalities occurred in instar one, four and the pupal stage (Table 4.1).

A possible explanation for the low percentage mortality is that instars two and three are "growth" instars i.e. enlargement is the prime function of these instars. Although instars one, four and the pupal stage are also in a sense "growth" instars, they have the added complications of escape from the eggshell (instar one), transformation from larvae to pupa (instar four) and metamorphosis from pupae to adult (pupal stage). The escape from the egg (instar one) may use up relatively more energy than the transition between the moults of instars two and three. Therefore, less energy is probably available for the active absorption or elimination of NaCl (Hudson, 1956). A similar situation probably exists in instar four and the pupal stage. Edwards (1982) has also shown that active control of osmosis by mosquito larvae occurs. Furthermore, as "active" control of osmosis implies the use of energy, the relative lack of energy may explain in part the high mortalities that occurred in instars one and four and the pupal stage.

There were significant differences in the percentage mortality at differing NaCl molarities ( $F = 9,27$ )

Table 4.1 The mean percentage mortality of the various instars irrespective of groups or NaCl molarity.

Instars	$\bar{x}$ % Mortality	S D	n
1	7,95	6,21	64
2	3,53	4,41	64
3	3,17	3,81	64
4	6,33	5,45	64
Pupae	6,20	6,44	64

Table 4.2 The mean percentage mortality with respect to NaCl molarity irrespective of instars and groups.

NaCl molarity	$\bar{x}$ % mortality	S D	n
0,00	4,14	4,92	40
0,02	3,90	4,35	40
0,04	4,73	4,51	40
0,08	4,07	3,39	40
0,10	3,35	3,38	40
0,12	7,06	6,66	40
0,14	6,47	5,81	40
0,16	9,78	7,79	40

irrespective of instars and groups (Table A 4.12). Tukey's test ( $Q_{0,05} = 3,5945$ ) showed that the highest percentage mortality occurred in 0,12M and 0,16M and the lowest in 0,02M and 0,10M NaCl solutions (Table 4.2). These data showed that there was a higher percentage mortality in both control and selected group with increasing molarity of the NaCl solutions. This supports work by a number of authors eg. Wallis (1954a), Parker (1979), Van der Linde (1984).

Tukey's test ( $Q_{0,05} = 4,2604$ ) applied to the instar x groups interaction indicated that significantly more instar one larvae died in the control group than in the selected group ( $F = 13,58$ ). On the other hand significantly more pupae died in the selected group than in the control group (Table 4.3 and Table A 4.12).

The reason for this is not clear. An explanation in terms of acclimatization does not seem appropriate. However, as the "fitness" of individuals is often a compromise of a number of conflicting selection pressures (Mayr, 1971), it is possible that the manifestation of this compromise is higher instar one mortality in the control group and higher pupal mortality in the selected group.

An analysis of variance ( $F = 5,73$ ) and Tukey's test ( $Q_{0,05} = 8,7557$ ) showed that for both the control and the selected group mortality of the pupal stage was higher than other

Table 4.3 The mean percentage mortality of the various instars in the various groups irrespective of NaCl molarity (Instar x groups interaction).

Instar Stage	Control group (0,02M NaCl strain)			Selected group (0,12M NaCl strain)		
	$\bar{x}$	S D	n	$\bar{x}$	SD	n
1	8,66	6,84	32	7,25	5,54	32
2	3,56	4,74	32	3,51	4,14	32
3	2,91	3,88	32	3,42	3,79	32
4	6,38	5,14	32	6,28	5,83	32
Pupae	5,60	5,84	32	6,81	7,03	32

Table 4.4 The mean percentage mortality of the various instars in each NaCl molarity irrespective of groups (i.e.: Instar x molarity interaction).

NaCl molarity	Instar/stage									x % Mortality								
	1			2			3			4			Pupae					
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n			
0,00	10,38	4,96	8	2,49	2,76	8	2,31	3,71	8	3,78	5,14	8	1,74	2,09	8			
0,02	5,38	4,96	8	1,66	1,87	8	2,94	2,68	8	3,65	2,83	8	5,88	6,95	8			
0,04	4,13	3,72	8	3,50	5,15	8	4,41	5,88	8	6,29	3,89	8	5,31	3,99	8			
0,08	4,75	3,54	8	2,29	2,39	8	2,33	2,46	8	5,00	3,75	8	5,98	3,60	8			
0,10	3,50	3,78	8	2,31	2,31	8	2,33	2,45	8	5,69	4,61	8	2,99	2,75	8			
0,12	11,00	8,45	8	4,39	4,39	8	3,26	3,12	8	7,94	6,92	8	8,48	7,39	8			
0,14	11,63	6,23	8	8,02	8,02	8	1,70	1,98	8	6,23	3,80	8	5,88	3,02	8			
0,16	12,88	5,30	8	4,03	4,03	8	6,05	5,77	8	12,08	7,82	8	13,40	10,92	8			

instars in the high molarities (Table 4.4 and Table A 4.12). This was not so for low molarities.

A possible reason for this is that increasing molarity of NaCl solutions has a more pronounced influence on metamorphosis during the pupal transition to adult than for moulting between the various instars.

Analysis of variance of the interaction ( $p < 0,05$ ) between groups and NaCl molarity showed significant differences ( $F = 15,96$ ) (Table A 4.12). With Tukey's test ( $Q_{0,05} = 5,4933$ ) it was shown that for the control group relatively more larvae and pupae died at high NaCl molarities. However, for the selected group relatively more died at low and at high NaCl molarities (Table 4.5 and Table A 4.12). Some selection for the tolerance of high NaCl molarities is thus indicated with this shift in percentage mortality.

It is noteworthy that the larvae reaching adulthood, remained fairly constant above 80 % for the control group up to 0,10M NaCl and the selected group up to 0,12M NaCl solution (Table 4.6). The drop in the percentage surviving larvae of the control group at a NaCl molarity of 0,12M is associated with an increase in the percentage mortality of instars one and four and the pupal stage.

This did not occur in the selected group (Fig. 4.7). The reason for this phenomenon in the control group is not clear, although factors associated with osmoregulation may be involved. The "switch" from hyporegulation in external

Table 4.5 The mean percentage mortality of all instars at each NaCl molarity for both the control and the selected groups (group x molarity interaction).

NaCl molarity	$\bar{x}$ % mortality control group (0,02M)			$\bar{x}$ % mortality selected group (0,12M)		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n
0,00	3,63	4,01	20	4,65	5,75	20
0,02	2,55	2,42	20	5,26	5,39	20
0,04	4,14	4,99	20	5,32	4,02	20
0,08	3,63	3,20	20	4,51	3,60	20
0,10	3,62	3,62	20	3,09	3,18	20
0,12	11,35	6,40	20	2,78	3,39	20
0,14	6,43	6,64	20	6,25	5,01	20
0,16	8,05	7,12	20	11,51	8,22	20

more dilute media than the haemolymph to hyporegulation in more concentrated media as occurs in some saline-water *Aedes* species (Garrett and Bradley, 1984) is probably not a tenable explanation. Wigglesworth, (1938 quoted by Garrett & Bradley, 1984) indicated that most freshwater culicines were obligate hyperregulators in fresh water. The tendency to reduced percentage survival with rising molarities of NaCl solutions agrees with results obtained by Van der Linde

Table 4.6 The numbers of adults and the percentage survival of instar one larvae to adulthood for the control group and the selected group of mosquitoes.

