THE ECOLOGY OF THE KANGAROO TICK *ORNITHODOROS GURNEYI* WARBURTON

A THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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DECLARATION

The work presented in this thesis is my own unless otherwise acknowledged, and has not previously been published or submitted to any university for the award of any degree.

B.M. Doube

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THE ECOLOGY OF ORNITHODOROS GURNEYI

Summary

This study is an attempt to elucidate some of the behavioural and physiological mechanisms which enable the kangaroo tick, Ornithodoros (Pavlovskyella) gurneyi (Warburton), to persist in some of Australia's most inhospitable country. In nature, when the tick is not feeding, it lies buried in the soil at the base of trees where its host, the red kangaroo, Megaleia rufa (Desmarest), is wont to lie in the shade during the heat of a summer's day. The kangaroo scrapes out a "wallow" in which it lies, and because of the kangaroo's movements, the soil in a wallow is usually soft and friable. Into this soil the tick can burrow easily.

The life-cycle stages are eggs, larvae, between three and five nymphal instars and adults. All stages except the egg feed, and females may feed and oviposit as many as six times. Moulting, mating and oviposition all take place in the soil.

Adults and late-instar nymphs remain attached to their host for 20 mins. to 2 hrs before detaching engorged. In contrast, early-instar nymphs may remain attached for several days, and larvae for up to twelve days before engorging and detaching. The timing of detachment in such ticks is regulated by a circadian rhythm within the tick, the phase of which can be set before or during attachment. This rhythm ensures that the engorged ticks detach during the middle

of the photo-phase. Because kangaroos are nocturnal and range widely, feeding at night but resting under shady trees by day, there is a high probability that the engorged ticks will detach while the kangaroo is resting and so will be in a place where the probability of finding another meal is relatively high. Because kangaroos are semi-nomadic, the prolonged period of attachment serves as a dispersal mechanism, possibly the only one.

Morphogenesis begins at temperatures between 15 and 20°C, depending on the instar. The earlier instars have lower developmental thresholds. The temperature thresholds for moulting are several degrees above the developmental threshold for each instar. High temperatures (40°C) inhibit morphogenesis; several days at 45°C is lethal. Observations on the rate of development in the field showed that laboratory data, in conjunction with meteorological records, could be used to predict the amount of development that would occur in the field during a particular period. The amount of development which could occur during each month of an average year was calculated. Most development occurs during the summer months and very little occurs during winter. If food were abundant, then the mean duration of the life-cycle in the field would be between four and ten months, depending on the season of the year. However in nature food is so scarce that the rate of development rarely, if ever, limits the rate of growth of

the population. Humidity had only a slight effect on the rate of morphogenesis.

An examination of the susceptibility to desiccation of the different developmental stages showed that solitary larvae died after only a few days' exposure to high saturation deficit, whereas nymphs and adults were very resistant and could survive at least several months of similar conditions. The longevity of larvae was enhanced when the eggs and subsequent larvae were "brooded" by their mother, but they were still very vulnerable. Larvae can rehydrate, but this ability did not increase their chance of survival. Nymphs and adults, on the other hand are very tolerant of dry conditions. They dehydrate very slowly, can tolerate the loss of a relatively high proportion of their body weight and they can rehydrate by extracting water from unsaturated air. These abilities enable the tick, when occasionally rehydrated, to survive several years without feeding.

Examination of the climatological data for the inland areas studied suggest that if ticks do not find a meal, the larvae will die from desiccation, as will a proportion of the early-instar nymphs, while the late-instar nymphs and adults are so resistant that very few perish in this way. The ticks which survive desiccation may die from starvation over a period of several years.

Most eggs are laid in spring and early summer and few at other times of the year. This was shown to be due to the occurrence of a

diapause in the adult during which oogenesis did not occur. There is a seasonal cycle in the incidence of imaginal disease in which most females are in diapause between mid-summer (December) and mid-winter (July). High temperatures during early summer induce diapause in the late-instar nymphs and in a proportion of the imagos. The factors inducing diapause in the rest of the adult female population are enigmatic. Diapause development occurs at temperatures between 10 and 20°C and in nature, occurs during autumn and winter.

Kangaroos do not begin to spend most of the day in wallows until late spring, and so during early spring ticks are unlikely to find a meal. However, even if a female engorged in early spring, the amount of effective temperature is so small that little oogenesis occurs. In late spring and early summer (before diapause becomes manifest) kangaroos visit wallows more frequently, and temperatures are relatively high. This results in a flush of larvae at those times. Kangaroos continue to visit wallows until early autumn and so the larvae give rise to a flush of first instar nymphs from January to March; these may give rise to second instar nymphs from February to April.

January and February are the hottest and dryest months of the year. Reproductive diapause halts egg production at a time when eggs and larvae are most likely to perish from desiccation and so is of obvious adaptive value.

The density of kangaroos in the area that was studied appears to be about 4 to 8 per sq. mile (1.5 to 3 per square kilometre). During summer kangaroos do not, as a rule, lie under trees which provide sparse shade; those trees providing denser shade were visited, on the average, about once a month during summer.

Whether a population persists in an area will depend on the balance between the ability of the tick to survive protracted periods without feeding and the frequency with which kangaroos visit wallows. The way in which the density of kangaroos and the density of shade-trees affects the probability of survival of a local population is discussed.

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CHAPTER 1

Introduction

The kangaroo tick, Ornithodoros (Pavlovskyella) gurneyi (Warburton), has been recorded throughout much of the inland arid areas of the Australian mainland. The tick was first described from two specimens found by Gurney in 1922 in an old shed at Tibboburra, New South Wales. They were sent to Warburton at the British Museum who, in 1926, published their description but nothing about their biology. Twelve years later some notes were published on the distribution of the tick, its habitat and its hosts, and the reaction of men to its bite (Henry, 1938). Then, for 20 years, the tick went virtually unnoticed, except by stockmen and their dogs! In the mid-1950's further papers were published on its taxonomy (Pospelova-Shtrom, 1950, 1969; Clifford et al., 1964; Hoogstraal et al., 1968; Oliver, 1966) and its biology (Lavoipierre and Riek, 1955; Pope and Carley, 1956; Wilkinson, 1958; Browning, 1962). In addition the tick has been briefly referred to in manuals about parasites (Roberts, 1952; Fielding, 1926) and in a general article on the tick fauna of Australian caves (Kemp, unpublished).

The species of Ornithodoros about which most is known occur in Africa (O. moubata, O. savignyi, O. erraticus), America (O. hermsi, O. turicata), Asia (O. tholozani) and Russia (O. lahorensis). These species, however, have been studied chiefly because of, and with reference to, their medical and veterinary importance. These studies

have centered about the ability of the ticks to act as vectors of rickettsial, viral, bacterial, protozoan and spirochaet diseases of man and other mammals. They have given rise to an extensive literature on morphology, taxonomy, geographical distribution, ecology and biology of Ornithodoros species. Much of this literature has been reviewed by Hoogstraal (1956) and Arthur (1962).

Many of these studies were utilitarian and directed towards solving problems about the epidemiology of disease. Because the information which was necessary to solve these problems was pragmatic, it was not the kind which could, of itself, significantly alter the theories of ecology which had been emerging over the last thirty years. Thus distribution and abundance were often measured but their explanation was only guessed at. The need to measure the microclimate in which the animals lived was seldom recognised and so few experiments were carried out to test the effects of changes in various components of the environment on the individual or the population. For example, O. moubata was said "to occur commonly in the dust of earth floors of thatched houses ... of African natives." (Hoogstraal, 1956). Geigy and Mooser (1955) measured the micro-environment of O. moubata but did not test whether the parameters measured were of significance to the tick. In short most of the work is natural history rather than ecology.

The studies of the sheep tick Ixodes ricinus by Lees (1946) et seq.) and Milne (1950 et seq.) heralded a new approach to tick population dynamics. The experimental physiology of Lees

complemented Milne's ecological data and the integration of their results produced for the first time an overall picture of many of the factors which influence the ecology of a tick population under natural conditions. Their description of a tick population was readily incorporated into at least one system of ecological classification (Andrewartha and Birch, 1954; Browning, 1963). Their approach differs from that of earlier workers in that, as well as measuring abundance and distribution, various specific aspects of the behaviour and physiology of the tick, its hosts and its predators were examined experimentally in the laboratory and in the field, and the influence of these factors on the chances of the tick surviving, reproducing and dispersing was estimated.

Because this approach seemed to be particularly useful for the study of population dynamics of other animals too, I used it when studying O. gurneyi to try to understand the mechanisms which allow this parasite to persist in some of Australia's most inhospitable country. Furthermore it is possible that this study may contribute to an understanding of the general problems of population ecology in arid regions.

From the literature and from hearsay it appeared that O. gurneyi was a relatively scarce desert-dwelling argasid tick whose principal host was a nomadic, nocturnal kangaroo. The tick appeared to be restricted to caves frequented by euros and wallabies and to the soil underneath shady trees in desert areas where trees are sparse and there is a resident, though possibly small, population of

the red kangaroo (*Megaelia rufa*). There have been no reports of parasites or predators on *O. gurneyi*. Hence *prima facie* there were four questions to be answered if we were to understand the ecology of this tick. They were:-

(i) How do ticks locate a host after each moult?

(ii) How do ticks survive the period between meals in the xerotic desert climate?

(iii) How do ticks disperse?

(iv) How are breeding and development regulated?

My first task, then, was to choose a study area where both ticks and kangaroos were present, and then to develop a laboratory culture of the tick. In October 1968, after much searching, I discovered a suitable study area on Moralana Station, 260 miles North of Adelaide. It had a large tick population and, because professional kangaroo-shooting was not permitted, there was a relatively stable population of kangaroos. This area was studied over the next three years. At intervals of about two months I ventured into the field where, in addition to sampling the tick population, I attempted to measure the influence of different components of the tick's environment on its chances of survival and reproduction. The seasonal and daily behaviour of the kangaroo was also studied. Later in the thesis an attempt is made to build a probabilistic model of the relationship between the survival of the ticks where they occur and their chances

of finding a host.

In the laboratory, four specific aspects of the biology of the tick were examined in detail, and where possible, field experiments were carried out.

The four aspects were:-

(i) The factors determining the time when the engorged tick detaches from the host.

(ii) The factors affecting larval and nymphal development.

(iii) The factors determining longevity.

(iv) The factors regulating imaginal diapause.

Distribution, Hosts and Taxonomy

O. gurneyi was said to occur in the soil underneath bushes, trees, etc., in "kangaroo camps" and in "wallaby caves", throughout inland New South Wales and Queensland (Henry, 1938). Browning (1962) noted that the tick was found under shade trees in open plains but not in adjacent woodlands, even though the red kangaroo was present in both areas; and he suggested a behavioural explanation for this distribution. The species has also been taken from rabbit burrows near Malki, near Rockhampton, Queensland (Wilkinson, 1958) and from an echidna (Tachyglossus aceulatus) on Mileura sheep station, Western Australia (Kaiser, personal communication). Browning (1962) and D.C. Swan (Waite Tick collection) have collected the tick from many inland areas of South Australia. I have found it in caves in hills in the Northern Territory.

The tick can feed on man, horses, cattle, dogs, laboratory rats and mice, and has even been taken from the bearded dragon, Amphibolurus barbatus (Smyth, personal communication), but its principal hosts in nature are the red kangaroo, Megaleia rufa (Henry, 1938; Browning, 1962) and the euro, Macropus robustus. Browning (1962) maintained and fed the tick in the laboratory and states that "when fed on rats' blood, adults and nymphs feed fairly rapidly, many completing their feed within two hours, but larvae require three days, on average, to complete their meal." I have shown that man, dogs, rats, rabbits and kangaroos are suitable hosts.

Lavoipierre and Riek (1955) divided the species of Ornithodoros into three groups according to the rate of feeding and the macroscopic and microscopic lesions which they produced in the skin. O. gurneyi was placed in the O. moubata group. The characteristics of individuals of this group are (i) they are slower to attach themselves to their hosts than are those of the O. erraticus group and they usually require up to $1\frac{1}{2}$ hours to engorge completely (cf. 20-30 mins for the O. erraticus group); (ii) they produce a considerable superficial haemorrhage which infiltrates the dermal tissue. The degree of haemorrhage is intermediate between the vicious bite of members of the O. erraticus group, and the mild effect of the third group, the O. savignyi group.

The effect of the bite of O. gurneyi on man is difficult to

assess, for, although it usually induces only a persistent localized irritation, there are cases of the bite inducing vomiting, temporary blindness or "blackout" from which most recipients recover one or two hours after having been bitten (Henry, 1938), although some have been confined to bed for several days. The usual rapid recovery and the observation that the sensitivity to the bite increases with each attack suggests that the bite may induce a severe allergic response - anaphylactic shock.

Pope and Carley (1956) mentioned O. gurneyi as a possible vector of the spirochaete Borrelia in native rodents, and Roberts (1962) stated that the tick could transmit Q-fever (Coxiella burnetti) between laboratory rats.

Pospelova-Shtrom (1950) revised the systematics of the genus Ornithodoros and placed O. gurneyi in the subgenus Pavlovskyella whose type species is O. tholozani. There is, however, little in common between this grouping of species and that produced by Lavoipierre and Riek (1955). Subsequent taxonomists (Clifford et al., 1964) have generally confirmed the classification of Pospelova-Shtrom. Oliver (1966) studied the cystogenetics of two Australian argasids, O. gurneyi and O. macmillani and found them to have chromosome numbers of twelve and sixteen respectively, twelve being the lowest number reported for any tick. This discovery puts O. gurneyi in a special position in the subgenus Pavlovskyella, for

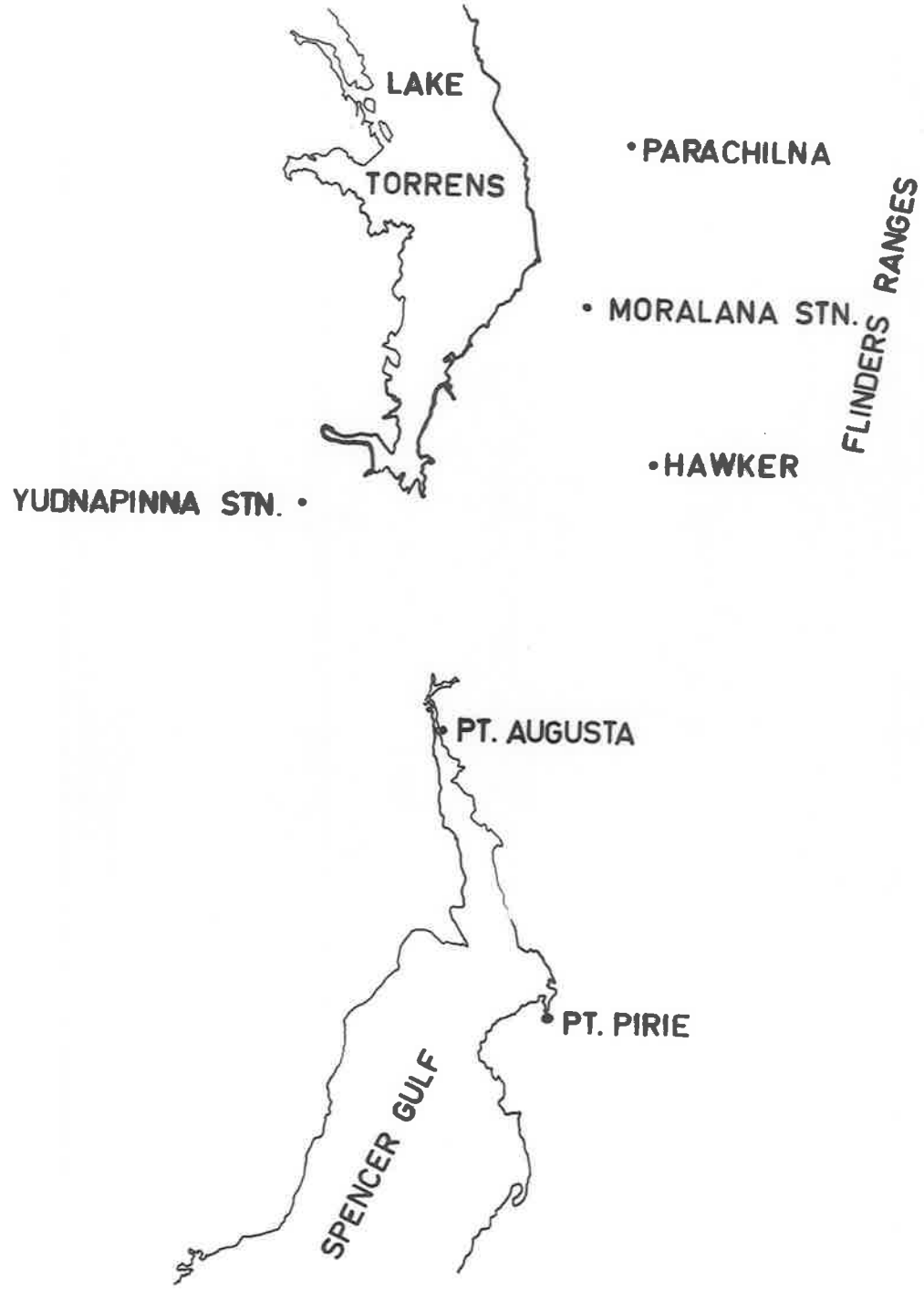
all but one other of the species in this subgenus which have been studied have had a chromosome number of sixteen; the other had a number of 32 and may have been tetraploid. Kohls (Oliver, 1966) believes that O. gurneyi differs markedly in appearance from the other members of the subgenus. Binnington (personal communication) has found rudimentary "eyes" in O. gurneyi, which had previously been considered eyeless. Thus the taxonomic status of O. gurneyi, at the subgeneric level, is somewhat uncertain. Hoogstraal (1968) has published a key to the Australian argasid fauna, including O. gurneyi.

There are few argasid species native to Australia and O. gurneyi and O. macmillani are the only two known Ornithodoros species that are restricted to the Australian mainland. O. macmillani was described in 1966 by Hoogstraal and Kohls but to date little is known of its biology.

The Study Area

The study area lies in a 50 square mile paddock on Moralana station (30 miles north of Hawker, South Australia) which is part of a belt of land, about 30 miles wide, known as the "sandy country". This land lies between the Flinders Ranges to the east, and Lake Torrens (a dry salt pan) to the west (Figure 1.1). The sandy country is known as some of the most harsh grazing country in Australia because, although in good seasons grazing is plentiful, in harsh dry seasons the combined pressure of scorching sun, strong winds and continued grazing

Figure 1.1 The mid-north of South Australia showing the areas in which the field work was carried out.


LAKE
TORRENS
PARACHILNA
FLINDERS RANGES
MORALANA STN.
HAWKER
YUDNAPINNA STN.
PT. AUGUSTA
PT. PIRIE
SPENCER GULF

causes the country to degenerate rapidly into a series of drifting sand-dunes. In fact, during a severe sand storm in January 1969, parts of Moralana were pointed out to me through the window of the Hawker Public House.

The sand-dunes run east-west between the mountains and the lake and are moderately stable close to the mountains, becoming more unstable towards the lake until they peter-out about 4 miles from its edge. The study area lay about half-way between the mountains and the lake and consisted of about one square mile between two relatively permanent sand-dunes, the peaks of which ran parallel about $1\frac{1}{2}$ miles apart.

The sand-dunes were covered with small shrubs (Chenopods and Acacias) and there were frequent patches of black oaks (Casuarina) on top of the dunes. The slopes of the dunes were scattered with shrubs and bullock bushes (Heterodendrum) and on the plains between the dunes were scattered bullock bushes, mulgas (Acacia aneura), a species of Hakea, various Acacia bushes and assorted shrubs and grasses. Another feature was occasional dry mud-flats which were found in the lowest areas between the dunes. These were invariably covered with prickly Acacia, Acacia victoriae. Wallows were found under most trees but those under large trees with dense canopies appeared to be preferred by the kangaroos.

There was no known source of permanent water for ten miles in any direction and yet on every sampling trip I saw kangaroos on or near the study area.

CHAPTER 2

2.1 The Life History of the Tick

The stages of the life-cycle of the tick consist of eggs, larvae, between three and five nymphal stages, and the adult males and females. Mating and oviposition take place in the sand, and all stages live there, except when feeding. Larvae and nymphs usually engorge once before moulting to the next stage. Usually each stage attaches only once, but if ticks are disturbed while feeding and detach, they will reattach and continue feeding until fully engorged.

The duration of the life-cycle will have a profound effect on the biology of the tick in nature because, with fecundity, it determines the rate of increase of the species. The factors determining the duration of the life-cycle of an Argaria, such as O. gurneyi, are:-

(i) The period spent attached to the host.

(ii) The number of developmental stages.

(iii) The time required to complete each developmental stage and be ready to feed again.

(iv) The interval between being ready to feed and feeding.