Group	NaCl molarity	Initial no's of larvae	Numbers of adults		% Survival	Group	NaCl molarity	Initial no's of larvae	Numbers of adults		% Survival
			♂	♀					♂	♀	
Control group (0,02M)	0,00	80	40	30	87,5	Selected group (0,12M)	0,00	80	33	33	82,5
	0,02	80	38	36	92,5		0,02	80	32	32	80,00
	0,04	80	29	39	85,0		0,04	80	28	36	80,00
	0,08	80	36	34	87,5		0,08	80	33	33	82,50
	0,10	80	34	36	87,5		0,10	80	35	37	90,00
	0,12	80	19	27	57,5		0,12	80	33	40	91,30
	0,14	80	27	33	75,0		0,14	80	27	33	75,0
	0,16	80	28	26	76,5		0,16	80	25	20	56,30
		640	251	261	80 %		640	246	264	79,7 %	
			Σ =	512				Σ =	510		



(1984) on *Cx. theileri*. Similar results have been recorded for other species of mosquitoes e.g. *Ae. dorsalis* (Parker, 1979), *An. albimanus* (Bailey *et al.*, 1981) and *An. merus* (Mosha & Mutero, 1982). Figs. 4.6 and 4.7 indicate a "shift" in tolerance to higher molarities in the selected group, but the total numbers of mosquitoes reaching adulthood did not differ significantly in the control (80 %) and selected groups (79,7 %) (Table 4.6).

#### 4.3.6 Head capsule widths

The analysis of variance of data in Table A 4.13 showed that the head capsule widths were significantly different ( $p < 0,05$ ) in some of the NaCl solutions for the control ( $F = 42,56$ ) and the selected ( $F = 12,46$ ) groups (Table A 4.14 and Table A 4.15). Thus, the lack of NaCl as well as an excess of NaCl salts in solution adversely influenced the growth of fourth instar *Cx. theileri* larvae (Fig. 4.8).

With Tukey's test it was shown that in the control group the head capsule widths of fourth instar larvae were significantly larger ( $p < 0,05$ ) ( $F = 42,56$ ) in 0,02M NaCl molarities than in all the others except 0,04M (Fig. A 4.2) Head capsule widths of larvae reared in NaCl solutions of 0,04M, 0,08M and 0,10M were also significantly larger ( $p < 0,05$ ) than those reared in 0,0M (distilled water), 0,12M, 0,14 and 0,16M (Fig. A 4.3).

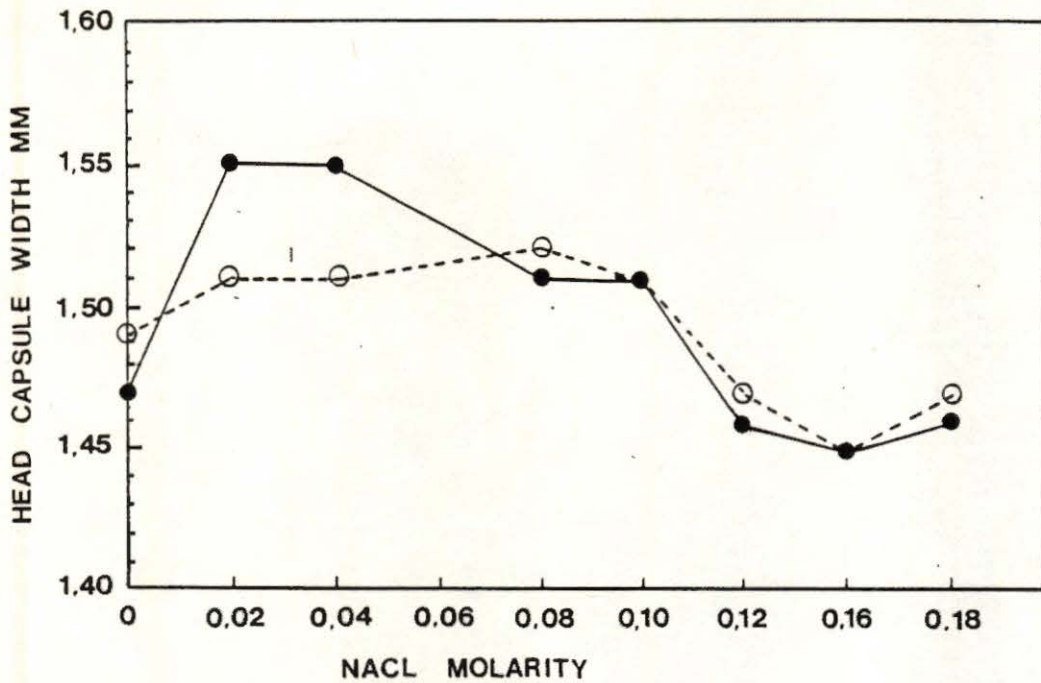


Fig. 4.8 The influence of NaCl solutions of different molarities on the mean head capsule widths of instar four *Cx. theileri*. Control group = ●—●, Selected group = ○---○.

In the selected group, Tukey's test showed that in the range 0,02M to 0,10M NaCl solutions the head capsule widths of larvae were significantly larger ( $p < 0,05$ ) than those reared in 0,12M, 0,14M and 0,16M NaCl solutions (Fig. A 4.3). Larvae reared in 0,0M (distilled water) also had significantly larger ( $p < 0,05$ ) head capsules than those in 0,14M NaCl solution (Fig. A 4.3).

#### 4.4 Conclusion

Some selection for the tolerance of high NaCl salinities was shown. The selected group developed significantly faster and generally laid greater numbers of eggs per raft as a whole than the control group in the NaCl solutions tested. Except for the differential effect of various NaCl solutions on the percentage mortality of the various instars, their ability to survive has largely been a compromise as the total numbers of adults that eventuated from both groups were practically the same (Table 4.6). Mayr (1971) has stated that selection in nature, even in the smallest populations, is primarily for overall fitness. Thus traits will be selected that enhance overall fitness.

The results obtained in the present study indicated that a tolerance to high concentrations of NaCl solutions is a general characteristic of *Cx. theileri*. This allows *Cx. theileri* to very effectively survive in the western Orange Free State with its relatively low rainfall and its variable climate. Furthermore, this wide tolerance of various NaCl salinities and by implication, habitats with different salinities, explains in part the dominant position of *Cx. theileri* in this area. However, highly tolerant strains of this species, though possible, are unlikely to develop in the field.

## 5.0 General conclusions

It had been long suspected that *Cx. theileri* and probably *Cx. pipiens* survived the winter in the immature stages (Jupp, 1969, 1975; Van der Linde, 1984). The field pool survey provided evidence that this is indeed so in the Orange Free State. If the total numbers of larvae of each species sampled during July (mid-winter) are expressed as a percentage of the numbers collected during the peak April (autumn) populations (section 2.3.1.5), then approximately 0,6 % and 1,2 % of *Cx. theileri* and *Cx. pipiens* survive the winter.

The most important factor affecting these two species in winter is the low mean temperatures. The low temperatures probably reduce flight activity of adults and feeding and in *Cx. theileri* also reduce oögenesis and oviposition (Van der Linde, 1984). Furthermore, a high mortality in the immature stages was also demonstrated in this study. However, the larvae that survived were probably better able to adapt physiologically to low mean variable temperatures in the laboratory and in the field. The threshold of development of these survivors in the field would probably approach 0°C as indicated in the laboratory trials in this study. It was significant that unseasonal rainfall recorded in June (section 2.3.1.5) had no effect on immature population numbers sampled at the time. Temperature, not rainfall, was

most certainly the limiting factor in winter. However, this may not be true for adult mosquitoes. Bloodfed *Cx. theileri* survived longer at low temperatures, but were greatly affected by high saturation deficits, even at low temperatures (section 3.3.5). The  $LT_{50}$ -values were approximately 23 days and 9 days respectively for saturation deficits of 0.32 kPa and 1,10 kPa at 10°C. Although adult *Cx. theileri* are unlikely to survive the whole winter period of approximately 80 days given an unrestricted choice of sites in which to take refuge, and a carbohydrate source, they may well survive considerable longer than suggested above.

During summer, the most important parameters limiting populations of mosquito larvae included rainfall, probably intra- and interspecific competition, and predation (White, 1980). Peak populations of immature mosquitoes were closely correlated with periods of peak rainfall in this study. Work by other authors eg. Hewitt *et al.*, (1982), Van der Linde (1984) have shown a similar close correlations of peak adult populations with peak rainfall and showed a time lag of approximately one week for adult *Cx. theileri* following good rains in summer. The time lag of approximately one month for the immature stages is probably the result of sampling being conducted at monthly intervals in the present study. With shorter periods between larval collections eg. weekly or fortnightly, a more accurate estimate of the lag

period between larval population peaks and rainfall would have been established.

Working with field populations of *Cx. tarsalis* White (1980) has also suggested that high summer temperatures and high saturation deficits are likely to cause considerable mortality in adult mosquito populations. Alternatively, he suggested that high temperatures may stimulate adults to take temporary refuge. This would reduce oviposition and contribute to limiting populations of mosquitoes in summer.

With respect to population peaks of immature *Cx. tarsalis* in the field, White (1980) has shown that distinctive cohorts laid in succession during progressively warmer periods eg. spring through summer, merge, resulting in a large population peak. What this probably means is that population peaks do not necessarily represent distinctive generations. For example, five population peaks in a season may in fact represent eight to twelve distinctive generations or cohorts. Thus one should be circumspect in attributing population peaks to distinctive generations. Furthermore, the latter author also suggested that the population "crashes" that often occur later in summer when climatic conditions would be expected to be optimal for mosquitoes was probably related to intrinsic density-dependent factors (eg. overcrowding, competition, predation, etc.). In addition, extrinsic factors such as low rainfall

(in summer) and progressively lower temperatures in autumn through winter probably explains the drastic reduction in field populations during this time.

The consistent numerical dominance of *Cx. theileri* in the western Orange Free State reflects to a large extent their ability to overwinter as larvae (sections 2.3.1.5 and 3.3.4), their ability to utilize virtually all available permanent and temporary water bodies (section 2.3.1.2) in which the salinity of the water does not exceed 0,16M NaCl (sections 4.0 to 4.4). With respect to the latter, some selection for the tolerance to high NaCl molarities is in evidence in *Cx. theileri* in that a higher fecundity occurs. However, as the total numbers that eventuated were essentially the same whether they were selected for high NaCl tolerance or not, no real selection was considered to have occurred. This means that all habitats, excluding the commercial salt pans are potential breeding sites of this species. However, it is noteworthy that the highest numbers of *Cx. theileri* were collected in the temporary pools during this field survey (section 2.3.1.2). This may reflect the opportunistic ability of this species to locate and utilize new field pools that occur after widespread rainfall in the region.

The analysis of temporal and spatial co-abundance of species (section 2.3.1.5) reflects largely the importance of

temperature and rainfall in the former and oviposition and habitat requirements in the latter. However, the exception is the co-existence of *Ae. hirsutus* and *Ae. caballus* in space and time. Their continued co-existence is probably due to the partitioning of the micro-habitat.

Aspects requiring further research indicated in this study include: the influence of other organisms sharing habitats with immature field populations of *Cx. theileri*, the influence of low temperature on the physiology and biochemistry of larvae and adults, especially the phenomenon of "cold hardiness" and acclimatization at sub-lethal temperatures. The effect of photo- and thermoperiodic cues on "conditioning" of adults and larvae to approaching inclement conditions, the effect of variable mean temperatures and saturation deficits on the longevity and survival of the adults, and the influence of various salts alone and in various combinations on the breeding of *Cx. theileri* in the laboratory. Finally, the sources of Rift valley fever virus, of which *Cx. theileri* is a vector, during the inter-epidemic periods needs to be ascertained.



## 6.0 Summary

As little was known of the seasonal abundance of immature mosquitoes in the Western Orange Free State, as well as the influence of variable temperatures and high NaCl salinities on the development and survival of the immature stages of the mosquito, *Cx. theileri*, these aspects were addressed in this study.

A monthly survey of 18 field pools over a period of 12 months yielded 14 species of which *Cx. theileri* (46,15 %), *Cx. pipiens*, (32,43 %) and *Cx. univittatus* (10,76 %) were the most abundant. It also showed that the former two species overwinter in low numbers in all the immature stages and were common during the rest of the year. *Cx. theileri* inhabited 89 % of sites. *Cx. pipiens* and *Cx. univittatus* occurred in 50 % and 56 % of the pools respectively, showing a preference for pools with more than 30 % vegetational cover.

*Cx. theileri* and *Cx. pipiens* had three larval population peaks per annum, the largest in autumn, while *Cx. univittatus* had two of equal magnitude in early summer and autumn. Population peaks are closely associated with rainfall peaks, especially regarding the *Aedes* species.

The major factor limiting mosquito populations in winter was

low temperature, which reduced flight and oviposition activity of adults and caused high mortality in larval populations. In mid-summer, the major limiting factors were low rainfall, availability of breeding sites, and density-dependant factors eg. overcrowding, competition and predation.

Temporal and spatial co-abundance analyses of nine species were done. A close ( $p > 5,5$ ) temporal association occurred between *Cx. theileri*, *Cx. pipiens* and *Cx. univittatus* on the one hand and *Ae. caballus*, *Ae. hirsutus* and *Ae. juppi* on the other, reflecting temperature and rainfall effects on seasonal abundance of these species. *Cx. salisburyensis*, *Cs. longiareolata* and *An. squamosus* were not correlated in time with each other or with the other species, because they occurred more often during colder times of the year. A close ( $p > 4,8$ ) spatial association was shown for *Cx. theileri* and *Cx. salisburyensis*, for *Ae. caballus* and *Ae. hirsutus*, and for *Ae. juppi* and *Cx. univittatus*, which reflected oviposition and habitat requirements in acceptable habitats. *Cx. pipiens*, *Cs. longiareolata* and *An. squamosus* were not closely associated in space (pool sites) with each other or with the other species because they had different habitat preferences.

In variable temperature trials, developmental time of 24 hour old larvae to adulthood took 50 and 11 days in the

laboratory at mean temperatures of 10°C and 24,1°C and 80 and 11 days in field trials at 8,2°C and 22,5°C respectively.

In field trials, 96 % mortality occurred at 8,2°C in the experimental larval populations while in the laboratory trials 85 % died at 10°C. The higher mortalities in field were attributed to the occasional freezing of surface water that occurred on some winter mornings for periods of one to three hours.

Head capsule widths of fourth instar larvae, which reflect growth rate of the larvae, were significantly larger at a mean developmental temperature of 10°C than at 24,1°C in the laboratory trials, but not significantly larger in field trials, due probably to erratic temperature changes that occur in nature.

Trials involving various fluctuating temperatures and saturation deficits showed that blood-fed females lived the longest ( $LT_{50} = 23,9$  days) at low mean temperatures (10°C) and low mean saturation deficits (0,33 kPa).