The first two factors are largely determined by the nature of the tick, but (iii) and (iv) are functions of the environment of the tick as well. Factor (iv) is a function of the behaviour of the

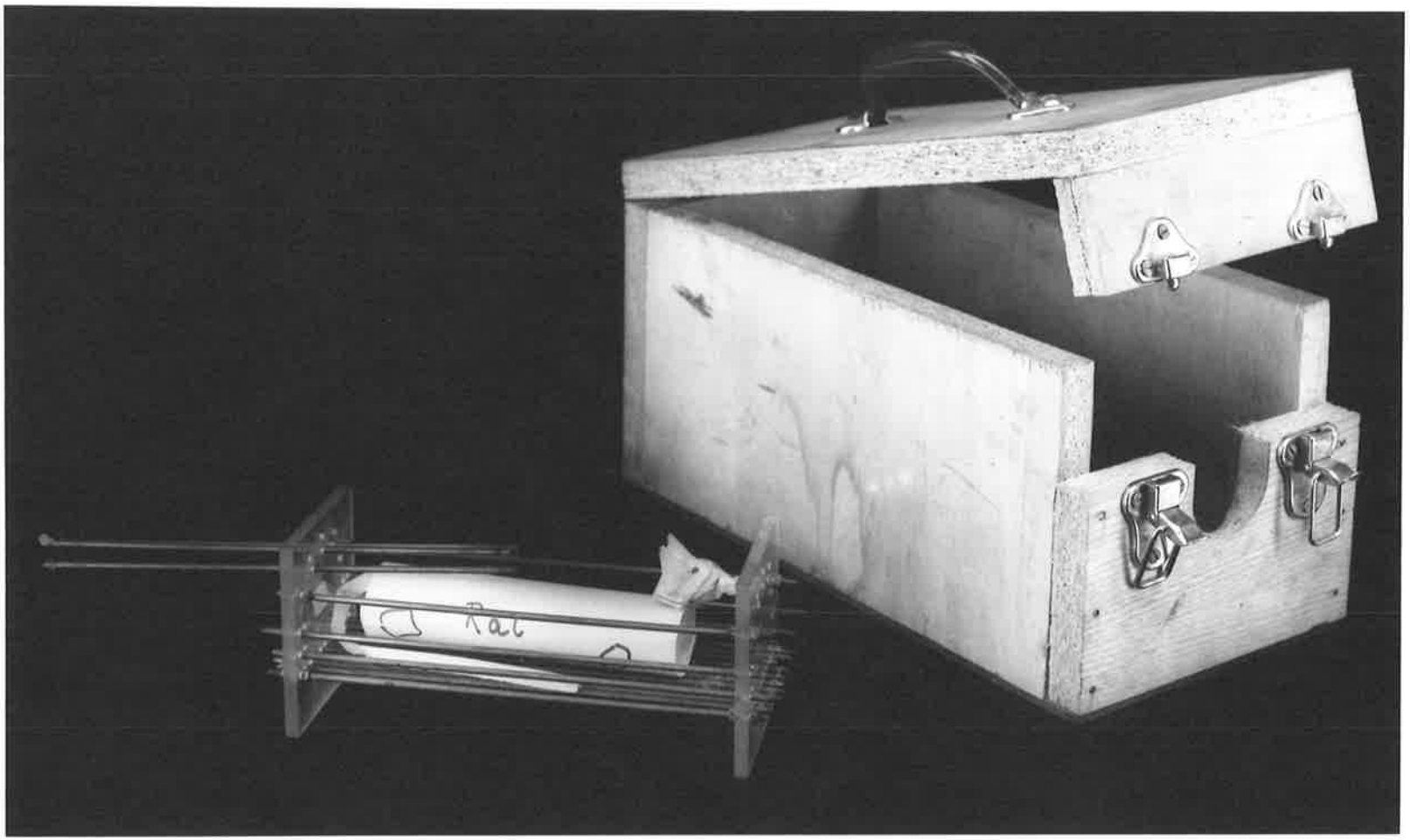
host, while (iii) is affected significantly by many factors principal amongst which are likely to be temperature, humidity, quality of food, and possibly photoperiod. However, before examining the reaction of the tick to these factors, I shall discuss some aspects of the feeding and breeding of the tick in the laboratory.

2.2 Laboratory Breeding and Feeding Methods

The animals used in the laboratory as hosts to the tick were virgin female white rats and 'New Zealand White' rabbits. Each animal was used only two or three times because on each successive occasion the proportion of ticks that attached and engorged decreased; this was thought to be a form of immunity. This type of interaction has been noted when other species of tick have fed on laboratory hosts (Traeger, 1939; Arthur and Snow, 1966). Larvae and the first and second instar nymphs were fed on rats and all other stages were fed on rabbits.

The rats were confined in cages which were placed over trays into which the engorged ticks fell when they detached. These cages, made of brass rods and perspex (see Figure 2.1), allowed the ticks to drop from their hosts while restraining the rats and preventing them biting or scratching themselves. Very few ticks survived to detach when the rats were not so constrained because the rats removed most by preening. The rats were fed and watered during the night. When ticks were to be fed, the rats were confined in their cages, allowed to calm

Figure 2.1 Cages in which Hosts were Confined while ticks fed.


Rat

down for an hour or so, and then the larvae or nymphs were poured onto the backs of the rats. Most of those that were to attach had done so within 20 mins.

The rabbits were confined in wooden cages (Figure 2.1) with a guillotine arrangement so that the head protruded. Ticks were placed in bags made of paper towelling which were placed over the ear and secured with sticky-tape. When the ticks had engorged, usually only two to three hours later, the towelling bags were removed and the rabbit released.

Engorged juvenile ticks were sorted, placed in petri dishes 6 cm in diameter and 1 cm deep and covered with fine dry sand. These dishes were then placed in constant temperature cabinets. Engorged females were placed in tubes with males, covered with sand and placed in constant temperature cabinets, the relative humidity of which fluctuated between 10 and 30%. They were examined at intervals and the day of moulting and the day when oviposition began were noted.

2.3 Feeding

i) The Readiness of Ticks to Feed

Usually *O. gurneyi* will not attach and feed until a few days after moulting.

The interval of fasting between emergence and feeding in three-host ixodid ticks has been referred to as "hardening-off"

(Greegson, 1966). Loomis (1961) reported that for the cuticle of Amblyomma americanum to harden after moulting, 4-7 days was required by larvae, 4-6 days by nymphs and 3-6 days by adults. Gladney et al. (1970) confirmed this, but they found that the imagos needed 7-8 days for hardening. These parameters have been estimated for four other ixodid species (Gladney et al., 1970). The period is characterized by hardening and darkening of the cuticle and possibly the mouthparts, preparatory to feeding, and by excretion of waste material. To my knowledge, Ornithodoros grangeri is the only Argasid in which this process has been reported: 2-3 days were required for hardening.

An experiment on the rate of development (Chapter 4) also provided data which allowed me to assess the readiness of O. gurneyi to feed at different intervals after moulting. A group of third instar nymphs was fed and placed at 30°C (40-50% R.H.) where they began to moult to fourth instar nymphs or adults ten days later. The ticks were examined every two days and those which had moulted during each interval were separated into nymphs and adults and kept under the same conditions. Nineteen days after moulting all the ticks which had moulted were given an opportunity to feed. The proportion in each group which engorged is shown in Table 2.1.

It is clear that most newly-moulted fourth instar nymphs and adults do not attach and engorge for at least the first three days

Table 2.1

The proportion of *O. gurneyi* that fed at different intervals after moulting

Days after moulting at 30°C	Females		Fourth nymphs	
	Percent feeding	Sample size	Percent feeding	Sample size
< 1	0	10	0	24
1, 2	8	12	19	43
3, 4	92	12	77	44
5, 6	83	12	79	67
7, 8	73	15	79	134
9-12	70	27	62	135
13-16	90	52	79	124

after moulting. Similar observations have been made on the first and second instar nymphs and larvae of O. gurneyi and this hardening period, at 30°C, is about four days.

It is also noteworthy that in no instance did all the ticks of a sample feed. Even amongst those ticks which were given several opportunities to feed, there remained a proportion (usually at least 10%) which did not feed. The explanation of this may lie in the nature of the tick (i.e. it cannot feed at that time) or in the interaction between the tick and the host (some form of immunity). This phenomenon has been reported for Dermacentor variabilis (Sonenshine and Atwood, 1967) where 80-90% usually fed, and for Amblylomma americanum (Gladney et al., 1970) where 60-90% usually fed.

ii) Duration of Attachment and Feeding Periods

The larvae of Argasids usually require longer periods on the host than do the corresponding nymphs and adults. Thus the larvae of Argas persicus and A. replexus feed for 5-10 days, A. brumpti for 6-15 days, A. boutei for 16-25 days (Arthur, 1962), A. brevipes for 6-11 days (Clifford and Kohls, 1963), Ornithodoros grangeri for 1-2 days (Heisch and Harvey, 1953), O. kellyi for 9-20 days and O. delanoiacinus for 7-14 days (Soneshine and Anaston, 1960). Nymphs and adults of all these ticks, except Argas brumpti, engorge and detach within 6 and 60 mins of attaching to their host. An exception is the nymphs and adults of A. brumpti which take 90 and 18-21 days

respectively before detaching, engorged.

Laboratory studies indicate that, in nature, O. gurneyi detaches only during the day (Chapter 3). Larvae remain attached for between 3 and 12 days (mean, 5.4) before engorging and detaching, whereas nymphs and adults remain attached for a much shorter period (see Table 2.2). In this O. gurneyi is similar to other species of Ornithodoros which have been studied. The amount of 'blood' ingested by nymphal and adult soft ticks is usually three or four times their weight before feeding, whereas in ixodid ticks this factor may vary from 23 times to as much as 150 times (Arthur, 1962). This difference is due to the different processes which occur during attachment. In ixodids there is a synthesis of new cuticle during attachment while in Argasids the cuticle of nymphs and adults is formed before attachment and when feeding begins the body merely distends passively (stretches) (Lees, 1952). On the other hand the way in which larval Argasids feed is apparently similar to that of ixodids. Lees (1952) has demonstrated that in the larvae of Ornithodoros delanoi acinus there is an increase in the dry weight of the cuticle from 0.04 to 0.20 mg (a factor of five) and in live weight from 0.24 to 4.9 mg (a factor of 20). Thus it appears that the magnitude of the increase in live weight during feeding is an indication of whether or not new cuticle is being synthesized. Observations on A. brumpti suggest that this gradual process of cuticle formation during feeding may occur in all developmental stages.

Table 2.2

Percentage of total detaching during each day after attachment

Days after attachment	Larvae	Stage of development * 1NN	2NN	3NN	4NN	5NN	Adults
1	0	47.5	50.2	91.3	100	100	100
2	0	41.2	46.0	8.7			
3	4.2	8.5	3.8				
4	20.8	2.1					
5	35.5						
6	24.3						
7	7.6						
8	2.8						
9	2.0						
10	1.4						
11	1.1						
12	0.1						
Sample size	830	1080	240	1170	500	40	500

* Throughout this thesis the abbreviations 1NN to 5NN will be used for the first to the fifth nymphal instar.

Although I have not estimated the changes in weight of the cuticle of different stages of O. gurneyi during feeding, I have noted the change in live weight (Table 2.3). The weight of larvae increases thirteen fold during feeding whereas the weight of nymphs and adults increases only three to five times. This suggests that new cuticle is being synthesized during larval feeding but that when the nymphs and adults feed, the cuticle merely stretches. The possibility that cuticle was synthesized by those first and second instar nymphs which did not detach on day one (see Table 2.2) was checked by comparing the average engorged weight of ticks that detached on day one with those detaching on subsequent days. There was no significant difference (see Table 2.3).

It is noteworthy that those larvae or nymphs which are to detach on a particular day do not begin the final rapid feeding until the morning of that day; those that are not going to drop off on that day remain attached but only slightly engorged. This behaviour is similar to that found in ixodids. Ixodids feed and grow during the whole period of attachment, although the rate of engorgement increases dramatically on the last day (Wharton and Uteck, 1970).

2.4 Hatching and Moulting

Under laboratory conditions 90-95% of all eggs laid were fertile and hatched if stored in favourable conditions (Chapter 5). Many of the resulting hexapod larvae fed when given the opportunity. When

Table 2.3

The mean weight (mg) of different stages of *O. gurneyi*: S.D. in brackets

Stage		Unengorged	Engorged	Ratio $\frac{\text{Engorged}}{\text{Unengorged}}$
Eggs		1.22		
Larvae		0.89	11.7(1.3)	13 : 1
1NN	Day 1	8.28(2.12)	44.8(3.2)	5.4 : 1
	Days 2 & 3		42.1(3.5)	5.1 : 1
2NN	Day 1	21.74(5.95)	115.8(5.1)	5.3 : 1
	Days 2 & 3		119.9(4.9)	5.5 : 1
Females		752.2(216.2)	2276.1(26.6)	3 : 1
Males		213.9(53.8)	676.2(9.1)	3 : 1

the engorged tick detached it searched until it found soft soil, into which it burrowed, or litter, under which it hid. It remained in this situation, unless disturbed, until it moulted. Engorged nymphs behaved similarly.

Except in extreme circumstances, there is very little mortality during hatching and moulting; most of the eggs laid hatched into larvae and most engorged juveniles moulted into the next stage. There were three main causes of mortality in the laboratory:-

(i) death before feeding,

(ii) failure to feed when offered a host and subsequent starvation, and

(iii) failure to engorge when attached to a host.

These factors are discussed later in Chapter 4.

Ticks moult by splitting the exocuticle in a line around the margin of the body. The split begins at the anterior end of the tick and continues around the margin of the body but leaving the dorsal and ventral cuticle joined only at the posterior end. Once the split is complete the tick is able to walk out of the exuvia. At 30°C the moulting process takes about one day from the first split to freedom.

All larval and nymphal stages of O. gurneyi must feed before they can moult. This pattern is consistent with other Argasid species, e.g. Argas persicus, but other patterns also occur. For example, O. moubata and O. savignyi both have a non-feeding larval stage

(Hoogstraal, 1956) whereas O. kellyi and at least six other ornithodorid species have a first nymphal instar which does not feed but moults directly into the second nymphal instar. Other Argasids may sometimes feed several times before moulting, e.g. Argas persicus (Roberts, 1952), O. savignyi (Nevill, personal communication).

In O. gurneyi, as in other Argasids, there is a variable number of nymphal instars. Table 2.4 illustrates the proportion of nymphs, males and females, which each instar gives rise to. There can be three, four or five nymphal instars and there is a definite tendency for males to mature earlier.

Similar behaviour has been observed in other Argasids. For example, O. kellyi has two, three or four nymphal instars. The second instar gives rise to males and nymphs, the third to males, females and nymphs, and the fourth instar gives rise to females alone (Sonenshine and Anastos, 1960). Similar, but not identical behaviour has been reported for Argas brevipes (Clifford and Kohls, 1963). The ratio of males to females in this sample of kangaroo ticks was 4:6.

2.5 Mating and Oviposition

Mating may occur before or after the female has engorged and females may mate many times. Although one mating has been shown to provide sufficient sperm to fertilize at least three egg batches it was usual for females in the laboratory culture to mate several times.

Table 2.4

The proportions of nymphs and adults arising after the moult of each nymphal instar (sample size = 3,200 ticks)

Nymphal instar	Nymphs		Males	Females
Engorged second instar nymphs moult to	100%	3NN		
" third " " " "	70%	4NN	23%	7%
" fourth " " " "	14%	5NN	23%	63%
" fifth " " " "	0%		0%	100%

The number of times that mating had occurred was assessed by dissecting the females and counting the number of sperm capsules in each branch of the uterus. During each mating there are two capsules deposited, one in each branch of the uterus; similar processes have been found in other Ornithodorid species (Arthur, 1962).

Males were capable of mating soon after moulting and before they had fed.

Females have not been observed to oviposit without feeding as has been reported for other Argasid ticks, e.g. O. moubata (Hoogstraal, 1956) and Otobius megnini (Cooley and Kohls, 1944). However, parthenogenesis has been reported in several species of tick, e.g O. gurneyi females must be fertilized before they will oviposit. This was tested by taking engorged virgin females and placing some with males and leaving others isolated. All those females placed with males laid eggs but none without males had laid eggs within 90 days after feeding. They were dissected and oogenesis had not begun. Hence parthenogenesis is unlikely to occur in the kangaroo tick.

When an engorged female detaches from the host she searches for soft sand into which she burrows. If she has mated and is not in diapause (see Chapter 6) she should lay a batch of eggs within the next 40 days. Oviposition involves extruding an egg from the vulva, seizing it in the mouthparts and covering it with a waxy secretion from Gene's organ before depositing it onto the egg batch below the female.

Frequently the female 'broods' the egg mass and the subsequent larvae for many days. Often larvae remain in a tangled mass beneath their dam [Mother (usu. of beast*), Concise Oxford, 1946] until she is disturbed and even then they cling to her, hiding in the crevices of her wrinkled body. The implications of this as a survival mechanism are discussed later, in Chapter 5.

The factors influencing the rate of oogenesis and egg development are treated in Chapter 4, and the reproductive diapause is discussed in Chapter 6.

This chapter has examined the life history of the tick and has briefly discussed the feeding and moulting behaviour. The next two chapters examine the latter two topics in greater detail. Chapter 3 examines the effect of photoperiod on the feeding and detaching behaviour of larvae and nymphs, while Chapter 4 examines the effect of temperature (and other variables) on the moulting behaviour of engorged ticks.

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* beast: fearsome creature common to fairy tales.

CHAPTER 3

A Circadian Rhythm of Feeding and Detaching

3.1 Introduction

The feeding behaviour of many Arthropods is regulated by circadian rhythms; the phase of which is set by the incident photoperiod, e.g. the biting cycle of many species of blood-sucking flies (Corbet, 1960). Similar phenomena have been observed in several species of tick.

The feeding process of ticks can be divided into two distinct stages; during the first stage the tick feeds only slightly, during the second stage it engorges fully before detaching. The first stage may take many days but the second stage takes a maximum of a few hours (Arthur, 1962, p. 136; Wharton and Uteck, 1970). In ticks the circadian rhythm is manifest in the second stage, i.e. as a rhythm of detachment of engorged ticks preceded by a period of rapid feeding. This behaviour contrasts with other blood-sucking Arthropods (e.g. flies) in which the circadian rhythm is manifest as host-searching behaviour.

Balashov (1954) demonstrated daily feeding and detachment cycles in the tick, Ixodes persulcatus, when it was feeding on cattle. Hitchcock (1955) demonstrates similar behaviour in Boophilus microplus. Similarly Arthur (1962) stated that females, nymphs and larvae of Ixodes hexagonus drop off during daylight in the nests of hedgehogs. George (1964, 1965) studied the feeding behaviour of the rabbit tick,

Haemaphysalis laporipulustris. He found that the timing of detachment was regulated by two main factors. One was a response to a circadian rhythm within the host rabbit. The phase of this rhythm was set by the time at which the rabbit was fed and the incident photoperiod. The other factor was a circadian rhythm within the tick the phase of which was set by the incident photoperiod.

The feeding behaviour of the different developmental stages of O. gurneyi has already been discussed (Table 2.2). Usually adults and late-instar nymphs stay attached to the host for less than three hours, whereas other stages may take considerably longer. Most of the second instar nymphs drop off, engorged, within four hours of attaching to the host; there are some, however, which do not drop off until the afternoon of the following day. Similarly, about 50% of the first instar nymphs drop off, engorged, on the afternoon of the first day while the remainder drop off during the afternoons of the following two or three days. Engorged larvae, however, drop from their host during the afternoons of the third to the twelfth day after attachment.

In a preliminary experiment, the feeding and detaching behaviour of larvae was examined. A group of 200 larvae was placed on the backs of two caged rats on the laboratory bench. The laboratory temperature fluctuated between 24 and 30°C and there was no artificial lighting. Seventy five percent of the larvae were recovered engorged and nearly all fell off around midday of the third, fourth and fifth days after the

larvae attached to their host; very few fell off during the night.

The most likely factors controlling this periodic behaviour are:

(i) a direct response to light,

(ii) a direct response to temperature fluctuations,

(iii) a circadian rhythm within the tick, or

(iv) a circadian rhythm within the rat to which the tick responds.

3.2 Materials and Methods

Larvae and nymphs were bred in the laboratory from eggs laid by females captured in the field. The breeding females were kept at 30°C at 75% R.H. in continuous light. The resulting larvae experienced identical conditions until the experiments began.

The hosts were all virgin female white rats about three months old. The running activity of individual rats was determined by placing the rat in a light-tight cabinet with internal lighting and containing a running wheel connected to an activity recorder (Figure 3.1A). The frequency of use of the activity wheel was recorded on a chart; the timing of every cycle of the wheel was noted.

Rats are nocturnal and so their period of activity occurs during the dark phase (scotophase) of the photoperiodic cycle. Thus the

Figure 3.1A Activity cabinets in which circadian rhythms within rats were entrained so that the rhythms were in phase with the light regime within the cabinet.