The limits of *Cx. theileri* tolerance to NaCl solutions was assessed by comparing the control group which was reared in 0,02M to the selected group which was reared in 0,12M ( $F = 6$ ) prior to initiation of trials. The NaCl solutions tested

ranged from 0,0M (distilled water) to 0,16M for larval developmental trials and to 0,22M for oviposition trials. In all parameters tested, no significantly ( $p > 0,05$ ) increased tolerance to NaCl solutions, and therefore no true selection of a halophilic *Cx. theileri* strain was achieved. However, significant reductions ( $p < 0,05$ ) occurred in oviposition, percentage eggs hatched, and head capsule widths. While developmental time and percentage mortality were significantly increased ( $p < 0,05$ ) in all stages at high NaCl molarities in both groups.

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## 8.0 LIST OF ABBREVIATIONS

- n = Number of observations/individuals.
- nr = The number of ranks in each set (eg. the number of populations being compared) (Table A 4.1).
- ns = The number of sets of ranks (eg. the NaCl solutions of 0,0 M to 0,22 M) (Table A 4.1).
- $Q(0,05)$  = Tukey's critical difference between treatments for them to differ significantly, eg. at the 5 % level of significance.
- R = Regimes.
- RH = Relative humidity.
- S = Stages.
- SD = Standard deviation of the mean.
- sd = Saturation deficit.
- SEM = Standard error of the mean.
- Sx = Sex (as in male and female)

9.0 APPENDIX

Table A 3.1 The influence of fluctuating temperature on developmental time of immature *Cx. theileri* in the laboratory.

Regime:	1			2			3			4			5			6		
$\bar{x}$ temp. (°C)	10,0			11,6			12,8			15,3			22,5			24,1		
$\bar{x}$ max. temp.	14,3			15,8			18,1			19,2			29,6			29,7		
$\bar{x}$ min. temp.	5,3			7,4			7,0			7,0			14,1			18,5		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
Instar 1 male	7,0	1,36	7	7,33	2,26	15	5,67	5,67	39	3,5	0,80	42	1,58	0,54	45	1,24	0,52	50
	11,7	3,25	"	9,93	1,62	"	6,41	1,27	"	4,55	1,60	"	1,98	0,53	"	2,02	0,74	"
	13,14	2,04	"	10,07	1,87	"	7,10	1,17	"	5,98	1,14	"	2,16	0,37	"	2,18	0,60	"
	16,14	2,41	"	13,67	0,98	"	10,41	1,01	"	7,67	4,14	"	5,40	0,64	"	3,74	0,79	"
	8,71	1,25	"	8,67	0,82	"	7,72	0,86	"	6,05	1,01	"	2,29	0,51	"	2,38	0,78	"
	55,57	4,16	"	49,93	2,85	"	37,41	2,26	"	27,76	3,84	"	13,29	0,69	"	11,56	1,01	"
Instar 1 female	7,75	1,14	12	7,57	2,99	7	5,86	1,56	42	3,71	0,90	41	1,85	0,53	53	1,27	0,57	49
	11,75	2,83	"	7,57	2,99	"	6,86	1,20	"	4,71	1,47	"	1,96	0,71	"	2,12	0,48	"
	13,25	1,49	"	10,71	0,76	"	7,57	1,15	"	6,49	1,55	"	2,74	0,72	"	2,27	0,57	"
	16,00	3,19	"	14,14	1,07	"	11,71	1,00	"	8,12	0,81	"	6,06	1,08	"	4,51	0,71	"
	9,67	1,15	"	9,29	1,38	"	8,33	0,98	"	6,17	1,05	"	2,26	0,45	"	2,63	0,57	"
	59,50	7,14	"	51,71	2,81	"	40,33	3,08	"	29,29	3,47	"	14,91	1,42	"	12,78	0,82	"

Table A 3.2 Influence of seasonal change on the developmental time of *Cx. theileri* under natural climatic conditions in an artificial pool.

Regime	1	2	3	4	5	6	7	8	9
Starting dates	7/5/85	7/5/85	17/8/85	20/9/85	5/11/85	24/2/85	5/2/85	14/1/85	2/1/85
Temp. °C:									
$\bar{x}$	8,2	8,2	12,9	17,2	20,6	20,9	22,4	22,6	22,8
$\bar{x}$ max.	10,8	10,8	16,9	21,4	26,0	24,0	26,0	27,1	26,4
$\bar{x}$ min	5,4	5,4	7,9	12,9	17,9	16,6	17,2	19,0	18,6
	n = 1 $\bar{x}$ SD	n = 0 $\bar{x}$ SD	n = 47 $\bar{x}$ SD	n = 48 $\bar{x}$ SD	n = 63 $\bar{x}$ SD	n = 54 $\bar{x}$ SD	n = 43 $\bar{x}$ SD	n = 57 $\bar{x}$ SD	n = 63 $\bar{x}$ SD
Males:									
Ins. 1	16 0,0	- -	5,91 0,10	4,06 0,52	1,44 0,50	2,02 0,13	1,19 0,42	2,05 0,59	1,29 0,55
" 2	12 0,0	- -	5,21 0,75	3,15 0,55	2,05 0,66	1,41 0,53	1,93 0,46	1,26 0,44	1,67 0,49
" 3	15 0,0	- -	6,11 0,67	3,77 0,47	2,95 0,50	2,54 0,54	2,35 0,49	2,16 0,48	1,86 0,47
" 4	24 0,0	- -	7,43 0,54	5,06 0,85	3,22 0,46	4,41 0,47	3,81 0,55	3,53 0,60	3,22 0,49
" p	8 0,0	- -	4,79 0,52	3,77 0,42	1,89 0,79	3,41 0,57	2,14 0,35	2,02 0,28	2,22 0,42
" adult	76 0,0	- -	29,38 1,85	19,90 0,53	11,57 0,61	13,85 0,81	11,42 0,73	11,07 0,83	10,25 0,44
	n = 6 $\bar{x}$ SD	n = 2 $\bar{x}$ SD	n = 57 $\bar{x}$ SD	n = 62 $\bar{x}$ SD	n = 52 $\bar{x}$ SD	n = 61 $\bar{x}$ SD	n = 64 $\bar{x}$ SD	n = 53 $\bar{x}$ SD	n = 52 $\bar{x}$ SD
Females:									
Ins. 1	16,67 1,03	17,50 2,12	6,25 0,69	4,24 0,43	1,62 0,51	2,08 0,28	1,29 0,48	2,13 0,39	1,27 0,60
" 2	12,17 1,47	14,0 0,00	5,33 0,62	3,27 1,17	2,25 0,52	1,56 0,53	1,93 0,43	1,38 1,16	1,73 0,57
" 3	20,83 2,48	21,0 4,24	6,53 0,60	3,74 0,48	2,94 0,54	2,75 0,94	2,17 0,42	2,25 0,46	1,96 0,59
" 4	19,61 1,37	19,0 1,41	7,95 0,64	5,56 0,74	3,58 0,50	4,89 0,55	4,48 0,53	4,02 0,49	3,17 0,57
" p	7,67 1,37	9,0 1,41	5,30 0,78	3,95 0,56	2,00 0,49	3,44 0,56	2,11 0,31	2,02 0,23	2,73 0,45
" adult	77,0 2,45	80,50 0,71	31,37 1,87	20,63 0,81	12,38 0,70	14,72 0,12	11,94 0,47	11,64 0,96	11,0 0,95

Table A 3.3 Analysis of variance of the developmental time of immature *Cx. theileri* under laboratory conditions.