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trace is flat during the photophase and jagged during the scotophase. It is assumed that any rhythm within the rat to which the tick may be responding would bear, in a normal situation, a constant relation to the phase of the activity rhythm. The phase of the running activity of the rat was therefore taken as an indication of the phase of the physiological rhythm(s) within the rat. Thus the running activity of the rat was taken as the indicator of the effect of changing the components of the environment on the circadian rhythm(s) within the rat.

When a fresh rat is put into a cabinet in which the photoperiodic regime is set 12 hours out of phase with that regime to which the rat has been accustomed, the rat will run spasmodically throughout the light and the dark phases for the first few cycles. Shortly, however, it will adapt to the new light regime and, within seven or eight days, will again be running only during the scotophase. Thus the phase of the circadian rhythm(s) within the rat can be set at will.

Previous experiments had indicated that feeding by the ticks may make the rats poor hosts by inducing some form of immunity, and so new rats were used in each experiment.

During the following experiments the rats were confined in cages of such dimensions that the rats could revolve but could not scratch or lick themselves. Each cage consisted of twenty rods of

$\frac{1}{8}$ inch brass, six inches long, threaded through two 4x4 inch ends of $\frac{1}{4}$ inch perspex (Figure 2.1). When the rats were not confined in this way, they would scratch and bite, killing most of the ticks placed on them.

To begin an experiment each rat (whose rhythm had been set and checked in an activity cage) was confined in a feeding cage and, when the rat had become calm, the ticks were placed on its back. Most ticks would search for a short time (less than 10 mins) and then burrow into the fur to the skin and vanish from sight. Usually, within half an hour, very few ticks were visible on the fur of the rat. The confined rat, with ticks attached, was then placed in a tray out of which engorged ticks could not crawl, and the rat and tray were then placed in a cabinet at 30°C ($\pm$ 0.5°C) with a regulated photoperiod. The rats were provided with food and water. The light regime inside the cabinet was set using a Venner time switch.

Thus the variables which may influence the dropping rhythm of the ticks can be regulated. The temperature was kept constant at 30 $\pm$ 0.5°C and the photoperiod experienced by ticks and rats could be regulated.

3.3 The Rhythm of Detachment of Engorged Larvae

Experiment 3.31 NORMAL and INVERTED photoperiodic regimes

Larval ticks were hatched and stored in constant light at 30°C,

75% R.H. It was presumed that these larvae were arythmic. Two rats from the same litter were kept for ten days prior to the experiment, one in each of two activity cages. The light regime in Cage A was 12 hours out of phase with that in Cage B, and each day consisted of 16 hours light and 8 hours of darkness (L16 D8).

Cage A. Noon $\pm$ 8 hours light - NORMAL (N)

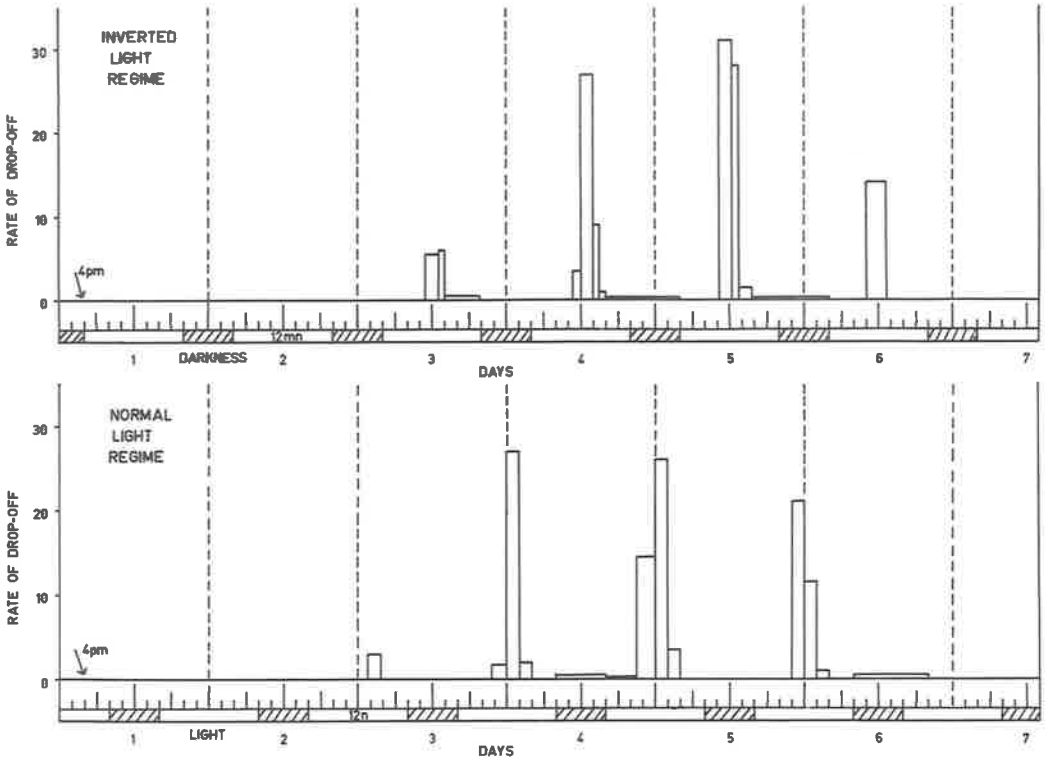
Cage B. Mid-night $\pm$ 4 hours light - INVERTED (I)

After ten days both rats were running in the scotophase of their respective incident photoperiods. Two constant temperature cabinets were set at 30°C with L16 D8, each cabinet 12 hours out of phase with the other.

At 4 p.m. on the tenth day after the rats had been placed in the activity cabinets, they were removed, confined, and 300 larvae were placed on the back of each. When the larvae had disappeared into the fur of their hosts, each rat was placed in the constant temperature cabinet in which the photoperiodic regime was in phase with that which it had experienced during the previous ten days. The trays, in which the caged rats stood, were searched at intervals and the engorged ticks, which had dropped from the host, were counted, removed and stored in soft sand.

The data was expressed as a rate of "drop-off" per hour and were plotted as a histogram (Figure 3.1B). The data for each day were pooled within each treatment and the treatments were compared.

Figure 3.1B The Pattern of Drop-off of Engorged Larvae of the Plains Variety in NORMAL and INVERTED light regimes.


INVERTED
LIGHT
REGIME
RATE OF DROP-OFF
30
20
10
0
4pm
12mn
1
DARKNESS
2
3
4
5
6
7
DAYS
NORMAL
LIGHT
REGIME
RATE OF DROP-OFF
30
20
10
0
4pm
12n
1
LIGHT
2
3
4
5
6
7
DAYS

From Table 3.1 and Figure 3.1B it is clear that ticks on both NORMAL and INVERTED regimes detached during the photophase. Furthermore there was a pronounced peak rate of detachment in the centre of the photophase. Since the drop off responses occur during the photophase and the responses are of equal proportions in each treatment, we can conclude that it is extremely unlikely that any influence from outside the cabinets was determining the time when the engorged ticks would leave their host.

The following experiment was designed to determine whether this drop-off behaviour was merely a direct response to light or whether it was controlled by a circadian rhythm. It was also designed to separate the effect of the rhythm within the tick from a response by the tick to a physiological rhythm within the host rat, if either rhythm existed.

Experiment 3.32 Drop-off in continuous darkness

Two groups of larvae from eggs laid, stored and hatched in continuous light, were subjected to L16 D8, one group 12 hours out of phase with the other. The light periods were centred around noon (N') and midnight (MN). The ticks were kept in glass tubes in this regime at 30°C and 75% R.H. for ten days, after which they were placed on the rats at 4 p.m. (day 0). The host rats had also been subjected to either a NORMAL or INVERTED LD regime for the previous ten days. Thus there were both NORMAL and INVERTED rats and ticks. Each group of ticks

Table 3.1

Detachment behaviour in NORMAL and INVERTED light regimes

Treatments	A		B	
	16L 8D Noon ± 8 hrs light NORMAL		16L 8D Noon ± 4 hrs darkness INVERTED	
Rats	NORMAL		INVERTED	
Ticks	Arhythmic		Arhythmic	
No. of ticks on	300		300	
Total off engorged	254		238	
% recovery	85		80	
	Between 1000 & 1600 6 hours	Between 1600 & 1000 18 hours	Between 2200 & 0400 6 hours	Between 0400 & 2200 18 hours
No. ticks off	242	12	228	10
% off in each period	95	5	96	4

was divided in half, one half being put onto a NORMAL rat and the other half onto an INVERTED rat. When the larvae had attached to their host, the confined rats were placed on individual trays, with a supply of food and water, and all four were put into one constant temperature cabinet set at 30°C. The lights inside the cabinet were then turned off.

Experimental Design

Treatment	1	2	3	4
No. rats	1	1	1	1
No. ticks ON	400	400	400	400
Photoperiod history				
Rats	N	N	I	I
Ticks	N	I	N	I

WHERE

I = Inverted = MN ± 8 hours light

N = Normal = N' ± 8 hours light

After two days the trays were examined regularly for engorged larvae. To do this, the room containing the cabinets was completely darkened; the cabinet was then opened and the trays on which the caged

rats stood were removed with the engorged ticks in them, and replaced with fresh trays. Since Arthropods, generally, are insensitive to red light (Danilevskii, 1965), a torch emitting red light was used while the trays were being changed. The cabinet was then closed and the ticks counted and stored.

From the previous experiment it appears that the significant periods during which ticks would be likely to drop off their host (if a rhythm was responsible) would be between 10 a.m. and 4 p.m. and between 10 p.m. and 4 a.m. To reduce the risk of even a red light interfering with the reactions of ticks and hosts, it was decided to sample only at 10 a.m. and 4 p.m. daily. This divided the day into two comparable periods, one 6 hours (10 a.m. to 4 p.m.), the other 18 hours (4 p.m. to 10 a.m.), but each containing one of the critical 6 hour periods (Table 3.2). Engorged ticks began falling off after 10 a.m. of the second day and had stopped by day seven (Figure 3.2).

Discussion

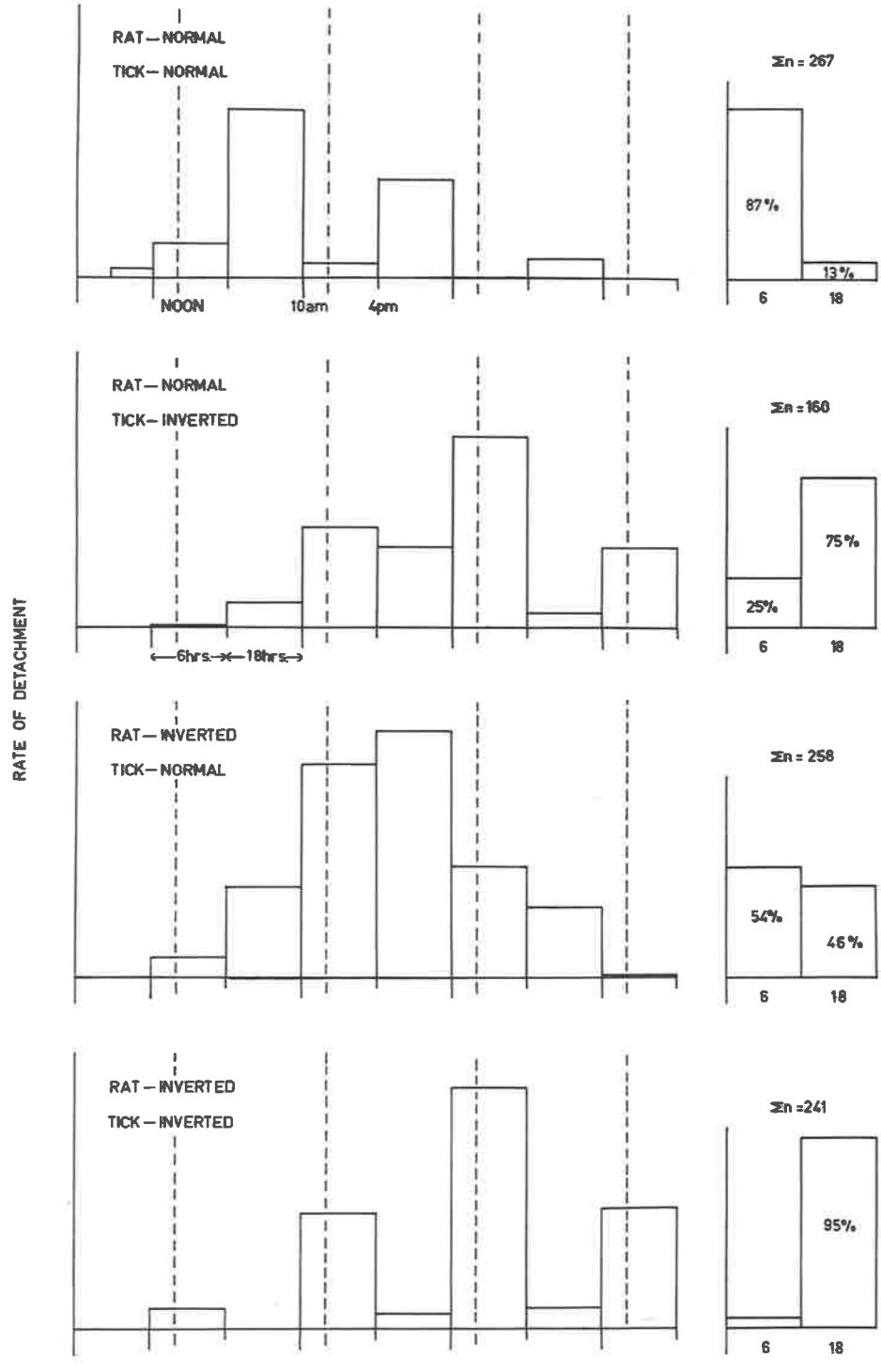
In treatments 1 and 4 the circadian rhythms of the rats and the ticks, if present, would be in phase, and thus their effect would be cumulative. In both cases it is evident that about 90% of the ticks detaching, dropped off during that period predicted if the ticks were detaching in response to a circadian rhythm. Therefore it is clear that the drop-off of the engorged ticks is regulated by a circadian rhythm and not by a direct response to light.

Table 3.2

Drop-off behaviour in continuous darkness

Treatment	1		2		3		4	
Rats	RN		RN		RI		RI	
Ticks	TN		TI		TN		TI	
Period Duration	6	18	6	18	6	18	6	18
Period Interval	1000-1600	1600-2200	1000-1600	1600-2200	1000-1600	1600-2200	1000-1600	1600-2200
Percent Recovery	87	13	24	76	54	46	7	93
Total Recovery (No. of Ticks)	267		160		258		241	

Figure 3.2 The Pattern of Drop-off of Engorged Larvae in Continuous Darkness.


RAT — NORMAL
TICK — NORMAL
NOON
10am
4pm
Σn = 267
87%
13%
6
18
RAT — NORMAL
TICK — INVERTED
←6hrs.→←18hrs.→
Σn = 160
25%
75%
6
18
RAT — INVERTED
TICK — NORMAL
Σn = 258
54%
46%
6
18
RAT — INVERTED
TICK — INVERTED
Σn = 241
95%
6
18
RATE OF DETACHMENT

In treatments 2 and 3 the rhythms of the ticks and the rats are twelve hours out of phase with each other and it is clear that both rhythms have contributed to determining when the ticks detached from their hosts. The proportion which detached during each of these two periods should give the effect of each rhythm relative to the other. A comparison of the 'drop-off response' during each of these two periods suggests that the rhythm within the tick controlled the detachment of about twice as many ticks as did the rhythm within the host. Thus the rhythm within the tick was more influential than that in the host, in determining when the engorged larvae detach in total darkness.

Experiment 3.33 Circadian rhythms within photoperiodic regimes

Circadian rhythms can determine when ticks detach in continuous darkness. This is not to say, however, that the larval drop-off is timed by these rhythms when the larvae feed in an LD regime because larval detachment may still be triggered by the lights-ON or -OFF signal.

Since circadian rhythms are present, there are two periods (entrainment periods) during which the larvae may pick up the phase of the rhythm; before feeding and during the two days of attachment before the drop-off commences.

An experiment was therefore designed to test whether circadian rhythms naturally determined drop-off, and also to test the relative strength of the two tick-rhythm entrainment periods.

Two groups of larvae were conditioned to L16 D8 for ten days, one group being 12 hours out of phase with the other. Each group was then placed on a rat which had been conditioned to a NORMAL L16 D8. When the ticks had attached, both rats were placed in a constant temperature cabinet set at 30°C, with a new light regime of NORMAL L8 D16 (Table 3.3). From Figure 3.3 it is clear that larvae in both treatments dropped off their host during the middle of the photophase.

Since engorged larvae from treatment 2 detach only during the photophase, even though they had been exposed during the previous ten days to an INVERTED L16 D8 regime, it is evident that the rhythm which the tick picks up before it attaches to the host, is completely over-ruled by that rhythm picked up during those first two days of attachment before drop-off commences. Thus the circadian rhythm within the tick determining drop-off, can be entirely reset by exposure for two days after attachment to an altered light regime.

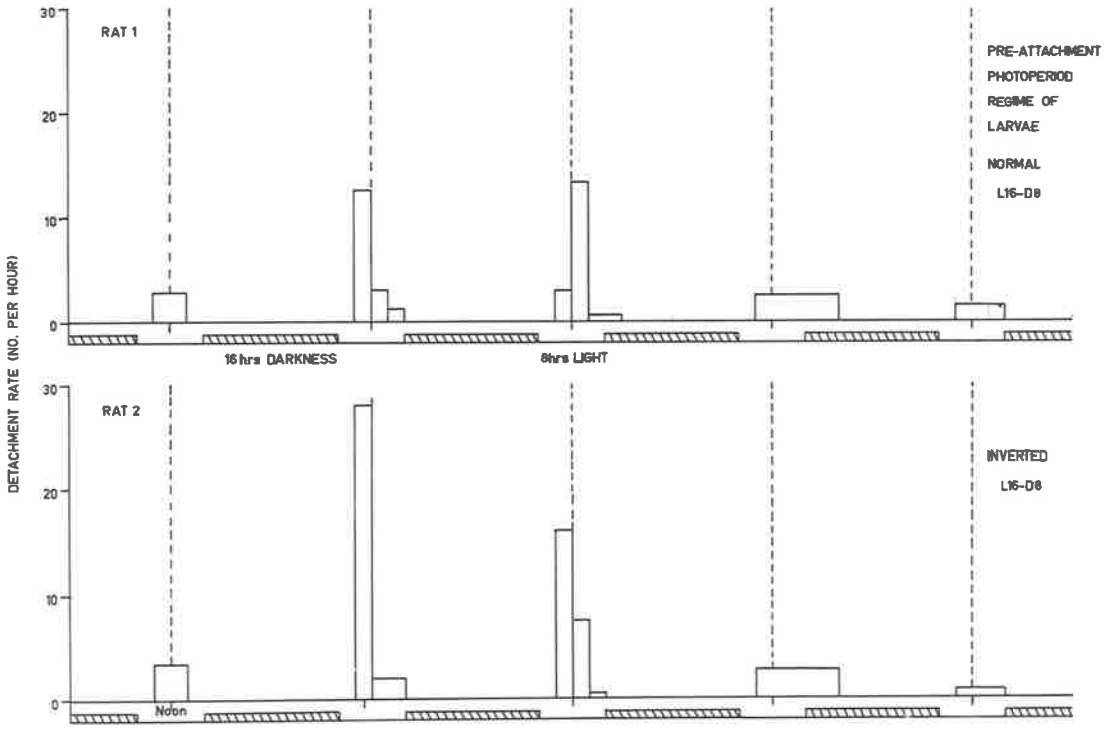
In view of this, and because the response to the rhythm entrained in the tick before attachment is about twice as powerful as the response to the rhythm within the rat, then it follows that the circadian rhythm within the rat, although it can influence the drop-off of engorged larvae, will have no significant effect in determining when engorged larvae will drop off host rats experiencing a photoperiod. When Figures 3.1 and 3.3 are compared, it is obvious that they are almost identical since the peak drop-off

Table 3.3

Effect of entrainment of larvae before attachment on the drop-off rhythm in short photoperiod

Treatment No.		1	2
History	Rats	NORMAL 16L 8D	NORMAL 16L 8D
	Ticks	NORMAL 16L 8D	INVERTED 16L 8D
Treatment		NORMAL 8L 16D	NORMAL 8L 16D
Engorged larvae off			
10 a.m. - 4 p.m. (photphase)		109	134
4 p.m. - 10 a.m.(scotophase)		8	8

Figure 3.3 The Effect of Experience of Photoperiod before Attaching, on the Drop-off Behavior of Engorged larvae in a NORMAL Light Regime.


RAT 1
RAT 2
30
20
10
0
DETACHMENT RATE (NO. PER HOUR)
16 hrs DARKNESS
8hrs LIGHT
Noon
PRE-ATTACHMENT
PHOTOPERIOD
REGIME OF
LARVAE
NORMAL
L16-D8
INVERTED
L16-D8

occurs daily between 10 a.m. and 4 p.m. in both cases, even though one treatment was in L16 D8 and the other in L8 D16. Virtually none dropped off during the scotophase in either case.

If the ticks had been detaching in response to lights-ON or OFF stimuli, the position of the histograms in Figures 3.1B and 3.3, relative to the transition from lights-ON to OFF or vice versa would have been quite different from that observed. In Figure 3.3 the peak rate of drop-off occurs eight hours after lights-ON and 8 hours before lights-OFF, whereas in Figure 3.1B the peak rate of drop-off occurs only four hours after lights-ON and 4 hours before lights-OFF. Thus it is clear that the ticks are not detaching in response to a direct light stimulus but that their behaviour is controlled by a circadian rhythm.

Experiment 3.34 The behaviour of larvae of the cave variety

The cave variety of O. gurneyi is discussed in Appendix III. Larvae of this variety which had hatched from eggs laid at 30°C 16L 8D, were placed in two groups, one on each of two rats. One rat had experienced a NORMAL 16L 8D and the other an INVERTED 16L 8D regime for the previous 8 days. The rats with ticks attached were placed into that photoperiod to which they had become accustomed. The engorged ticks which dropped off into the collecting tray were counted and removed at regular intervals as in previous experiments.

Figure 3.4 gives a comparison of drop-off rates within and between days and treatments. The results are shown in Table 3.4.

Discussion

The behaviour of larval cave ticks (Figure 3.4) is apparently identical to that of the larval plains ticks (Figure 3.1B). If we assume that a circadian rhythm of drop-off also occurs in cave larvae then the circadian rhythm which induces the ticks to drop off the host in the inverted LD regime had been entrained to a new LD regime during the two days spent attached to the host prior to dropping off.

Thus it is apparent that during the period of attachment to the host the larval ticks are very sensitive to the incident LD regime and that the rhythm entrained during this period overrides any previously entrained circadian rhythm.

Summary

1. The rhythmic daily drop-off of the engorged larvae of O. gurneyi is determined by a circadian rhythm within the tick.
2. This rhythm is entrained by the incident photoperiod during the two days the larvae spend attached to the host before they begin to drop off.
3. If there are no photoperiodic stimuli during these two days, then the drop-off can still be determined by a circadian

Figure 3.4 The Pattern of Drop-off of Engorged Larvae of the Cave Variety in NORMAL and INVERTED Light Regimes.

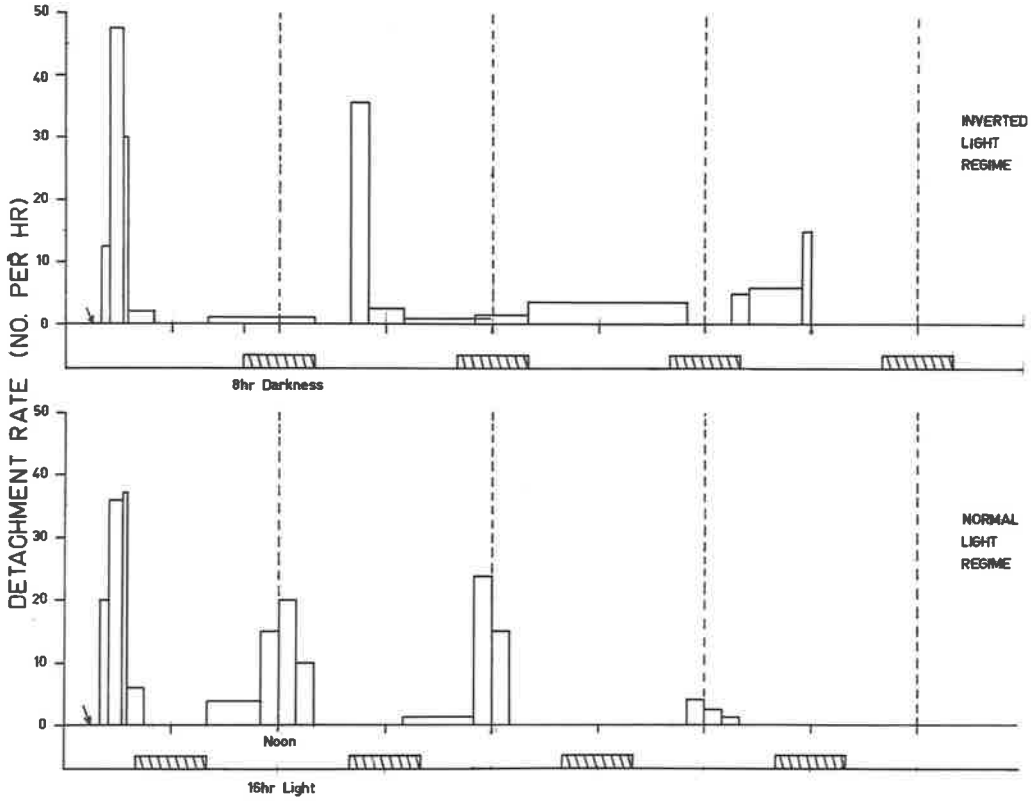

DETACHMENT RATE (NO. PER HR)
50
40
30
20
10
0
INVERTED
LIGHT
REGIME
8hr Darkness
NORMAL
LIGHT
REGIME
Noon
16hr Light

Table 3.4

Detachment behaviour of engorged larvae
(cave variety)

Treatment		16L 8D NORMAL	16L 8D INVERTED
History	Rats	16L 8D NORMAL	16L 8D INVERTED
	Ticks	NORMAL	NORMAL
No. ticks on		400	400
No. engorged ticks off		117	296
% recovery		29	77
10 a.m. - 4 p.m.		90% (photophase)	25%
4 p.m. - 10 a.m.		10%	75% (photophase)

rhythm. The rhythm influencing the drop-off in such a case are, firstly the rhythm within the tick entrained by photoperiod before attaching and, secondly, but less importantly, a circadian rhythm, within the host rat, to which the ticks respond.

4. The rhythm entrained before attachment and the response to the rhythm in the host rat are completely overruled by any rhythm entrained during the first two days of attachment.

3.4 The Rhythm of Feeding and Detaching of Engorged Nymphs

Engorged larvae from the previous experiments were stored in groups of 100 in constant light in glass vials with $\frac{1}{4}$ inch of sand at the bottom; larvae fed during NORMAL and INVERTED light regimes were mixed and not stored separately. At 30°C they moult within 10 days of dropping from their host (Chapter 4). These first nymphal instars (1NN) were used in the following experiments to attempt to elucidate the factors determining when the 1NN would drop from their host, engorged.

A preliminary observation indicated that about 50% of the engorged 1NN fell off on the day of attachment while the remaining ticks fell off during the afternoon of the subsequent two or three days (Table 2.2).

Experiment 3.41 INVERTED and NORMAL photoperiodic regimes

It was next decided to investigate what factors timed the drop-off of engorged nymphs.

Ticks were taken from constant light where they had moulted, and were exposed to one NORMAL cycle of L16 D8; they were then put onto two rats, one of which had been conditioned to an INVERTED 16L 8D regime, the other to a normal regime.

About one-third of these ticks dropped off engorged within three hours of attaching to the host. The rats, with food and water, were then placed one in each of two constant temperature cabinets set at 30°C. The light regimes, 16L 8D, within each cabinet were set 12 hours out of phase with each other. Rats were placed into the light regime to which they had become accustomed.

Table 3.5 shows that nearly all the ticks which drop off engorged, drop off during the photophase. But those which dropped off during the scotophase, dropped from the rat in the INVERTED regime. This group made up 14% of those which dropped from the INVERTED rat after day 0. Since no ticks dropped from the rats in the NORMAL regime during the scotophase I concluded that this 14% was not a random drop-off but rather was a response to a circadian rhythm which was entrained during the one initial LD cycle experienced before the ticks attached to their hosts. The drop-off of the remaining ticks may have been controlled by circadian rhythms or by a direct response to light.

Table 3.5

Detachment behaviour of engorged first instar nymphs

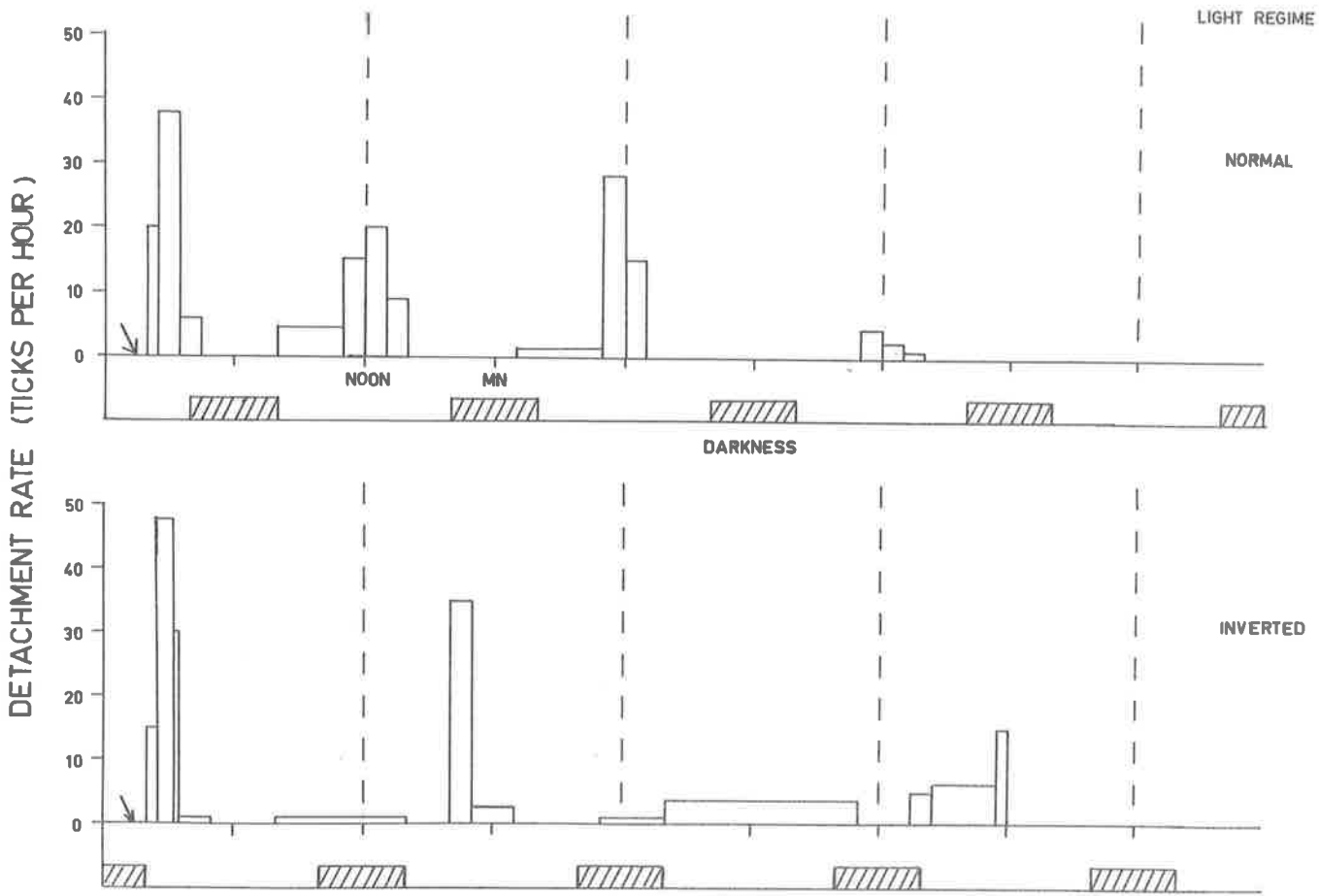
Incident LD regime	NORMAL		INVERTED	
Day 0 No ticks on	350		350	
Day 0 No ticks off engorged	103		105	
	Photophase	Scotophase	Photophase	Scotophase
Day 1	112	0	75	6
Day 2	66	0	53	6
Day 3	15	0	42	16
	193	0	170	28
Total off engorged	296		305	

In Figure 3.5 the drop-off of engorged larvae on day one in an INVERTED regime (histogram B) was delayed by 8 hours compared with those in a NORMAL regime (histogram A). This could be interpreted as the scotophase delaying the drop-off of engorged ticks (temporary inhibition). This explanation, however, seems unlikely since larvae detach in response to circadian rhythms even in total darkness (Figure 3.2). Another possible explanation is that ticks are merely responding to a lights-ON or -OFF stimulus. But in the NORMAL regime peak drop-off occurs 6 to 10 hours after lights-ON, whereas in the INVERTED regime the peak drop-off occurs 4 to 6 hours after lights-ON. Thus this explanation also appears unlikely. A further possibility is that the drop-off of engorged nymphs is regulated by a circadian rhythm the phase of which can be set by one LD cycle. In such a case the zietgeber would be either lights-ON or-OFF and so this explanation suffers the same defect as the previous one. Nevertheless, the latter two possibilities seem to be the only factors which could be regulating the drop-off of engorged nymphs, and so the following experiment was designed to test if either was responsible, and if so, to distinguish between them.

Experiment 3.42 Do circadian rhythms regulate drop-off of engorged nymphs?

Three groups of 200 nymphs each were taken from constant light and subjected to L8 D16, L16 D8 and L22 D2 respectively for 20 days.

Figure 3.5 The Drop-off Behaviour of Engorged 1NN in NORMAL and INVERTED Light Regimes.


LIGHT REGIME
NORMAL
INVERTED
DETACHMENT RATE (TICKS PER HOUR)
50
40
30
20
10
0
NOON
MN
DARKNESS

During the photophase of the 21st day they were placed on six (100 NN per rat, i.e. two replicates per treatment) rats which had been conditioned to a normal L16 D8 light regime. After 3 hours, when the first batch had stopped dropping off, the rats with ticks from L8 D16, were placed in L22 D2 and vice versa, while those from L16 D8 were left at L16 D8 (Figure 3.6).

From a comparison of histograms A, B and C in Graph 3.6 it is evident that the drop-off occurs principally in the middle of each day (photophase). I therefore conclude that, like engorged larvae, the periodic drop-off of engorged first nymphal instars is governed by a circadian rhythm. This rhythm may emanate from either the rat, the tick, or both. Previous experiments with larvae indicate that the rôle of the rat is likely to be minimal or non-existent.

Second Instar Nymphs

The detachment behaviour of second instar nymphs was examined on NORMAL rats in a NORMAL L16 D8 regime and again the nymphs detached during the middle of the photophase (Figure 3.7).

Summary

The drop-off rhythm of engorged first nymphal instars is determined by a circadian rhythm. This rhythm most probably emanates from within the tick.

A similar behaviour pattern occurs in the second instar nymphs.

Table 3.6

Detachment behaviour of engorged nymphs

Treatment		A	B	C
LD history	Rats	16L 8D	16L 8D	16L 8D
	Ticks	16L 8D	22L 2D	8L 16D
Incident photoperiod		16L 8D	8L 16D	22L 2D
Total No. ticks off engorged		47	29	110

Figure 3.6 The Drop-off Behaviour of Engorged 1NN in NORMAL Light Regimes with Photophases of 22, 16 and 8 hours.

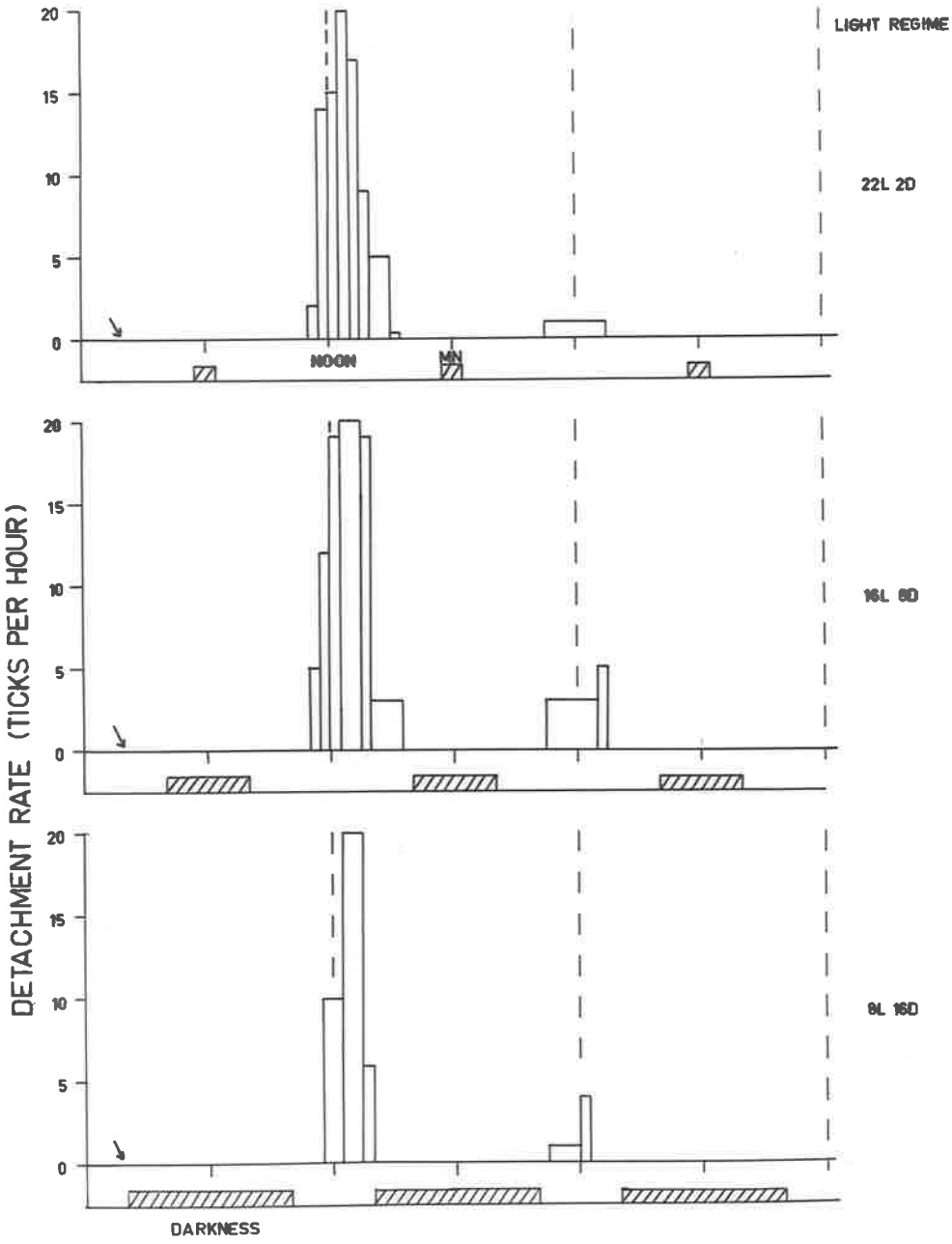
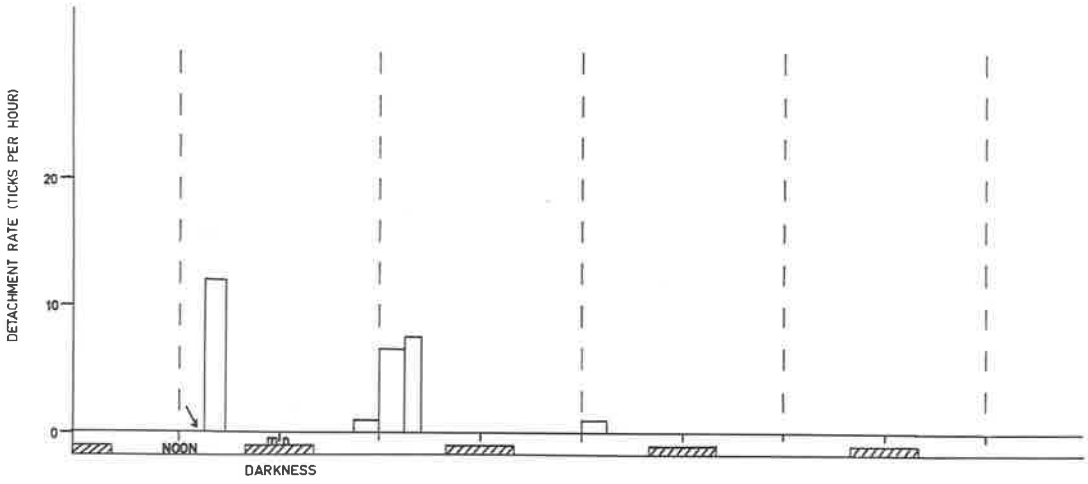

LIGHT REGIME
22L 2D
16L 8D
8L 16D
DETACHMENT RATE (TICKS PER HOUR)
20
15
10
5
0
NOON
MN
DARKNESS

Figure 3.7 The Drop-off Behavior of Engorged 2NN in a NORMAL Light Regime.