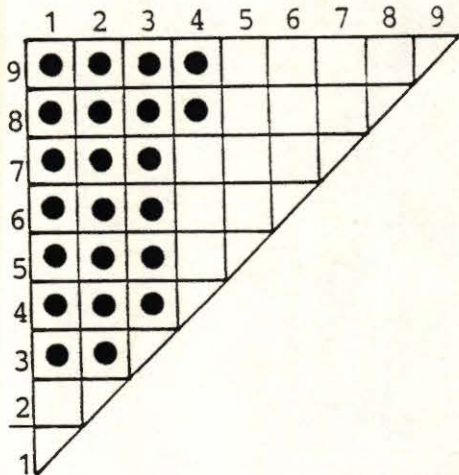
Source	df	MSS	F (0,05)
Stages (S)	5	29180,56	1441,30*
Regimes (R)	5	10544,06	520,80*
Sex (Sx)	1	134,28	6,63*
Error	2400	20,25	

Table A 3.4 Analysis of variance on the developmental time of *Cx. theileri* under natural climatic conditions in an artificial pool.

Source	df	MSS	F (0,05)
Stages (S)	5	23125,42	2638,38*
Regimes (R)	8	5937,42	677,40*
Sex (Sx)	1	749,98	85,57*
Error	4701	8,77	



Fig A 3.1 Tukey's test on significant differences in developmental time between regimes (mean temperatures)



Significant ( $p < 0,05$ )



Non significant ( $p > 0,05$ )

1 =	Regime 1	(x temp.	= 8,2°C)
2 =	"	2 (" "	= 8,2°C)
3 =	"	3 (" "	=12,9°C)
4 =	"	4 (" "	=17,2°C)
5 =	"	5 (" "	=20,6°C)
6 =	"	6 (" "	=20,9°C)
7 =	"	7 (" "	=22,4°C)
8 =	"	8 (" "	=22,6°C)
9 =	"	9 (" "	=22,8°C)

$Q_{0,05} = 2,8656$

Table A 3.5 The influence of fluctuating temperatures on the percentage mortality of immature *Cx. theileri* in the laboratory.

Regime	1			2			3			4			5			6		
Temp. °C:																		
$\bar{x}$ max.	14,3			15,8			18,1			19,2			29,6			29,7		
$\bar{x}$ min	5,3			7,4			7,0			7,0			14,1			18,5		
$\bar{x}$	10,0			11,6			12,8			15,3			22,5			24,1		
Stages	$\bar{x}\%$	SD	n	$\bar{x}\%$	SD	n	$\bar{x}\%$	SD	n	$\bar{x}\%$	SD	n	$\bar{x}\%$	SD	n	$\bar{x}\%$	SD	n
1	16,7	10,3	6	18,3	11,3	6	7,5	2,7	6	7,5	2,7	6	8,3	4,1	6	4,2	3,8	6
2	15,6	8,3	6	8,4	3,8	6	10,8	7,0	6	0,9	2,3	6	1,7	2,6	6	1,7	2,6	6
3	29,0	13,9	6	25,0	9,3	6	1,1	2,6	6	1,0	2,4	6	4,5	4,0	6	4,5	4,0	6
4	33,6	21,0	6	41,4	6,1	6	12,9	7,1	6	5,4	3,3	6	5,7	6,4	6	5,7	6,4	6
Pupa	41,4	28,7	6	33,7	22,9	6	4,7	3,7	6	3,8	4,6	6	2,8	4,7	6	2,8	4,7	6

Table A 3.6 Analysis of variance of the percentage mortality in immature *Cx. theileri*.

Source	df	MSS	F (0,05)
Regimes (R)	5	3579,27	36,69*
Stages (S)	4	680,25	6,97*
SXR interaction	20	263,42	2,70
Error	150	97,55	

Table A 3.7 The influence of natural diurnal temperature fluctuations on the percentage mortality of *Cx. theileri* larvae.

		Regimes:																										
		1			2			3			4			5			6			7			8			9		
Mean tempera- ture °C																												
	8,2			8,2			12,9			17,2			20,6			20,9			22,4			22,6			22,8			
In- stars	x̄% SD n			x̄% SD n			x̄% SD n			x̄% SD n			x̄% SD n			x̄% SD n			x̄% SD n			x̄% SD n			x̄% SD n			
	1	85,0	12,5	3	86,7	7,6	3	4,2	2,9	3	0,8	1,4	3	0,0	0,0	3	0,8	1,4	3	10,8	1,4	3	3,3	1,4	3	1,7	1,4	3
2	25,7	38,5	3	20,0	18,0	3	1,7	3,0	3	0,8	1,4	3	0,8	1,4	3	0,0	0,0	3	0,0	0,0	3	1,8	1,5	3	0,0	0,0	3	
3	7,0	6,4	3	33,3	31,5	3	1,8	3,2	3	0,0	0,0	3	1,8	1,4	3	0,8	1,4	3	0,0	0,0	3	0,0	0,0	3	0,0	0,0	3	
4	33,3	14,4	3	22,2	35,5	3	2,7	2,8	3	4,3	3,9	3	1,8	1,4	3	1,7	2,6	3	0,0	0,0	3	0,0	0,0	3	0,0	0,0	3	
Pupa	5,0	7,1	3	33,3	28,9	3	2,9	2,9	3	2,7	4,6	3	1,7	1,4	3	0,0	0,0	3	0,0	0,0	3	0,0	0,0	3	2,5	2,5	3	

Table A 3.8 Analysis of variance of percentage mortality of the immature stages of *Cx. theileri* under natural climatic conditions in an artificial pool.

Source	df	MSS	F (0,05)
Stages (S)	4	1349,85	10,50*
Regimes (R)	8	3476,42	27,03*
S x R interaction	32	493,49	3,84*
Error	89	128,59	

Table A 3.9 The influence of fluctuating temperatures on the head capsule widths of instar four *Cx. theileri* larvae in the laboratory.

Regime number	$\bar{x}$ Temperature °C	Head capsule width (mm)		
		$\bar{x}$	SD	n
1	10,0	1,54	0,06	19
2	11,6	1,53	0,03	22
3	12,8	1,53	0,06	60
4	15,3	1,51	0,02	60
5	22,5	1,50	0,04	60
6	24,1	1,50	0,05	60

Table 3.10      Analysis of variance of the influence of fluctuating temperatures in the laboratory on the head capsule widths of instar four *Cx. theileri* larvae.

Source	df	MSS	F (0,05)
Treatments	5	0,01	3,48*
Error	278	0,002873	

Table A 3.13 Analysis of variance of the mean survival times of blood-fed *Cx. theileri* in the various temperature and saturation deficit regimes.

Regime	Temperature (°C):			RH (%)	sd (kPa)	LT <sub>50</sub> -values			Polynomial regression coefficients: Y = a + bx + cx <sup>2</sup> + dx <sup>3</sup> + ex <sup>4</sup> + fx <sup>5</sup> + gx <sup>6</sup>	r
	$\bar{x}$	$\bar{x}$ max	$\bar{x}$ min			$\bar{x}$	SD	n		
A	20,0	26,5	13,5	38	1,44	4,76	1,06	59	Y = 225,13998-260,01786x + 184,95875x <sup>2</sup> - 57,4605x <sup>3</sup> + 7,72374x <sup>4</sup> - 0,37499x <sup>5</sup>	1,000
B	10,0	15,3	4,6	10	1,10	10,09	2,73	43	Y = 870,29467-595,35145x + 181,52749x <sup>2</sup> 27,8297x <sup>3</sup> + 2,24894x <sup>4</sup> -0,09186x <sup>5</sup> + 0,0015x <sup>6</sup>	0,9996
C	20,0	26,5	13,5	74	0,53	18,36	2,94	44	Y = 2398,26297-600,89273x + 57,85285x <sup>2</sup> - 2,42603x <sup>3</sup> + 0,03694x <sup>4</sup>	0,9915
D	10,0	15,3	4,6	77	0,32	22,75	5,51	36	Y = 919,02497-341,96943x + 58,12905x <sup>2</sup> - 5,14489x <sup>3</sup> + 0,24794x <sup>4</sup> -0,00615x <sup>5</sup> + 0,0006x <sup>6</sup>	0,9973

Table A 3.14 Analysis of variance of the mean survival times of blood-fed *Cx. theileri* in the various temperatures and saturation deficit regimes.