DETACHMENT RATE (TICKS PER HOUR)
20
10
0
NOON
m'n
DARKNESS

Discussion

It is clear that engorged larvae and first and second instar nymphs drop from their hosts between 10 a.m. and 4 p.m. when the host is exposed to a solar day. This drop-off behaviour is regulated by circadian rhythms within the tick which are entrained before and while the ticks are attached to their host. In nature, this behaviour would ensure that ticks detach from the host kangaroo during the day when the kangaroo would be lying most probably in a wallow under a shady tree, and not during the night when the kangaroo would be grazing out on the plain. Thus this mechanism ensures that most ticks drop into wallows where there is a far greater chance of finding another kangaroo (and thus another meal) than if they fell off while the kangaroo was feeding out over the plains, where the chance of finding another kangaroo would be very low indeed. Two LD cycles experienced while the larvae is attached to the host are sufficient to entrain the circadian rhythm. The biological significance of this mechanism is obvious. Larvae may hatch and live beneath the sand (in continuous darkness) until the day that they attach to their host. Thus the two days spent attached to the host, before the larvae begin to detach, can provide sufficient information to entrain the circadian rhythm (i.e. to ensure that it is in phase with the incident LD regime). On the other hand the significance of the ability to entrain the rhythm to the incident LD

regime before attaching to a host is less clear.

These observations suggest two lines of speculation. Firstly it would be of interest to know the minimum stimulus which could entrain the rhythm. This could be achieved by changing from NORMAL to INVERTED regimes (or vice versa) at different intervals after attaching to the host. However, this aspect was not pursued because it was not directly relevant to the ecology of the tick.

These data also suggest the questions, "what is the stimulus which acts as a zeitgeber?" and "how accurate is the biological clock?". These questions are relevant to the regulation of the circadian rhythm in the larvae and especially in the nymphs. When engorged larvae detach they bury themselves in sand and moult to first instar nymphs which may remain buried until a kangaroo visits the wallow (this period could be many weeks). The hungry first instar nymphs may emerge from the sand at any time during the day. Of those that attach, half will engorge and detach that day while a proportion will remain attached and then detach during the middle of the next day. This detachment is regulated by a circadian rhythm which was in phase with the solar day. There are two possible explanations for this: either the rhythm has persisted (from the larval stage) in phase with solar day, or the experience of the nymph after surfacing was sufficient to synchronize the circadian rhythm within the tick with the solar LD regime. Histogram B in Figure 3.5

suggests that one scotophase is sufficient to reset the rhythm in nymphs. With these problems in mind it would be revealing to examine the accuracy of the clock and look for a zietgeber. However, these questions are outside the scope of this thesis.

This chapter has considered the influence of photoperiod on feeding and detaching. The influence of various environmental factors (especially temperature) on the rate of development of engorged ticks will be considered in the next chapter.

CHAPTER 4

The Rate of Development

4.1 Summary

1. The minimum temperature threshold for development (the developmental zero) and the thermal constants were calculated for each stage.

2. Short exposures to temperatures $\geq 40^{\circ}C$ temporarily inhibit development; prolonged exposure inhibits moulting, but ticks can moult if they feed again.

3. Humidity has only a slight effect on the rate of development.

4. The temperature threshold for moulting is about $20^{\circ}C$.

5. The relationship between the cumulative percentage having moulted and day-degrees is presented.

6. Ticks were placed in the field at two-monthly intervals throughout the year. The percentage having moulted after each two-month period was measured and the number of day-degrees experienced was read from the graph of day-degrees against percentage having moulted. The number of day-degrees experienced was also calculated from temperature records taken from where the ticks were living in the field. The two estimates of the number of day-degrees experienced were in reasonable agreement.

7. These studies show that meteorological records can be used to estimate the day-degrees experienced by ticks in the field. Hence it is possible to calculate the amount of development that could occur in each season of the year provided there was abundant food.

8. The average duration of a life-cycle when food is not limiting can vary from four months to ten months depending upon the season of the year.

9. The differential rates of development of the different instars tend to synchronize annual development by causing the late instar nymphs to accumulate during autumn and winter.

4.2 Literature Review

The effect of temperature on the duration of the developmental stages in Arthropods has been the subject of an extensive literature. There have been two main themes. The first has been to develop a pragmatic understanding of the biology of pest species, in order to predict outbreaks and so to assist in their control. The concept of temperature summation has been the principal tool of these studies which have successfully predicted the timing of outbreaks of pests such as the codlin moth (Simpson, 1903), the Australian plague grasshopper, Austroicetes cruciata (Andrewartha, 1944) and the spruce bud-worm, Choristoneura tumiferana (Bean, 1961).

Nevertheless the concept of temperature summation must be used with care because it is based on several invalid assumptions. Namely:-

(i) that the relationship between temperature and rate of development is linear throughout the range of temperatures experienced in the field,

(ii) that the temperature threshold for development, calculated using this relationship, is a biological reality, and

(iii) that the reaction to temperature of laboratory and field populations is identical.

The second theme has been the attempts by various workers to discover a mathematical formula which accurately describes the relationship between temperature and the rate of development, and thus to establish a sound foundation to consolidate the present pragmatic approach (Davidson, 1942, 1944; Andrewartha and Birch, 1954; Browning, 1952). Various mathematical relationships have been proposed but there seems to be little basis for choice between them except what one is willing to accept. Howe (1967) and Watt (1968) have both reviewed in detail some of these attempts. Although the relationship between the rate of development and temperature has been precisely described for some species, attempts to fit curves to these data have not succeeded. The failure has been due in part to the

asymmetrical shape of the curve (which looks like an asymmetrical catenary, or a sigmoid turned over at the top) and in part to the fact that the statistics used to test the 'goodness of fit' of the data were so sensitive that slight experimental error (e.g. slight fluctuations in temperature) make small differences highly significant (Browning, 1952; Howe, 1967).

The present study is more concerned with the pragmatic relationship between the tick and its environment than with any attempt to examine the exact shape of the curve relating temperature to the rate of development.

Over a restricted portion of the temperature range, the medial range (Shelford, 1927), the relationship between the rate of development and temperature approximates to a straight line. This section of the curve is used when calculating "day-degrees" of development. The regression of temperature against rate of development is calculated, and from the regression equation one can calculate the "developmental zero" and then, using this, calculate the number of day-degrees necessary to complete any particular stage of development. This statistic is referred to as the "thermal constant" of that stage of development. This term was coined by Simpson in 1903 and the concept has been the much used tool of applied biologists ever since.

The biological meaning of developmental zero calculated in this

way has been strongly questioned (Andrewartha and Birch, 1954) but some authors still consider it to be a biological reality (Hunter-Jones, 1970). Although it may not be the developmental zero in the biological sense it is in the day-degree sense (provided the temperature remains in the medial zone). Whether or not it is a biological reality is academic for most purposes because the amount of development that occurs about that temperature is so slight as to be negligible.

One consequence of assuming a linear (rather than a sigmoid) relationship between temperature and rate of development is that the estimated thermal constant (in day-degrees) will always be too high if the temperature experienced by the animal remains near the lower temperature threshold for development and too low if the temperature remains above the optimum (Howe, 1967). Furthermore, experience of extreme temperatures may damage the animal in some way, inducing a 'refractory' period during which it recovers its normal response to temperature, and so extreme temperatures may cause the thermal constant to be underestimated. Thus the concepts of 'thermal summation' and 'developmental zero' are invalid as soon as the curve deviates from the calculated straight line. Nevertheless they have a practical use provided the temperature does not remain too long outside the linear zone.

Humidity is known to affect the rate of development of many

arthropods. Buxton (1932a) and Bursell (1964) have reviewed much of the relevant literature. The rate of development of some animals appears to be independent of the humidity experienced, while other animals can develop satisfactorily only within a limited range of humidities. The optimum range for some arthropods lies close to 100% R.H. and in such cases the speed of development decreases as the R.H. decreases, e.g. Ptinus and Lucilia (Bursell, 1964). In other arthropods, Locusta for instance, the optimum occurs in the middle of the humidity range and development is retarded at humidities outside this range. Those species which are unaffected by humidity are frequently those which live in dry places, e.g. Cimex and Thermobia (Bursell, 1964).

In addition to affecting the speed with which developmental stages are completed, the humidity may affect the proportion of individuals which complete development. But this aspect will be more conveniently dealt with in the section in which humidity and survival are discussed (Chapter 5).

One further problem is that of fluctuating temperatures. Because of technical problems most studies have been conducted at a series of constant temperatures. The problems of relating studies done at constant temperature to those done in fluctuating conditions have been discussed by Howe (1967) and he concludes that there is little evidence indicating that variable or alternate temperatures either

stimulate or retard the rate of development, provided that the mean of the fluctuating temperature is compared with the constant temperature. Messenger and Flitter (1959) made a very detailed study of the rate of development of the eggs of three species of Hawaiian fruit fly at constant and at regularly fluctuating temperatures. They found that if varying temperatures were represented by a mean, then for low temperatures development proceeds faster than expected, in the medial range it proceeds at the expected rate and at high temperatures development is slower than expected. Thus it appears that the model is accurate, but only when the temperature remains in the medial range.

4.3 Introduction

In order to analyse the seasonal cycle of activity of O. gurneyi it was necessary to trace the development of the different stages of the life-cycle of the tick in the field. This required a knowledge of the relationship between the rate of development of these stages and the various factors which might affect that rate (e.g. temperature, photoperiod, humidity and food). The data in this chapter are chiefly concerned with the effect of temperature on the rate of development of eggs, engorged larvae and nymphs, and of the eggs within the mother, but also briefly examines the effect of humidity on the rate of development.

From this data I have calculated the number of day-degrees needed by each stage to complete its development. The accuracy of this estimate was assessed by comparing it with estimates calculated from observations on the rate of development of engorged ticks in the field, where the temperature experienced by the ticks had been monitored. It should then be possible, using data from meteorological records, to calculate the duration of the life-cycle in the field, assuming that the ticks had abundant food. This, combined with knowledge about the behaviour of the red kangaroo, should give some insight into the seasonal cycle of activity of the tick in the field.

Three criteria were used to estimate the rate of development. For eggs, the interval between oviposition and hatching was taken as an index of the rate of development; for nymphs, the interval between detaching engorged and moulting to the next instar was used; and for females, the interval between feeding and the beginning of oviposition was used.

4.4 Larval and Nymphal Development

The period between engorging and the next moult (the pre-moult period) was measured and found to vary within a group of ticks raised and kept under constant conditions. The distribution of the pre-moult periods is approximately normal and so the mean (and variance) of the

group of ticks has been taken as an index of the rate of development of that group.

In the following section the effect of high, intermediate and low temperatures on the rate of development is examined. The rate of development at temperatures within the medial zone was used to calculate the developmental zero and the thermal constant for each stage. Field studies on the rate of development under field conditions examine the applicability of these statistics to field conditions. Using these statistics in conjunction with soil temperature records, the effect of seasonal changes in temperature on the rate of progression through the life-cycle is estimated.

4.41 The Effect of Temperature on the Rate of Development

Groups of ticks of all instars were fed, placed in petri dishes and covered with sand; these were then placed in a series of constant temperature cabinets at a range of temperatures between 12° and 45°C. The humidity inside the dishes was monitored using cobalt thiocyanate paper. The humidity was found to fluctuate between 10 and 30%. The ticks were examined at regular intervals and the pre-moult period for each tick was noted. From these data the mean pre-moult period for each instar over a range of temperatures was calculated (Table 4.1). At 12° and 15°C ticks did not moult, and only a proportion moulted at 20°C (Section 4.421). Furthermore at high temperatures there was a

Table 4.1

Mean Pre-moult Period (Days) of Engorged Ticks at a Range of Constant Temperatures

Instar	Temperature (°C)	20	25	27	30	32	34	35	40	45
Larvae	$\bar{x}$	17.1	6.9		4.9			4.1	4.9	-
	S.D.	3.6	0.6		0.6			0.4	1.5	
	n	175	227		199			158	23	
1NN	$\bar{x}$	25.9	11.2		8.1	7.4	6.9	6.7	6.9	-
	S.D.	5.8	1.0		1.3	1.4	0.7	3.0	1.6	-
	n	165	200		221	100	100	58	30	
2NN	$\bar{x}$	44.8	12.5		9.9			8.3	7.0	-
	S.D.	6.9	1.1		1.3			6.1	1.8	-
	n	17	100		201			99	47	
3NN	$\bar{x}$	*	28.7		17.5			13.4	9.8	
	S.D.		7.3		3.8			3.8	2.6	
	n		98		90			172	57	
4NN	$\bar{x}$	*	26.0	20.2	17.6			11.2	15.0	
	S.D.		7.8	3.0	5.7			9.8	4.0	
	n		34	50	52			53	3	
5NN	$\bar{x}$	*	30.9		25.5			13.0		
	S.D.		8.0		4.7			1.4		
	n	10	10		10			2		

\- died before moulting
* failed to moult at chart temperature (sample size = 50)
$\bar{x}$ mean pre-moult period
S.D. standard deviation
n number moulting

proportion of engorged ticks which failed to moult. This phenomenon is dealt with in Section 4.42.

When the rate of development of the engorged nymphs is plotted against temperature (the rate of development being calculated as 100 divided by the mean duration of the pre-moult phase in days, and hence expressing the percentage of the total pre-moult period completed each day) the data conform to the expected (sigmoid) curve (Figure 4.1). The estimates of the rate of development at those temperatures within the "medial range" (20° - 35°C) were used to calculate the regressions of temperatures against rate of development shown graphically in Figure 4.2. The equations calculated for each instar are set out in Table 4.2.

From these equations one can estimate the developmental zero for each instar. For example, the regression line for the first instar nymphs intersects the x-axis at 15.8 (y = 0), i.e. the developmental zero is 15.8°C (Table 4.2). Using the developmental zero I can now calculate the thermal constant for each instar. The product of the duration of the pre-oviposition period at any temperature (within the medial zone) and the effective temperature is a constant: the thermal constant.

For the first instar,

Thermal Constant = $d(x - 15.8) = 133$ day-degrees,

Figure 4.1 The Effect of Temperature on the rate of development of Engorged 1NN.

Duration of Pre-moult Phase (days) S.D. ————

The Relationship between Temperature and the Rate of Development (curve fitted by eye) ----------

Equation of the Regression Line $y = 0.73x + 14.3$

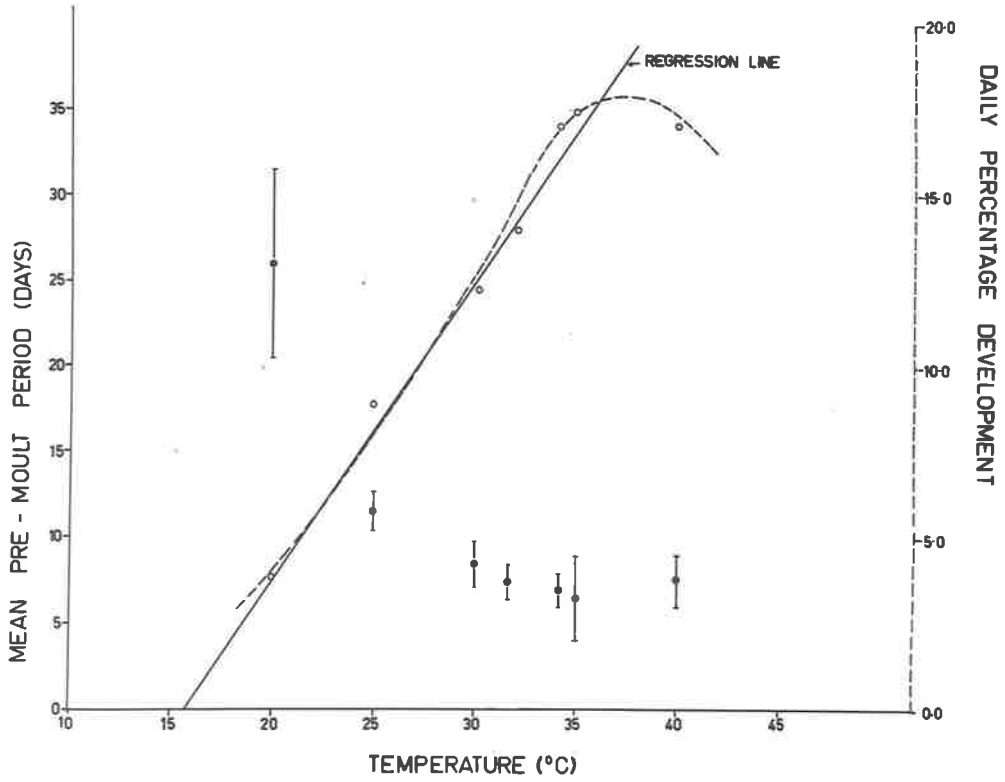

REGRESSION LINE
MEAN PRE - MOULT PERIOD (DAYS)
DAILY PERCENTAGE DEVELOPMENT
TEMPERATURE (°C)
0
5
10
15
20
25
30
35
10
15
20
25
30
35
40
45
0·0
5·0
10·0
15·0
20·0

Figure 4.2 The Regression Lines for the Rates of Development against Temperature for the Different Instars.

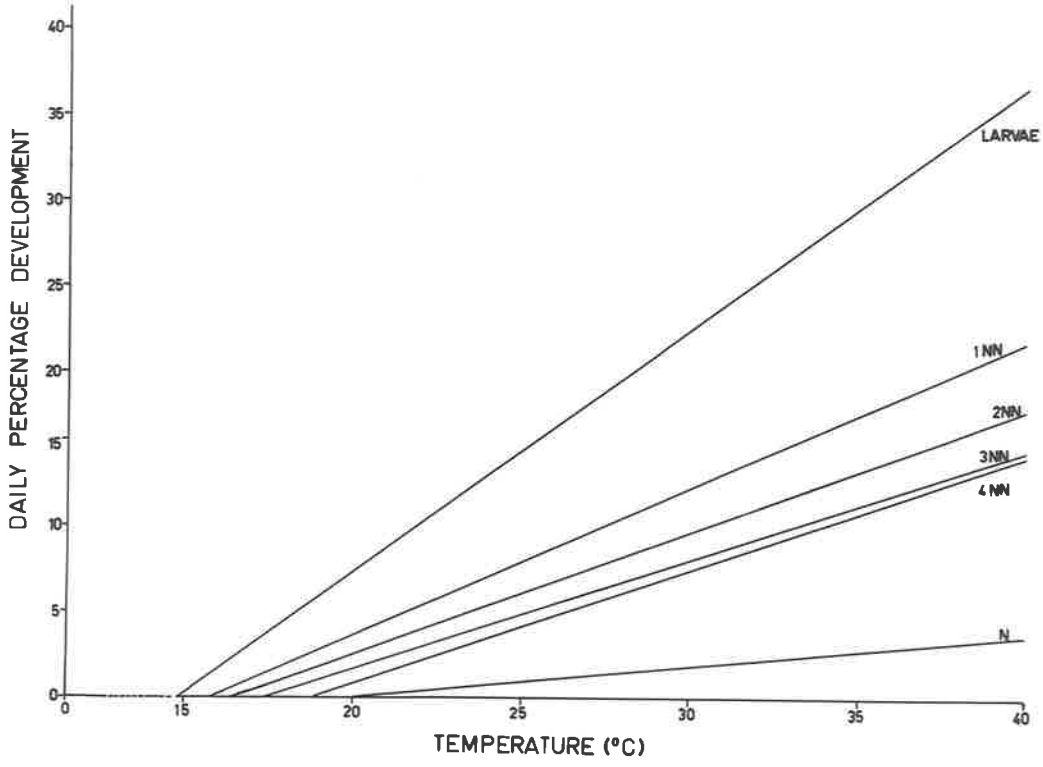

DAILY PERCENTAGE DEVELOPMENT
40
35
30
25
20
15
10
5
0
0
15
20
25
30
35
40
TEMPERATURE (°C)
LARVAE
1 NN
2NN
3NN
4 NN
N

Table 4.2

Regression Equations, Developmental Zeros and Thermal Constants for the Juvenile Stages

Stage	Regression Equation	Developmental Zero (°C)	Thermal Constant (Day-degrees) From Regression	From Section 4.43
Larvae	y = 1.47x - 22.03	14.68	69	83 (10)
First instar	y = 0.88x - 13.9	15.8	115	133 (10)
Second instar	y = 0.71x - 11.68	16.5	141	169 (41)
Third instar	y = 0.70x - 12.18	17.4	160	220 (8)
Fourth instar	y = 0.68x - 12.70	18.7	147	210 (13)
Fifth instar	y = 0.46x - 14.80	19.8	202	195 (21)

where d is the mean duration of the pre-moult period and x is the incubation temperature (^{o}C) (Table 4.2).