Source	df	MSS	F (0,05)
Treatments	3	3017,20	296,16*
Error	178	10,19	



Table A 3.15 Mass loss as a percentage of the original live mass of blood-fed *Cx. theileri* females.

Regimes	A			B			C			D		
temp. $\bar{x}$ sd $\bar{x}$ RH	20°C 1,44kPa 38%			10°C 1,10kPa 10%			20°C 0,53kPa 74%			10°C 0,32 kPa 77%		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
Mass lost (%)	76,09	5,18	52	65,14	12,45	40	70,40	10,03	41	96,00	3,15	30

Table A 3.16 Analysis of variance of the % mass loss by blood-fed *Cx. theileri* females.

Source	df	MSS	F (0,05)
Treatments	3	1062,67	14,41*
Error	159	73,73	

Table A 4.1 The overall similarity of response in the ovipositional behaviour of various populations of *Cx. theileri* to different NaCl solutions as indicated by Kendall's coefficient of rank correlation (Yeomans, 1970).

Group	Method	ns	nr	Ranked data compared	Kendall's X <sup>2</sup> value	X <sup>2</sup> table value	Kendall's coef. of concordance (w)
Control	Petri-dishes	12	4	% Egg rafts	0,65	7,815	0,01806
Selected	Petri-dishes	12	4	% Egg rafts	0,95	7,815	0,02639
Control	Apex vials	12	3	% Egg rafts	1,66	5,991	0,04861
Selected	Apex vials	12	3	% Egg rafts	0,13	5,991	0,00521

Table A 4.2 95% Confidence interval for oviposition in petri-dishes.

Group	Variable	Limits	
		Upper	Lower
Control	Constant	18,03186	12,32883
	x	267,45485	32,52725
	x <sup>2</sup>	-1586,77826	-4136,71514
	x <sup>3</sup>	12383,67376	4776,88286
Selected	Constant	16,23344	5,08701
	x	554,41288	95,25309
	x <sup>2</sup>	-1883,77536	-6867,55958
	x <sup>3</sup>	19751,52828	4884,25655

Table A 4.3 95% Confidence interval for oviposition in Apex vials.

Group	Variable	Limits	
		Upper	Lower
Control	Constant	12,20612	6,42920
	x	254,82364	16,85217
	x <sup>2</sup>	-279,07341	-2862,04893
	x <sup>3</sup>	7527,86305	-177,48641
Selected	Constant	12,66794	7,78663
	x	102,10741	-1,04330
	x <sup>2</sup>	-215,97892	-667,74589

Table A 4.4 The average number of eggs/raft laid in the various NaCl solutions by both the control and the selected group of mosquitoes.

Treat NaCl Molarity	Black Petri-dishes						Oviposition vials					
	Control group			Selected group			Control group			Selected group		
	X	SD	n	X	SD	n	X	SD	n	X	SD	n
0,00	88,85	25,45	55	93,36	31,91	25	99,36	29,38	14	96,65	23,41	17
0,02	85,33	31,57	73	90,61	32,15	31	93,85	26,10	20	99,18	27,98	17
0,04	91,47	27,84	60	89,61	40,25	33	96,45	27,62	22	97,36	31,01	22
0,06	79,23	27,92	62	114,18	29,61	45	96,06	31,06	17	92,86	27,70	21
0,08	67,63	21,24	35	92,88	35,04	25	102,91	24,73	22	110,00	29,64	19
0,10	80,93	22,89	40	98,66	38,38	22	95,61	25,43	18	101,29	25,21	17
0,12	90,67	31,63	18	94,06	20,91	18	80,43	26,80	14	94,69	34,14	16
0,14	90,55	35,60	11	86,00	24,43	3	98,20	29,83	15	113,07	24,27	15
0,16	77,00	-	1	78,60	24,93	5	74,4	29,59	10	87,71	25,21	14
0,18	36,00	-	1	59,00	-	1	95,33	42,58	6	95,14	39,82	7
0,20	56,00	-	1	83,00	-	1	85,67	37,36	6	117,33	25,54	3
0,22		-	-		-	-	51,25	37,99	4	100,50	81,32	2

Note: - = no eggs laid or only 1 egg raft laid but no SD (standard deviation) calculated.

Table A 4.5 Analysis of variance of the number of eggs/raft laid in black petri-dishes by the control and the selected groups.

Source	df	MSS	F. (0,05)
Treatments	11	1481,20	1,61
Groups	1	22107,66	24,07*
Error	553	918,48	

Table A 4.6 Analysis of variance of the numbers of eggs/raft laid in oviposition vials by the control and the selected group of mosquitoes.

Source	df	MSS	F. (0,05)
Treatments	11	1731,21	2,08*
Groups	1	3626,99	4,35*
Error	325	833,03	

Table A 4.7 The percentage of eggs/raft that hatched in the various NaCl solutions on which the control and selected groups of mosquitoes oviposited.

Treatment (NaCl molarity)	Oviposition vials:					
	Control group			Selected group		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n
0,00	94,42	10,02	14	85,71	29,09	17
0,02	88,56	21,90	20	76,00	34,23	17
0,04	80,37	38,07	22	92,05	21,28	22
0,06	95,59	6,58	17	95,86	6,31	21
0,08	94,18	8,42	22	96,85	4,29	19
0,10	96,28	2,87	18	91,48	13,02	17
0,12	82,57	27,74	14	85,27	25,02	16
0,14	83,42	21,38	15	87,89	18,67	15
0,16	50,99	27,92	8	45,81	39,74	14
0,18	0,73	1,80	6	1,76	4,65	7
0,20	0,00	0,00	6	0,00	0,00	3
0,22	0,00	0,00	4	0,00	0,00	2

Table A 4.8 Analysis of variance of the percentage of eggs/raft hatched in the various NaCl solutions in oviposition vials.

Source	df	MSS	F (0,05)
Treatments (NaCl molarities)	11	21032,58	45,47*
Groups	1	30,34	0,07
Error	323	463,17	