4.42 The Effect of Sub-lethal Doses of High Temperature on Development (or the Failure of Nymphs to Moult)

When gathering the data for the previous section, I noticed that at high temperatures many nymphs did not moult. The failure of arthropods to moult, even though they are in apparently favourable conditions for development has frequently been taken as a sign of diapause (Danilevskii, 1961; Belozerov, 1968; Kemp, 1968). Nymphal diapause has been observed in at least six species of Ixodid tick, and in every case either photoperiod, temperature, or both, have been implicated as diapause inducing factors. On the other hand, there is a possibility that high temperature merely acts to delay or inhibit morphogenesis. Thus Mellanby (1954) showed that exposure of larvae of Aedes aegypti and Tenebrio molitor to high temperature for brief periods interrupts development, but after a delay - perhaps a month in Aedes - normal pupation occurs and adults are produced after the usual interval. Okasha (1968) has shown that brief exposure of any nymphal stage of Rhodnius prolixus to high temperature either delays or inhibits moulting, depending on the severity of the temperature and the period of exposure. Exposing Rhodnius to high temperature either before or after feeding produces similar effects. Brief exposures to low

temperatures have also been shown to slow down development in Culex pipiens (Moulinier, 1969) and Tribolum castaneum (Howe, 1962).

Thus a series of experiments was designed, firstly to assess the extent of the failure to moult, and then to distinguish between the two possible causes; diapause or high temperature inhibition of development.

The criterion by which I intend to distinguish between the two possibilities was the fate of those ticks which did not moult. If diapause is responsible for the failure to moult then the ticks should moult once diapause development has been completed. On present knowledge the stimulus encouraging diapause development would almost certainly be either temperature or photoperiod or both, and that temperature or photoperiod would be different from that which induced diapause. Thus a period of exposure to low temperature and either short or long photoperiod should "break" the diapause and the ticks should moult. On the other hand, if the phenomenon is not diapause but rather high temperature inhibition of development, then exposure to those conditions which promote diapause development should have little or no effect. However there will always remain the possibility that the stimuli which promote diapause development have not been discovered. Usually it is not possible to distinguish between the two possibilities on the basis of resumption of development in favourable conditions because the manifestations of a transitory diapause and that of temporary

inhibition of morphogenesis are frequently identical. However, there are several instances (in the Lepidoptera) where this is not the case (Masaki, personal communication).

4.421 The Failure of Engorged Juveniles to Moult at Constant Temperatures

The reactions of engorged ticks in all juvenile instars to a range of temperatures at both long and short photoperiod (L16 D8 and L8 D16 respectively) were examined.

Up until the time of the experiment the ticks were kept at 30°C in long photoperiod. They were then fed and the newly-engorged ticks of each instar were assigned at random to groups, placed into petri dishes, covered with dry sand and allotted to constant temperature cabinets at a range of temperatures from 12° to 45°C at long and short photoperiods. In addition, at 30° and 35°C some fourth instar nymphs were covered with small glass beads (glass sand) to ensure that they were exposed to the incident light photoperiod.

Moulting was considered to be complete when 99% of the ticks which were to moult, had done so. This 'cut-off time' (tm) was calculated and corrected to the nearest day for each instar at each temperature by adding to the mean moulting period, 2.576 times the standard deviation of the mean (t at probability level of 0.01 is 2.576). For example, the mean pre-moult period of fourth instar nymphs at 30°C is 17.6 days (S.D. = 5.7) (Table 4.1). Theoretically,

99% of those nymphs which were to have moulted would have done so 32.3 days (= 17.6 + (5.7 x 2.576)) after engorging, i.e. the cut-off time is 32 days after engorging. These calculations were used when estimating the proportion which did not moult. Figure 4.3 illustrates the relationship between moulting and the failure to moult at high temperature; Figure 4.4 illustrates it for low temperature.

It is clear from the results in Table 4.4 that the incident photoperiod did not affect the proportion which moult. Similar data showing that photperiod had little effect on moulting can be cited for all instars at 25° and 30°C.

The effect of temperature however is pronounced, as can be seen in Table 4.3. It appears that as temperature increases, an increasing proportion of ticks fail to moult. In addition the later nymphal instars are more susceptible to the effect of temperature than are the earlier ones. The failure to moult at low temperatures is attributed to the cessation of morphological processes at those temperatures. Ticks which fail to moult at low temperatures, moult readily when returned to 30°C.

4.422 Moulting Behaviour in Changing Temperatures

Although moulting of some engorged nymphs is inhibited at constant high temperature, the effect of varying the temperature experienced by the tick was unknown. Such information is more relevant

Figure 4.3 The Moulting Behavior of Engorged 3NN at 40° C.

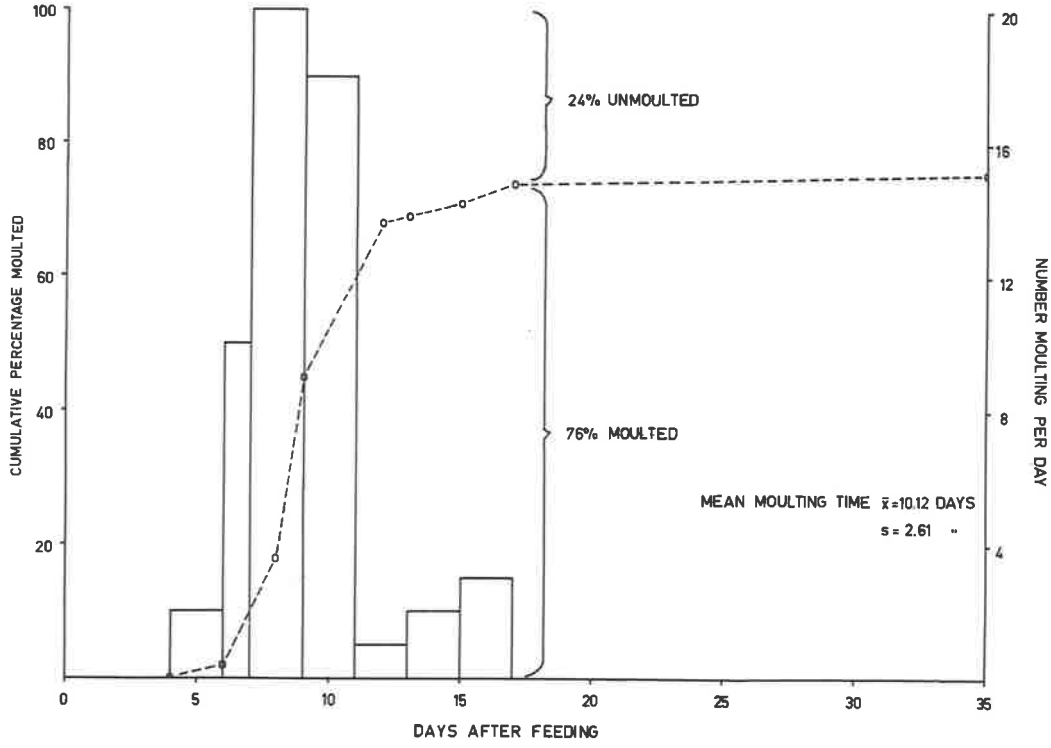

100
80
60
40
20
0
5
10
15
20
25
30
35
20
16
12
8
4
CUMULATIVE PERCENTAGE MOULTED
NUMBER MOULTING PER DAY
DAYS AFTER FEEDING
24% UNMOULTED
76% MOULTED
MEAN MOULTING TIME x̄ =10.12 DAYS
s = 2.61 "

Figure 4.4 The Moulting Behaviour of Engorged Larvae, 1NN and 2NN at 20° C.

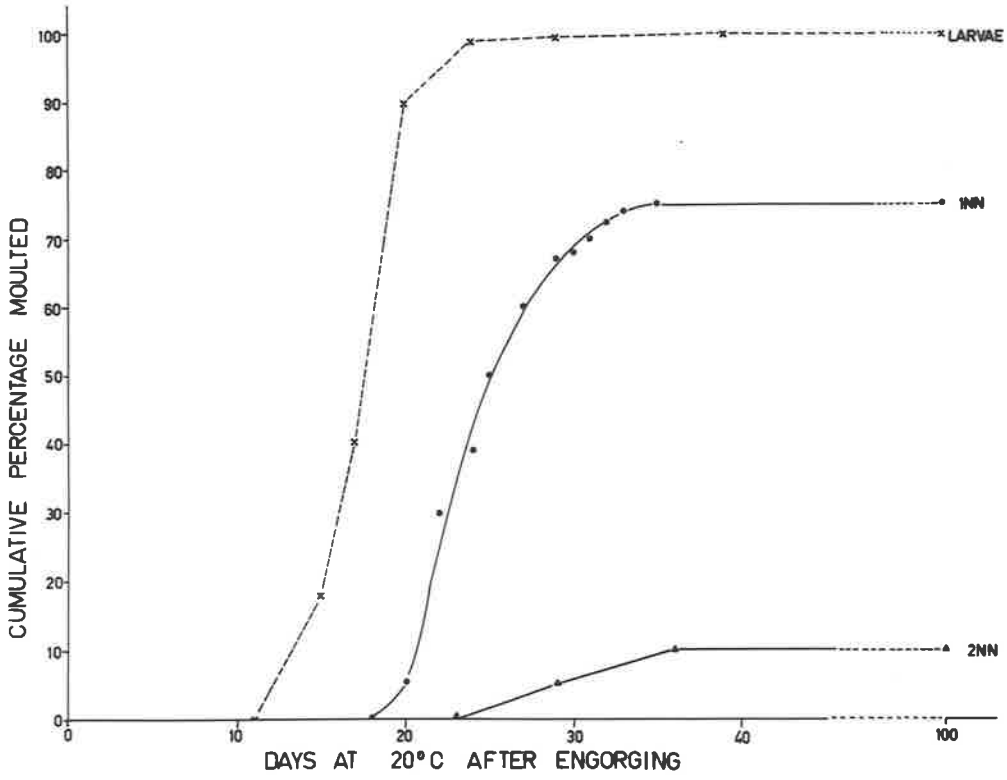

CUMULATIVE PERCENTAGE MOULTED
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
100
DAYS AT 20°C AFTER ENGORGING
LARVAE
1NN
2NN

Table 4.3

The Moulting Behaviour of Engorged Nymphs at a Range of Constant Temperatures

Instar	Larvae			1NN			2NN			3NN			4NN			5NN		
Temperature	% failing to moult	tm (days)	Sample size (n)	% failing to moult	tm (days)	Sample size (n)	% failing to moult	tm (days)	Sample size (n)	% failing to moult	tm (days)	Sample size (n)	% failing to moult	tm (days)	Sample size (n)	% failing to moult	tm (days)	Sample size (n)
20°C	2	26	200	20	41	50	90	63	60	100			100			100		
25°C	1	8	200	0	14	100	0	15	110	3	48	30	23	46	190	17	51	12
30°C	0	7	100	1	11	100	1	13	160	6	27	90	9	32	130	23	37.5	13
35°C	0	5	70	10	14	60	3	11	65	7	23	90	15	36	130	83	17.0	12
40°C	30	9	40	45	11	70	21	12	50	20	17	70	62	25	130	100		13
45°C	100		80	100														

TABLE 4.4

The Moulting Behaviour in Long and Short Photoperiod
(The Number of Fourth Instar Nymphs Moulted
t_m Days after Engorging)

Temperature	Photoperiod	Medium	Number Moulted	Number Not Moulted
30°C	16-8	Sand	69	1
	8-16	Sand	76	1
	16-8	Glass Sand	25	2
	8-16	Glass Sand	24	3
35°C	16-8	Sand	74	3
	8-16	Sand	76	5
	16-8	Glass Sand	22	3
	8-16	Glass Sand	22	2

to the ecology of the tick than are studies at constant temperature, and so the effect of exposing ticks to high temperatures for a variable number of days was examined next. Engorged fourth instar nymphs were placed at 40°C for 1, 2, 5, 9, 17 and 40 days before being removed to 30°C. The effect of this treatment on both the rate of development (expressed by the mean pre-moulting period) and the proportion which moulted was examined.

The previous experiment has shown that continuous temperatures of 30° and 40°C inhibit moulting in 9% and 60% of engorged fourth instar nymphs respectively. In Table 4.5 the moulting behaviour of each group has been expressed in two ways; firstly as the mean pre-moult period, and secondly as the mean of the period between when the animals were placed at 30° and moulting. It is clear that although an increasing period of exposure to a high temperature inhibits moulting in an increasing proportion of nymphs, the proportion not moulted which was characteristic of continuous 40°C (i.e. 60%) was not attained by 17 days of exposure; thus after 17 days at 40°C there are still some nymphs that would moult if placed at 30°C but would not moult at continuous 40°C. However it is also clear from Table 4.4 that when such ticks are returned to 30°C the moulting process is more protracted than it would be if they had not experienced the high temperature. For example, after one day's exposure to 40°C the duration at 30°C is 16.0 days, whereas after 17 days at 40°C it is 24.1 days.

Table 4.5

The Effect of Different Periods of Exposure to 40°C on the Moulting Behaviour of Nymphs

Days spent at 40°C before transferring to 30°C	1	2	5	9	17	40
Mean pre-oviposition period (days) ($\bar{x}$)	17.0	18.0	21.9	27.7	41.1	15.0
S.D.	5.1	5.6	7.5	15.5	23.9	5.0
Duration at 30°C ($\bar{x}$ - days at 40°C)	16.0	16.0	16.9	18.7	24.1	
Number moulting	48	44	40	39	32	24
Percentage not moulting	2	4	15	20	45	60
Percentage dead after 60 days	0	0	0	0	6	10

Thus short doses of high temperature induce a delay in morphogenesis in some engorged ticks and the duration of this delay is slightly greater than the period spent at high temperature.

In addition, as the period of exposure to 40°C lengthens, the proportion of ticks which fail to moult also increases. The fate of these unmoulted ticks is examined in Section 4.424; but firstly I shall examine the influence of high temperature on ticks which have not fed.

4.423 The Influence of High Temperature on Unfed Ticks

It is possible that unfed nymphs are also sensitive to high temperature. This has been found in Rhodnius prolixus, where exposure of larvae to high temperatures before feeding delayed and sometimes inhibited moulting once a bug had engorged (Okasha, 1968). An experiment was designed to test the sensitivity of unfed ticks to high temperature.

Groups of unfed fourth instar nymphs were exposed for one or three weeks to temperatures of 35°, 40° and 45°C and then returned to 30°C. Controls were kept at 30°C. The effect of this treatment on the proportion of nymphs which fed and moulted was determined.

Table 4.6 shows that temperature experienced before feeding affects neither the proportion which feeds nor the proportion which moults after feeding. However at high temperature there was

Table 4.6

The Effect of Exposing Unengorged Fourth Instar Nymphs to High Temperature

Temperature (°C)	30	35	40	45
1 week Sample Size	16	33	29	30
% surviving (number)	100(16)	100(33)	90(26)	7(2)
% feeding (number)	68(11)	61(20)	55(14)	0
% not moulting (number)	9(1)	5(1)	0(0)	-
3 weeks Sample Size	28	25	35	30
% surviving (number)	100(28)	100(25)	80(28)	0
% feeding (number)	46(13)	44(11)	54(15)	-
% not moulting (number)	8(1)	9(1)	7(1)	-

considerable mortality, e.g. only 5% survived one week at 45°C.

A similar experiment in which groups of unengorged third instar nymphs were placed in L16 D8 at 12°, 25° and 35°C for eight weeks before being fed and placed at 30°C, L16 D8, yielded similar results (Table 4.7).

In summary, it appears that only engorged nymphs are sensitive to the moult-inhibiting influence of high temperature. Short exposures to high temperature delay moulting in some ticks, the proportion affected depending on the instar exposed, the temperature and the duration of exposure. There are, however, some ticks which do not moult even if returned to favourable conditions. Thus, so far, either diapause or high temperature inhibition of development could explain this behaviour, and so I carried out an experiment designed to separate the two possibilities.

4.424 Is it Nymphal Diapause?

Two groups of engorged fourth instar nymphs which had not moulted forty days after feeding, were placed at 12°C in long and in short photoperiod. These conditions were considered most likely to promote diapause development (Section 4.42). In addition 12°C is known to encourage diapause development in the imagos of O. gurneyi (Section 6.9). After six or twelve weeks' exposure the nymphs were removed to 30°C, long photoperiod, where they were observed regularly and the proportion which moulted was noted. The nymphs which

TABLE 4.7

Effect of Pre-feed Exposure to High and to Low Temperatures on the Feeding and Moulting Behaviour of Third Instar Nymphs

Eight weeks' exposure to:-	Percentage Engorging	Percentage Not Moulting	Sample Size
12°C	58	19	96
25°C	57	22	82
35°C	60	12	68

had not moulted after 30 days were given an opportunity to feed again and then they were returned to 30°C and the proportion which moulted was again noted. A control group was kept at 30°C L16 D8. The data in Table 4.8 show that exposure of unengorged nymphs to low temperature had little effect on the tendency to moult when returned to 30°C.

When the ticks were offered a meal after 30 days at 30°C many of them engorged (80%, n = 40), and most of those which engorged for the second time moulted, while those which did not feed again did not moult.

Another group of non-moulted nymphs from 30°C L16 D8 was offered a meal and the moulting behaviour of those which engorged was examined and compared with that of nymphs of the same instar which moulted after one meal. The mean moulting periods were 16.9 (S.D. = 3.7 days and 17. (S.D. = 4.0 days) respectively, and so it appears that the ticks in which moulting was inhibited after the first feed nevertheless moulted successfully and normally after the second feed.

There are three pieces of evidence which bear on the problem of whether this phenomenon is diapause or high temperature inhibition of development. Firstly, diapause-inducing stimuli are usually not lethal, except in extremes, and so, because high temperatures (40° and 45°C) were injurious to O. gurneyi, one might argue that the phenomenon was not diapause. However this argument by itself, is not convincing.

Table 4.8

The Effect of Exposure to Low Temperature on Moulting Behaviour

Weeks at 12°C	6		12		Control
	L16 D8	L8 D16	L16 D8	L8 D16	
% moulting within 30 days at 30°C	0	0	0	8	5
(Sample size)	13	16	17	16	50

Secondly, nymphal diapause in ticks is manifest as the failure of engorged nymphs to moult. When diapause development is complete a tick will moult (in favourable conditions) without feeding again. However O. gurneyi nymphs resume normal development after a second meal. This is contrary to the pattern normally found in nymphal diapause in ticks. Thus the ability of nymphs to resume normal development after a second meal suggests that the nymphs do not undergo diapause development. It could be argued that a second meal is the special stimulus necessary to complete diapause development but this seems unlikely.

Thirdly, prolonged exposure to low temperature and to long or short photoperiod failed to make the engorged ticks ready to moult when returned to 30°C and so the nymphs were indifferent to stimuli which normally promote diapause development in other species.

Thus this phenomenon is not diapause in the ordinary sense, but it may be a special case. If it were, then I doubt if it would be worthwhile drawing the distinction between the two explanations. Nevertheless, although the evidence is not unequivocal, it suggests that the phenomenon is high temperature inhibition of development.

4.43 Temperature Thresholds for Moulting

There are temperatures several degrees above developmental zeros at which moulting was not observed (e.g. fourth instar nymphs at 20°C). This is obvious when the developmental zeros (Table 4.2) are compared

with the temperatures at which ticks were observed to moult (Table 4.1). Thus there is a possibility that there are two critical temperatures; one for morphogenesis and another for moulting. Similar situations have been found in other Arthropods. Johnson (1940) recognised that in Cimex the threshold for hatching was 8°C but the developmental threshold was 4°C. Lin et al. (1954) found that the minimum temperature for hatching was 15°C in Oncopeltus and they recognised egg development below this level. Similarly Hunter-Jones (1970) showed that the minimum temperature at which the eggs of the desert locust, Schistocerca gregaria, would hatch was 20°-24°C, while the minimum developmental temperature was about 15°C.

The minimum temperature at which each instar of O. gurneyi moults has not been determined directly, but the moulting behaviour of the ticks at 15°, 20° and 25°C sheds some light on this aspect of the tick's biology. In any sample, the minimum temperatures at which individual ticks moult will vary. As the temperature increases through the critical range, the proportion of individuals in the sample able to moult increases until all are able to moult.

The moulting behaviour of engorged larvae and nymphs has been extracted from Table 4.3 and is presented in Table 4.9.

If the values of percentage moult are plotted on probability paper, limits can be placed on the mean moulting temperature (that temperature at which 50% of the engorged ticks are capable of moulting;

Table 4.9

The Effect of Temperature on the Proportion of Engorged Ticks which Moult

Temperature	Percentage which moult at constant temperature					
Instar	Larvae	1NN	2NN	3NN	4NN	5NN
15°C	0	0	0	0	0	0
20°C	100	75	10	0	0	0
25°C	100	100	100	100	100	100

assuming that the response follows the normal curve). These limits are 15° to 20°C, 19.3° to 20°C, and 20° to 21.3°C for larvae, 1NN and 2NN respectively. The limits for the 3NN, 4NN and 5NN are 20° to 25°C. The developmental zero, as a mathematical device is discussed in Section 4.41. One consequence of the way in which developmental zeros are calculated is that some morphogenesis usually occurs at temperatures below the developmental zero (Table 4.2). Because developmental zeros are several degrees lower than the mean moulting temperature of each instar it is clear that development can occur at temperatures below those at which moulting can occur.

4.44 Effect of Humidity on Nymphal Development

Engorged larvae were placed in petri dishes containing one cm. of sand. These were placed in humidity chambers (at 12, 32, 75, 95% R.H.) at 25°C, and examined periodically for moulted ticks. The day that each tick moulted was noted and the mean pre-moult period for each treatment was calculated. From the data in Table 4.10 it is clear that nymphal development is unaffected by humidity, except at high humidities where development is slightly delayed.

4.45 Day-degrees and Moulting Behaviour

Many workers have queried the value of using laboratory data to estimate the rate of development in the field (in conjunction with meteorological records). Estimates of the developmental zeros and the

Table 4.10

The Effect of Humidity on the Moulting Behaviour of Engorged Nymphs

Relative humidity (%)	12	32	75	95
Mean pre-moult period (days)	6.9	6.9	7.2	8.0
S.D.	0.63	0.52	0.69	0.81

thermal constants for the developmental stages of the tick have been calculated from laboratory data. However before they could be used meaningfully to interpret development in the field (using meteorological records), their applicability to field conditions needed to be tested. To this end ticks were exposed to field conditions which were monitored, and the amount of development observed was compared with that amount which was predicted from the temperature records.

Engorged larvae and nymphs were placed in the field at intervals of about two months between March and December 1969 and the proportion of each stage which had moulted after two months was recorded. The results thus obtained were in the form of 'x% of an instar had moulted y days after being placed in the field'. The temperature experienced by the ticks during this period was monitored at hourly intervals by a battery-powered temperature recorder. Using the estimates of thresholds for morphological development derived from the laboratory studies, the number of day-degrees experienced by each instar during the period was calculated.

A second estimate of the number of day-degrees experienced by each instar during each two-monthly period was read from a graph of cumulative percentage having moulted against day-degrees experienced (Figure 4.6). The graph was developed from laboratory data using the mean pre-moult period and its variance to describe the moulting behaviour of each instar at each temperature. Hence at each temperature

Figure 4.5 The Relationship between the Cumulative Percentage which have Moulted and the Mean Number of Day-degrees experienced by Engorged Larvae and 1NN (± S.D.).

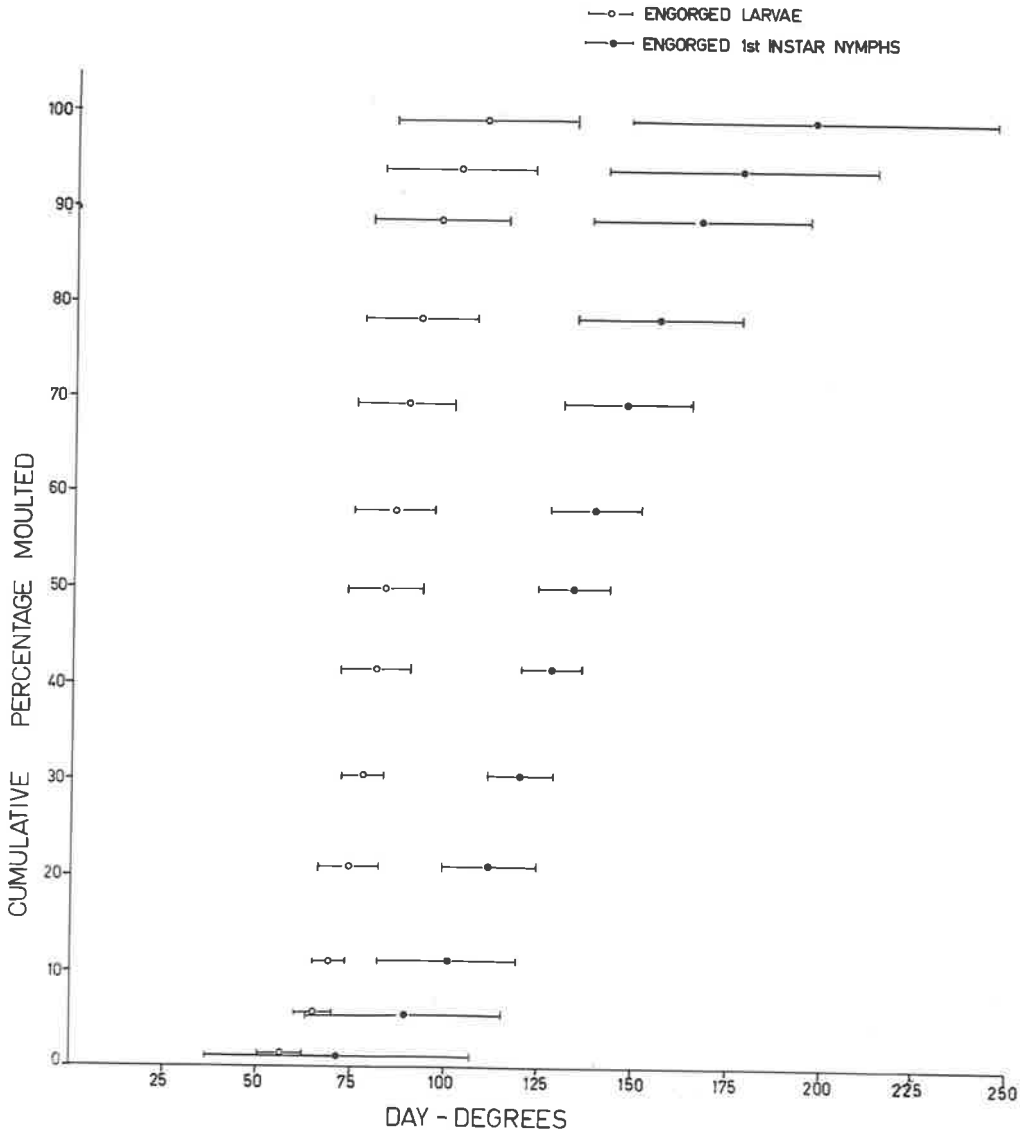
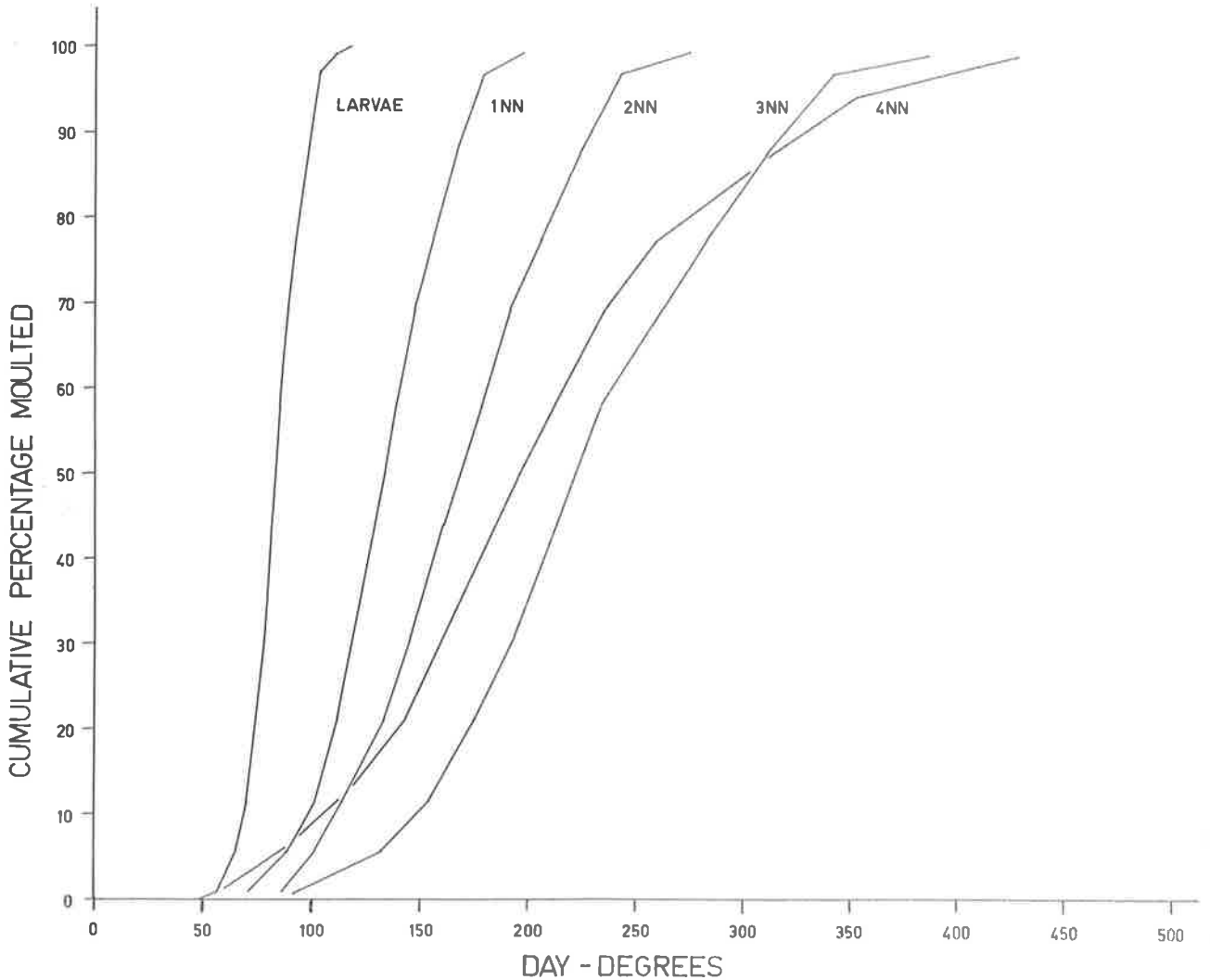

ENGORGED LARVAE
ENGORGED 1st INSTAR NYMPHS
CUMULATIVE PERCENTAGE MOULTED
100
90
80
70
60
50
40
30
20
10
0
25
50
75
100
125
150
175
200
225
250
DAY - DEGREES

Figure 4.6 The Relationship between the Cumulative Percentage which have Moulted and the Mean Number of Day-degrees Experienced by engorged Larvae, 1NN, 2NN, 3NN and 4NN.


CUMULATIVE PERCENTAGE MOULTED
100
90
80
70
60
50
40
30
20
10
0
LARVAE
1NN
2NN
3NN
4NN
0
50
100
150
200
250
300
350
400
450
500
DAY - DEGREES

one can develop the curve of the cumulative percentage having moulted against the time spent at a particular temperature. Knowing the developmental zero for that instar and the temperature at which the experiment was performed, the time axis can be transformed into day-degrees, thus producing a cumulative curve of percentage which have moulted against day-degrees experienced. For each instar the curve should be independent of the temperature at which the experiment was conducted. Thus for each instar there are as many replicates of the curve as there were temperatures examined. The replicates of each curve were used to calculate the mean curve for that instar (and the variance of the curve).

If the two estimates of day-degrees agree, then the amount of development which occurred in the field will be the same as that amount predicted using laboratory data on development in conjunction with the temperature records from the field; so temperature records and the laboratory studies should allow me to produce a relatively accurate account of the amount of development which could occur in the field, given abundant food.

In order to illustrate the technique I shall recount the process for the first instar nymphs. At 20°C the mean pre-moult period was 25.9 days (S.D., 5.8). Table 4.12 (after Li, 1964) shows the relationship (for a normal curve) between the proportion of the

Table 4.11

The Relationship between Cumulative Percentage having Moulted and the Number of Day-degrees Experienced by Engorged 1NN at a Range of Temperatures

Temperature (°C)	Mean pre-oviposition period (days)	S.D.	Estimate of number of day-degrees for a particular proportion to develop												
		Percentage	1.1	5.5	11.5	21.2	30.6	42.0	50.0	57.9	69.2	78.8	88.5	94.5	98.9
20	25.9	5.8	71.6	94.7	108.0	121.2	131.1	141.0	147.6	154.2	164.2	174.1	187.3	200.5	222.7
25	11.2	0.96	96.2	103.4	107.5	111.6	114.7	117.8	119.8	121.9	125.0	128.1	132.2	136.3	143.5
30	8.1	1.3	80.2	94.5	102.7	110.8	117.0	123.1	127.2	131.3	137.4	143.5	151.7	159.8	174.1
32	7.4	1.4	74.0	91.3	101.2	111.2	118.6	126.0	131.0	135.9	143.4	150.8	160.7	170.6	188.0
34	6.9	0.73	102.9	112.9	118.7	124.4	128.7	133.1	135.9	138.8	143.1	147.4	153.2	158.9	169.0
35	6.7	3.0	4.1	39.3	64.1	89.0	107.6	126.3	138.7	151.1	169.7	188.4	213.3	238.5	281.5

Table 4.12

The Relationship Between the Proportion of a Population Contained within Certain Limits and the Factor, n, by which One must Multiply the S.D. to Determine where the Limits Lie

Percentage occurring beneath the ogive	Factor (n) by which one multiplies the S.D.
0.1	3.0
1.1	2.3
5.5	1.6
11.5	1.2
21.2	0.8
30.6	0.5
40.1	0.2

population contained within certain limits and the factor, n, by which one must multiply the S.D. to determine where the limits lie (limits = mean $\pm$ (n x S.D.). For example, the mean 2.3 S.D. will give two values, being those points at which 1.1 and 98.9% of the population is to be found beneath that portion of the curve. Thus after 73.1 and 227.4 day-degrees of exposure (i.e. 25.9 $\pm$ (2.3x5.8) where 15.8°C is the developmental zero) 1.1 and 98.9% of the first instar nymphs would have moulted at 20°C. Similarly one can calculate the number of day-degrees of exposure at which other proportions of the population should have moulted. An analogous set of data can be calculated for each temperature at which the development of the tick was studied. Thus in the case of the first nymphal instar, there were six temperatures at which the rate of development was studied and so there were six estimates of the number of day-degrees needed for each % moult (Table 4.11).

The mean and the variance for each of these points was calculated and an example of the process is shown in Table 4.11. The means and the S.D.s for the engorged larvae and first instar nymphs are plotted in Figure 4.5 and the means alone for all the instars are plotted in Figure 4.6.

From these data one can estimate the mean number of day-degrees of exposure (and its variance) which corresponds to a particular percentage having moulted. Thus when exposure to field conditions for

a period of time results in a particular proportion of the ticks moulting, the number of day-degrees which would have been experienced by a group of ticks at constant temperature can be read from the graph.

The point at which 50% of the ticks have moulted is the thermal constant for that instar (if the curves are normal). It is noteworthy that the estimates of the thermal constants for each instar derived by use of the regression equations (Section 4.41) are from 10 to 30% lower than the estimates read from the graphs of day-degrees against percent which have moulted (at 50%) (Table 4.2). This suggests that the distribution of the pre-moult periods is slightly scewed or that the inclusion of results from 40°C (where the rate of development is maximal and is not altered greatly by changes in temperature) might have scewed the curves in Figure 4.6.

4.46 Field Experiments on the Rate of Development

In March 1970 groups of engorged larvae, first instar nymphs and fourth instar nymphs were placed in field cages* and buried in wallows in the study area on Moralana station. About two months later the proportion which had moulted was noted. Using the graph of

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* The field cages consisted of screw-top PVC one-pint canisters. Each end was covered with wire gauze which was so fine that the ticks could not escape; nevertheless it permitted vertical passage of water and gas, when the canisters were buried vertically.

cumulative percent which have moulted against day-degrees experienced (Figures 4.5 and 4.6), the number of day-degrees which the ticks would have experienced at constant temperature was estimated.

Another group of larvae and nymphs (which had recently engorged) were then placed in the field cages which were replaced in the wallow; two months later the proportion of ticks which had moulted was again assessed and another group of engorged ticks placed in the cages. These results acted as a 'bio-assay' of the number of day-degrees experienced. Table 4.13 shows the moulting behaviour of each instar during the different seasons of the year.

The temperature of the soil inside one field cage was recorded at hourly intervals on a chart recorder so that another estimate of the number of day-degrees experienced could be calculated. The comparison between the two estimates of day-degrees experienced is set out in Table 4.14 for the two periods, May 17 until August 2, and August 2 until October 15, 1970. It is clear from Table 4.13 that development virtually ceases during winter (between May and August) and that it proceeds relatively slowly during early spring (between August and mid-October). During the rest of the year the rate of development is much faster, but the device used to measure that rate was not sensitive enough to differentiate between the rates of development during the different periods.

The two estimates of the amount of effective temperature

Table 4.13

Moulting Behaviour of Engorged Nymphs when Exposed to Field Conditions during Different Seasons of the Year

Sample Size in Brackets

Period of Exposure	Percent Moult (n in brackets)		
	Larvae	1NN	4NN
16.3.70 - 17.5.70	100 (300)	99 (300)	96 (400)
17.5.70 - 2.8.70	25.3 (80)	0 (50)	0 (50)
2.8.70 - 15.10.70	64.6 (200)	0 (100)	0 (50)
15.10.70 - 7.1.71	100 (90)	100 (90)	43 (200)
7.1.71 - 13.3.71	100 (100)	100 (100)	91.2 (300)

Table 4.14

Number of Day Degrees Experienced by Ticks in the Field

Period of Exposure	Instar Exposed		
	Engorged Larvae	Engorged 1NN	Engorged 4NN
17.5.70 - 2.8.70			
Percent moulted	25	0	0
Estimated number of day-degrees experienced			
1. From Graph (95% limit)	74±15	<70	<40
2. From Temperature Records from field on Moralana	65	50	8
3. From Meteorological Records of Yudnapinna	91	62	12
2.8.70 - 15.10.70			
Percent moulted	65	0	0
Estimated number of day-degrees experienced			
1. From Graph	86±26	<70	<40
2. From Temperature Records on Moralana	90	75	40
3. From Meteorological Records of Yudnapinna	370	320	150

experienced during winter and early spring are in relatively close agreement. Thus it appears that the amount of development which occurs in the field can be calculated from field temperature records in conjunction with laboratory data on the development of the tick.

A third estimate of the number of day-degrees experienced during those periods of the year was calculated from the meteorological records from Yudnapinna station*. These values are the mean monthly maxima and minima at a depth of 1 inch for an eight year period. Because they are the average of an eight year period, the amount of development will be typical of an average year rather than a particular year. As can be seen in Table 4.14 the estimated amount of development calculated from these records for the period May 17 to August 2 is in relatively close agreement with the estimate of the amount of development which occurred during 1970 but that estimate for the period August 2 to October 15 is considerably greater than the amount of development which occurred during that period during 1970.

Thus the data from Yudnapinna give an over-estimate of the amount of effective temperature experienced. It must be noted, however, that these temperature records were taken from an area which was not shaded and so the temperatures are likely to be higher than those experienced in a shaded wallow. Nevertheless these temperature records can give an approximate, though exaggerated, estimate of the amount

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* Yudnapinna station is 40 miles south-west of Moralana and has a very similar climate.

of effective temperature experienced during an average year (Section 3.7).

4.5 Incubation Period

The effect of temperature and humidity on the rate of development of eggs of O. gurneyi was studied. The eggs used were all laid within one 24-hour period at 30°C. The eggs were then allotted to humidity chambers (described in Section 5.42) and placed at a range of temperatures. There were 100 eggs per treatment. The eggs were examined daily and the day each egg hatched was recorded. Four humidities (10, 75, 95 and 100%) at each of four temperatures (20°, 25°, 30° and 35°C) were examined.

The time taken for the eggs in each treatment to develop was expressed as the mean of the interval between the day that the eggs were assigned to the treatments and the mean day of hatching, i.e. the mean incubation period. The results are set out in Table 4.15.

All the embryos at 10% R.H. died from desiccation before they hatched. However it is clear from the rest of the data that within the limits studied, humidity had no effect on the rate of embryogenesis.

The mean incubation period was used as an index of the rate of embryogenesis. The regression of rate of development against temperature was calculated and the equation was $y = 0.95x - 26.1$ where y is the rate of development and x is the temperature (°C).

Table 4.15

The Effect of Temperature and Humidity on the Rate of Development of Eggs

	Mean Incubation Period (S.D. in brackets)			
Temperature (°C)	20	25	30	35
Relative Humidity (%)				
100	29.5(5.3)	14.2(2.7)	7.5(0.6)	5.8(0.7)
95	33.3(4.7)	15.0(3.4)	7.3(1.2)	6.0(0.9)
75	29.3(4.0)	14.0(3.4)	7.4(0.9)	5.8(0.7)
10	-	-	-	-

Thus the developmental zero was 16.7°C and the thermal constant, 104.5 day-degrees.

4.6 The Rate of Oogenesis

Engorged females do not begin to oviposit until a 'batch' of eggs has matured. Once begun, oviposition continues for a number of days. Thus the mean pre-oviposition period has been used as an index of the rate of oogenesis. However, even at one temperature, this rate has been found to vary widely from group to group and only some of the factors causing this variation have been elucidated; they include humidity and the incidence of diapause.