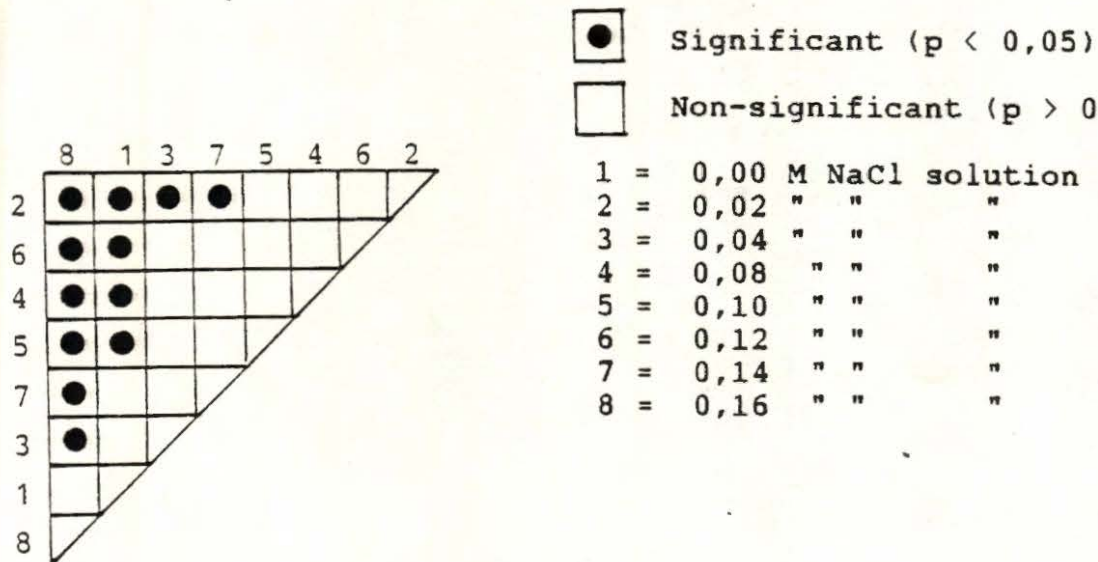
Table A 4.9 The influence of NaCl solutions on the mean total developmental time (days) of immature *Cx. theileri* for the control group (0,02 M) and the selected group (0,12 M).

Treatment NaCl molarity	Control group : (0,02 M)						Selected group : (0,12 M)					
	$\bar{x}$	males SD	n	$\bar{x}$	females SD	n	$\bar{x}$	males SD	n	$\bar{x}$	females SD	n
0,00	11,65	1,10	40	12,80	1,32	30	10,67	0,51	33	11,47	0,96	33
0,02	10,21	1,95	38	11,72	1,26	36	11,28	1,02	32	12,94	1,11	32
0,04	11,34	0,55	29	12,00	0,46	39	10,96	0,96	28	11,69	0,86	36
0,08	10,78	0,80	36	11,82	1,06	34	10,33	0,85	33	11,36	0,65	33
0,10	10,91	0,75	34	12,08	0,81	36	10,74	0,61	35	11,89	0,84	37
0,12	10,63	0,68	19	11,59	1,65	27	10,12	1,02	33	11,85	1,03	40
0,14	11,07	0,83	27	12,24	0,56	33	11,26	0,71	27	12,70	1,16	33
0,16	12,21	0,99	28	13,69	1,16	26	11,16	1,28	25	11,90	1,17	20
$\Sigma$	251			261			246			264		



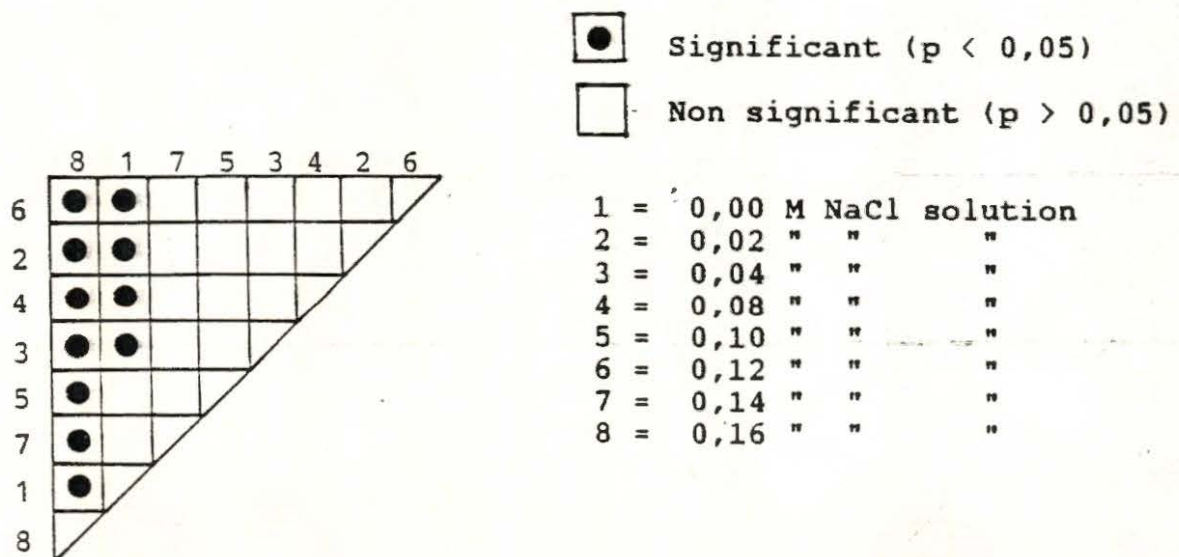
Fig. A 4.1 Tukey's test of significant differences in developmental time at various NaCl molarities for:

Fig. A 4.1.1 Control group males



$$Q_{0,05} = 0,8261$$

Fig. A 4.1.2 Control group females

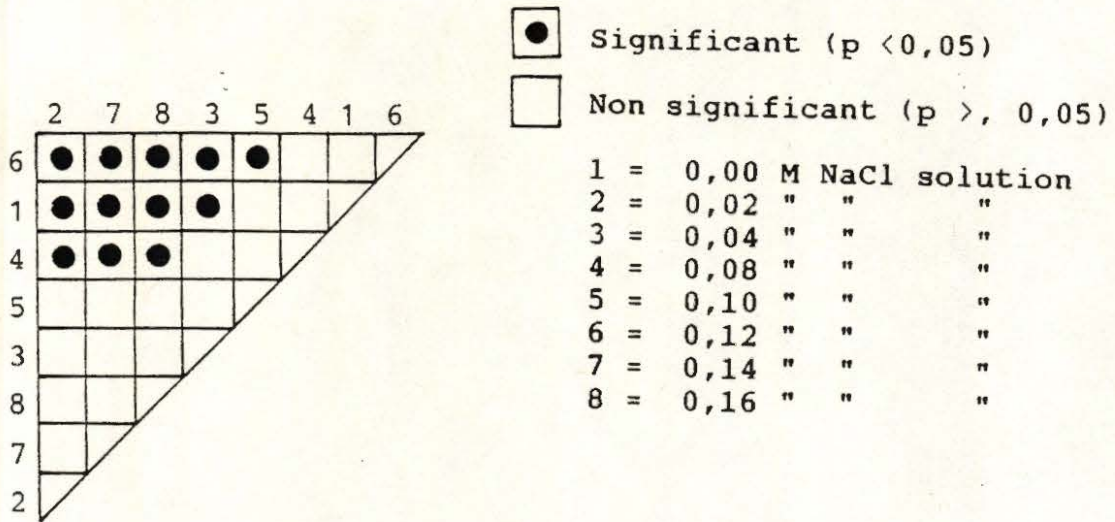


$$Q_{0,05} = 0,7798$$

Table A 4.10 Analysis of variance of the developmental time for *Cx. theileri* adults for both the control and the selected groups.

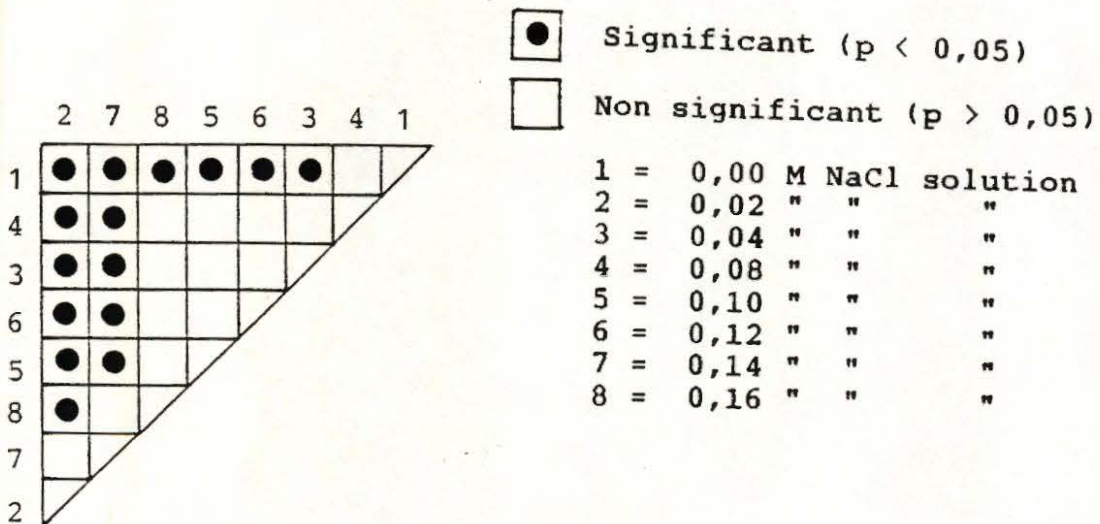
Source	df	MSS	F (0,05)
Treatments (molarities)	7	18,07	15,39*
Groups	1	31,36	26,71*
Generations	1	2,87	2,44
Sex	1	341,40	290,75*
Error	1011	1,17	

Fig. A 4.1.3 Selected group males



$$Q_{0,05} = 0,8186$$

Fig. A 4.1.4 Selected group females



$$Q_{0,05} = 0,8005$$

Table A 4.11 Mortality of *Cx. theileri* larvae expressed as a percentage of those entering each stage.

Control Group Instar	NaCl molarity															
	0,00		0,02		0,04		0,08		0,10		0,12		0,14		0,16	
	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD
1	6,25	2,50	2,50	2,89	3,75	4,79	3,75	2,50	2,50	5,00	16,25	6,29	12,50	6,45	13,75	6,29
2	0,00	0,00	0,00	0,00	0,00	0,00	2,58	2,98	2,50	2,89	6,03	4,84	5,25	10,50	4,10	5,09
3	2,63	5,25	1,25	2,50	4,18	5,35	0,00	0,00	1,33	2,65	4,52	3,02	1,40	2,80	6,00	0,00
4	2,78	5,55	3,98	2,65	6,38	4,92	3,95	5,03	4,10	5,29	11,38	5,86	3,05	3,53	7,48	5,62
p	1,48	2,95	0,00	0,00	1,40	2,80	2,88	3,32	2,65	3,07	13,55	4,61	4,93	3,29	9,90	8,65
Selected Group Instar	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD	$\bar{x}\%$	SD
1	12,50	5,00	6,25	6,29	2,5	2,98	3,75	4,79	2,50	2,89	3,75	4,79	8,75	6,29	10,00	4,08
2	2,97	3,45	1,33	2,65	5,00	7,07	0,00	0,0	0,00	0,00	1,25	2,50	6,63	6,21	2,88	3,32
3	0,00	0,00	2,63	3,04	2,65	3,06	2,65	3,07	1,33	2,65	0,00	0,00	0,00	0,00	10,10	3,11
4	2,78	5,55	1,33	2,65	4,20	2,81	4,05	2,73	5,28	4,54	2,50	5,00	7,40	2,95	14,68	8,76
p	0,00	0,00	9,75	7,03	7,23	2,60	7,08	2,72	1,33	2,65	1,40	2,80	4,83	3,24	14,90	13,67

Table A 4.12 Analysis of variance of the percentage mortality of immature *Cx. theileri* for the control and the selected groups.

Source	df	MSS	F (0,05)
Instars (I)	4	264,13	12,85*
Groups (G)	1	0,08	0,00
NaCl molarity (M)	7	190,51	9,27*
I x G	4	279,04	13,58*
I x M	28	117,67	5,73*
G x M	7	327,94	15,96*
I x G x M	28	30,84	1,50
Error	240	20,55	

Table A 4.13 The influence of various NaCl solutions on head capsule widths of instar four *Cx. theileri* larvae for the control and the selected groups.

Treatment (molarity)	Control group			Selected group		
	x	SD	n	x	SD	n
0,00	1,47	0,06	60	1,49	0,05	60
0,02	1,55	0,04	60	1,51	0,06	60
0,04	1,55	0,03	60	1,51	0,04	60
0,08	1,51	0,05	60	1,52	0,06	60
0,10	1,51	0,04	60	1,51	0,04	60
0,12	1,46	0,05	46	1,47	0,06	60
0,14	1,45	0,04	60	1,45	0,05	59
0,16	1,46	0,05	60	1,47	0,06	45

**Table A 4.14** Analysis of variance of the influence of salinity on the head capsule width in fourth instar *Cx. theileri* larvae of the Control group.

Source	df	MSS	F
Treatments (molarity)	7	0,095714	42,56
Error	458	0,002844	

**Table A 4.15** Analysis of variance of the influence of salinity on head capsule width of fourth instar *Cx. theileri* of the Selected Group.

Source	df	MSS	F
Treatments (molarity)	7	0,031429	12,46
Error	456	0,0025223	

Fig A 4.2 Tukey's test of significant differences between head capsule widths in various sodium chloride solutions (Control group):

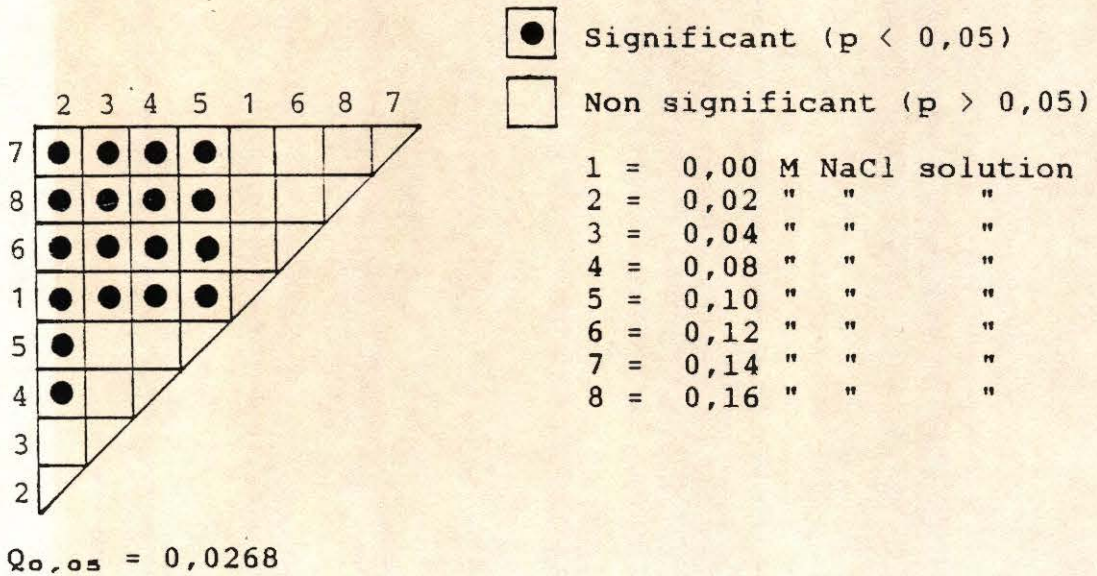


Fig A 4.3 Tukey's test of significant differences between head capsule widths in various sodium chloride solutions (Selected group):