The effect of temperature on the rate of oogenesis was examined using a group of females which had oviposited at 30°C and had remained there until they were fed. After feeding they were divided into sub-groups of 50 to be placed at a series of constant temperatures between 15 and 40°C. Each sub-group of engorged females was divided into ten groups of five and placed in plastic vials 2" by 1" diameter, each containing 1 to $1\frac{1}{2}$ cm of sand and five male ticks. All treatments were kept in long photoperiod and between 10 and 20% R.H. The ticks were examined periodically and those which had oviposited (i.e. were astride an egg-mass) were removed and noted.

The oviposition behaviour of each group was expressed as a rate (the number of females beginning to oviposit per day) and it was plotted

against time. The histograms in Figure 4.7 are clearly bimodal and so there are two oviposition behaviour patterns within each group of females. The pre-oviposition period of the first peak decreases as temperature increases but the behaviour of the second group of peaks was erratic and did not appear to follow any trend. Thus the first peak was used when estimating the effect of temperature on the rate of oogenesis. The existence of this bimodality has been substantiated by the oviposition patterns of two other groups of ticks at 30°C which are also bimodal (Figure 4.8, Histograms A and B). However, many other groups of females did not show any obvious bimodality (Figure 4.8, Histograms C and D) and so considerable variability exists in both the timing and the relative proportions of the two peaks.

The mean pre-oviposition period for the first peak of the data in Figure 4.7 (the limits of the first peak were decided by eye) was used to calculate the regression of rate of development against temperature (°C). This equation gave a developmental zero of 25.8°C. This estimate is approximately what one would expect because the ticks oviposit at 20°C but not at 15°C.

At 30°C the mean pre-oviposition period for the first peak has been found to vary from 6.2 (S.D. = 2.3) days to 30.0 (S.D. = 18.0) days for different groups (Figure 4.9). Thus the thermal constant varies from 90 to 440 day-degrees depending on the group. In doing this I have assumed that the developmental zero does not change even

Figure 4.7 The Oviposition Behavior at a Range of Constant Temperatures.

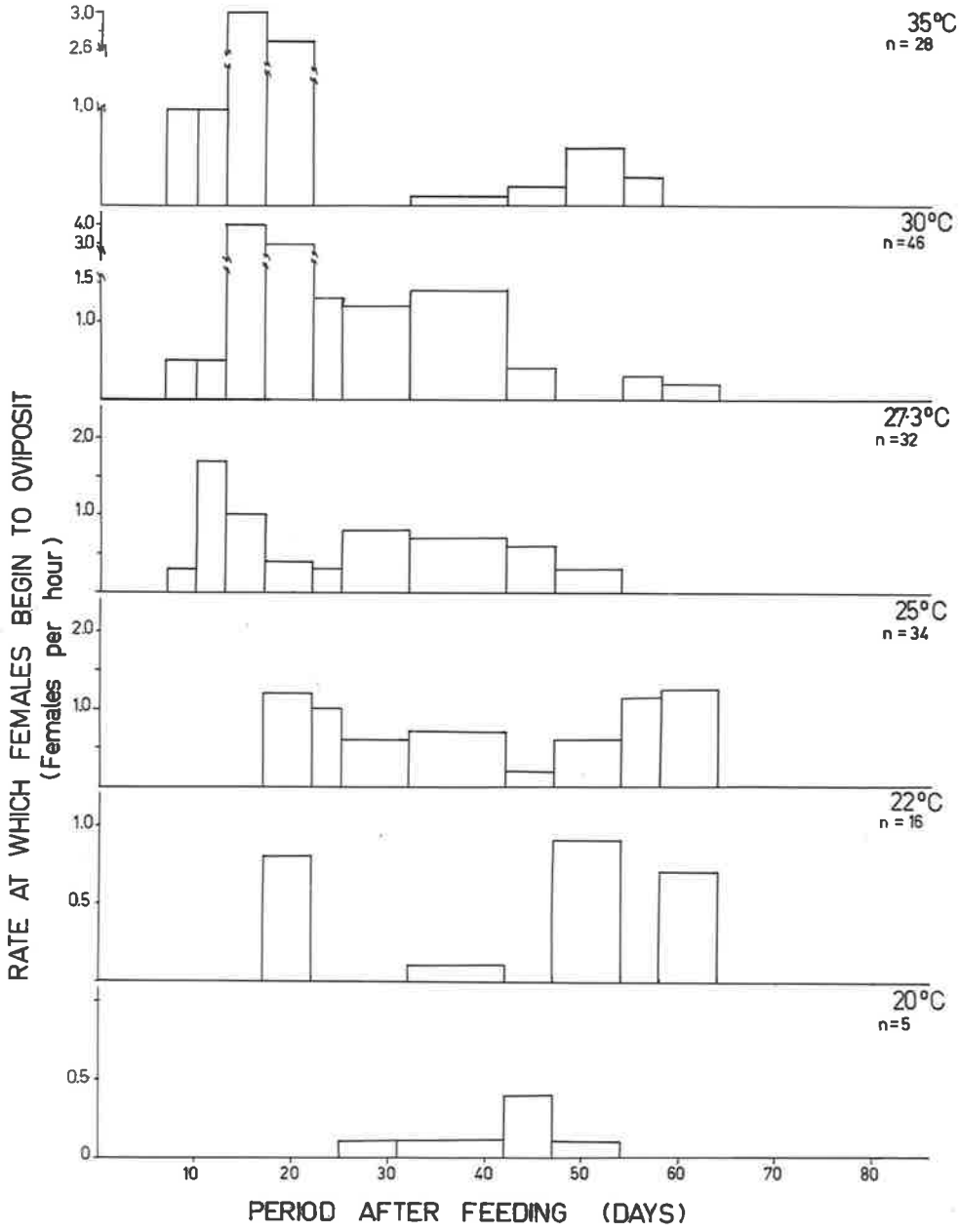

35°C
n = 28
30°C
n = 46
27·3°C
n = 32
25°C
n = 34
22°C
n = 16
20°C
n = 5
3.0
2.6
1.0
4.0
3.0
1.5
1.0
2.0
1.0
2.0
1.0
1.0
0.5
0.5
0
10
20
30
40
50
60
70
80
RATE AT WHICH FEMALES BEGIN TO OVIPOSIT
(Females per hour)
PERIOD AFTER FEEDING (DAYS)

Figure 4.8 The Oviposition Behavior of four Groups of Females at 30°C.

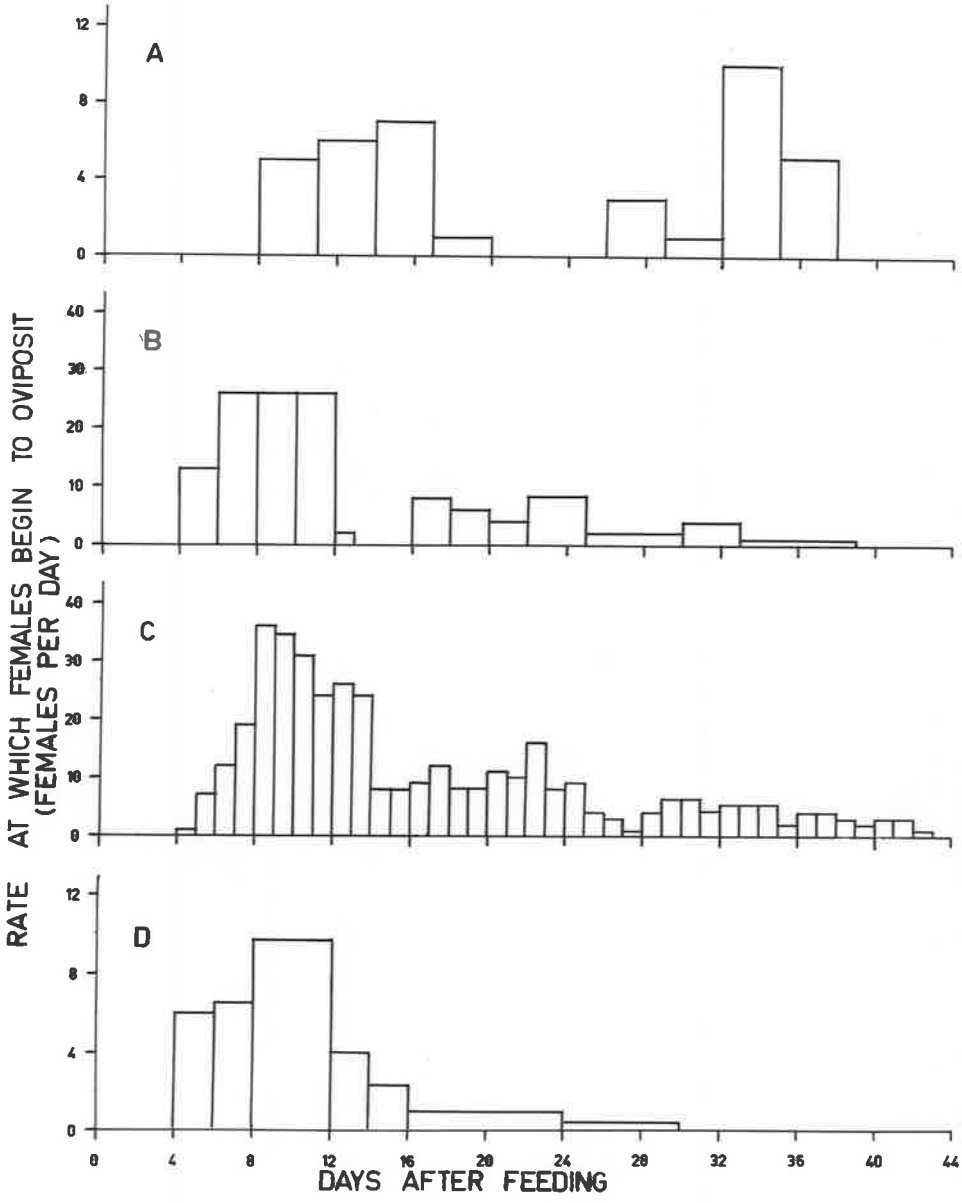
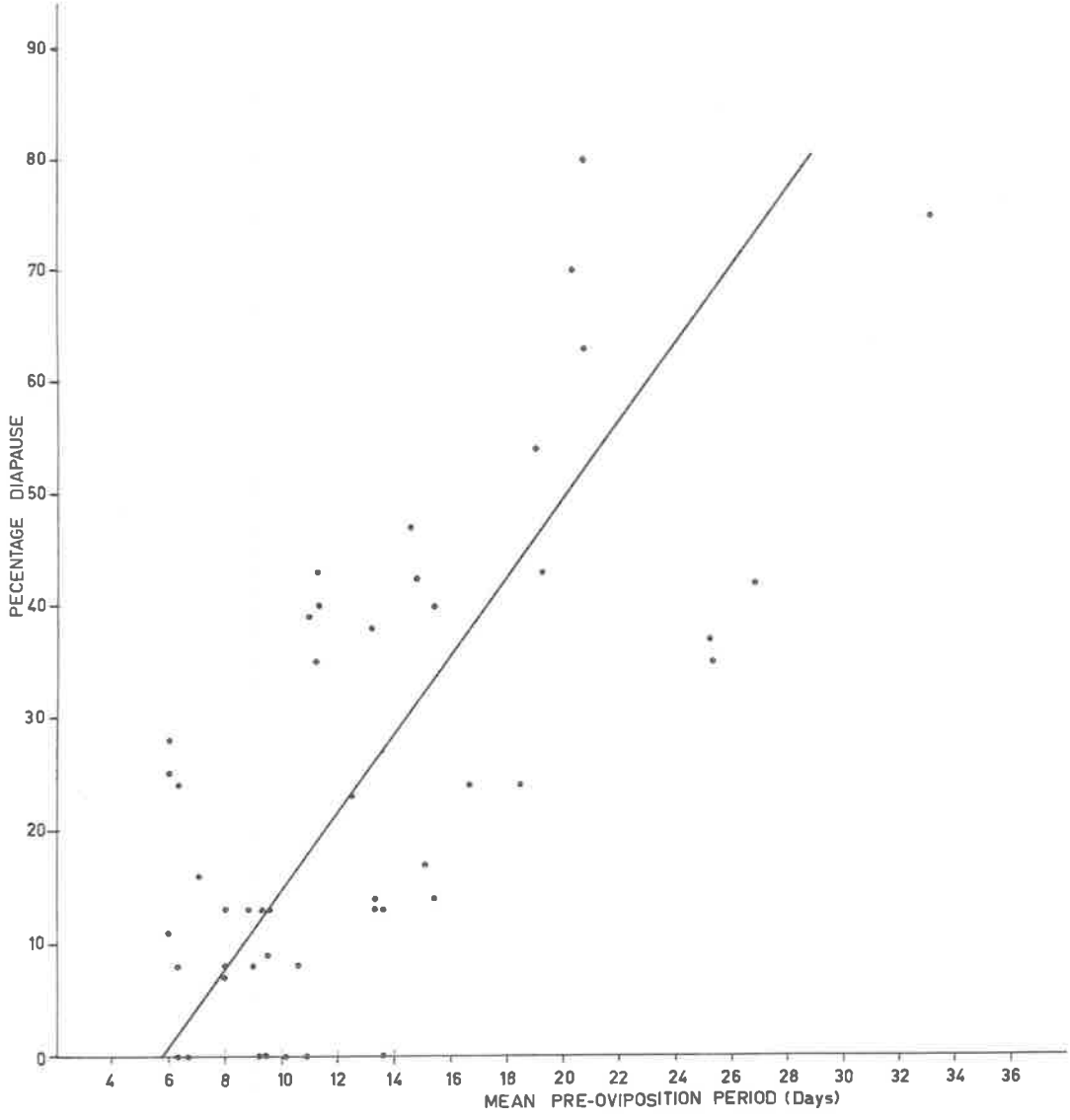

A
B
C
D
RATE AT WHICH FEMALES BEGIN TO OVIPOSIT (FEMALES PER DAY)
DAYS AFTER FEEDING
0
4
8
12
16
20
24
28
32
36
40
44

Figure 4.9 The Relationship between the Incidence of Diapause and the Mean Pre-oviposition Period at 30° C.

Regression Equation $y = 3.7x - 26.0$


PECENTAGE DIAPAUSE
0
10
20
30
40
50
60
70
80
90
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
MEAN PRE-OVIPOSITION PERIOD (Days)

though the rate of development does. A similar situation has been found by Hodek (1971a) for the bug Pyrrhocoris apterus. This bug has a reproductive diapause, and the mean pre-oviposition period varies from about 8 to 30 days at 30°C depending upon the season of the year. Similarly Masaki (1963) found that the rate of development (and hence the thermal constant) of the emma field cricket Teleogryllus emma varied with the incident photoperiod.

This prompted an examination of the relationship between the incidence of imaginal diapause (Chapter 6) and the mean pre-oviposition period. From the scatter diagram (Figure 4.9) it is clear that as the incidence of diapause in a group increased so does the mean pre-oviposition period. This trend was further analysed by calculating the regression of percentage diapause against mean pre-oviposition period. The regression was highly significant ($F = 41.6$, $P < 0.001$). Furthermore, as the mean pre-oviposition period increased so did the variance of the mean. This association was also analysed by a regression which was highly significant ($F = 87.3$, $P < 0.001$, $r = 0.95$).

This behaviour means that the thermal constant for groups of ticks varies according to the incidence of diapause in that group. In nature this will have the effect of slowing the rate of oogenesis during those months when the incidence of diapause is high. In order to investigate this possibility, the oviposition behaviour of females

taken from the field at different times of the year was assessed.

Section 6.4 deals with the changing incidence of diapause throughout a year. In Figure 4.10 the mean pre-oviposition period at 30°C is plotted against the time of the year when the ticks were captured. From these data it is clear that the mean pre-oviposition period of field ticks varies considerably throughout the year. A curve was fitted by eye through the points (Figure 4.10). From the curve it is possible to estimate the mean pre-oviposition period which was characteristic of each month. Using this and the developmental zero for oogenesis, I calculated the thermal constant characteristic of each month; it was found to vary from 170 to 440 day-degrees (Table 4.20). The way in which this phenomenon affects the life-cycle of the tick emerges in the discussion (Section 4.7).

4.62 Effect of Relative Humidity on the Rate of Oogenesis

A group of females was fed and divided into sub-groups which were placed at each of four relative humidities at 30°C. The oviposition behaviour was noted and the mean pre-oviposition period for each group was calculated. The results are shown in Table 4.16.

It is clear from these data that oogenesis proceeds more rapidly at high than at low humidity.

There is a possibility that the rate of oogenesis for a subsequent batch of eggs may have been affected also by the relative humidity experienced during and after a previous oviposition. To

Figure 4.10 The Relationship between the Mean Pre-oviposition Period (at 30°C) and the Season of the Year. (Curve fitted by eye)

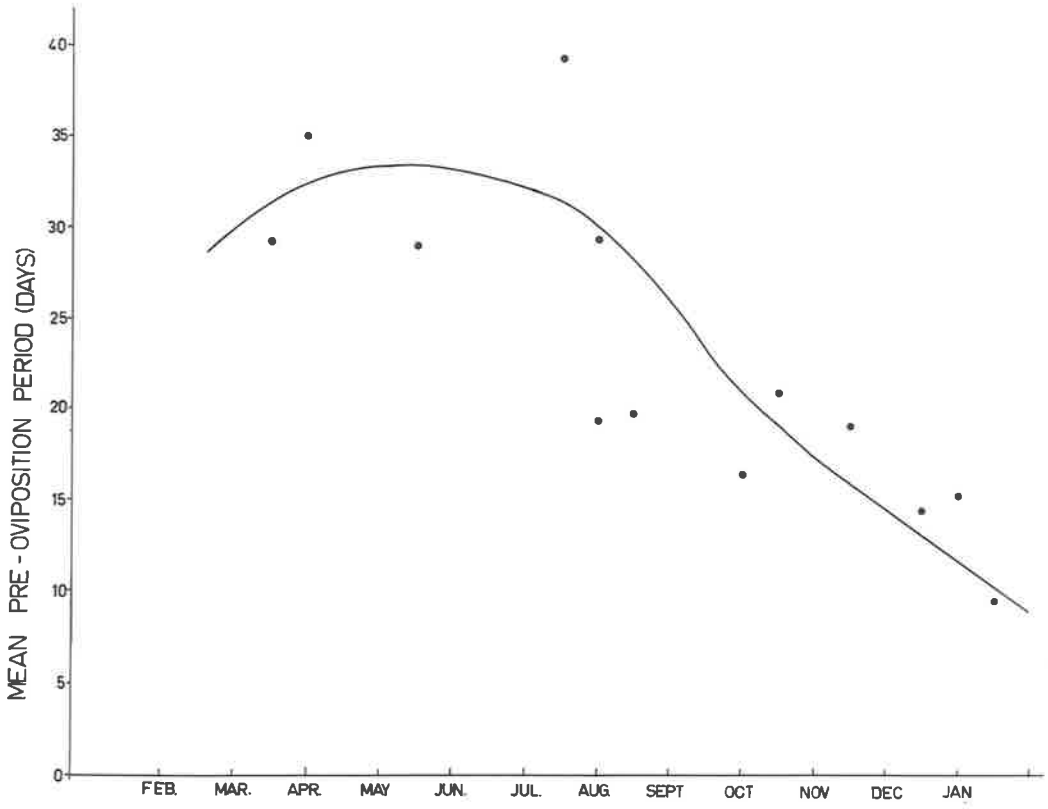

MEAN PRE-OVIPOSITION PERIOD (DAYS)
40
35
30
25
20
15
10
5
0
FEB.
MAR.
APR.
MAY
JUN.
JUL.
AUG.
SEPT
OCT
NOV
DEC
JAN

Table 4.16

The Effect of Relative Humidity on the Pre-oviposition Period of Engorged Females

Relative Humidity (%)	10	32.5	75	95
Mean Pre-oviposition Period (Days)	13.6	11.4	11.1	9.2
S.D.	6.0	4.9	4.5	3.7
Sample Size	37	33	33	31
Day-degrees	194	160	155	130

Table 4.20

The Thermal Constant Characteristic
of Each Month

January	February	March	April	May	June
170	280	370	440	440	440

July	August	September	October	November	December
440	370	310	300	270	240

test this, females which had oviposited, from the above experiment, were fed again and the rate of oogenesis was assessed at 30°C and 95% R.H. Those from 10 and 32.5% R.H. were pooled and compared with those from 76 and 95% R.H. As can be seen from Table 4.17, there was no effect of humidity.

4.7 Discussion

Temperature and humidity (to a lesser extent) have been shown to influence the rate of development and, in nature, these effects will help to determine the duration of the life-cycle. However, the duration of the life-cycle is also influenced by the availability of food; sometimes meals are separated by long periods. In this discussion, I consider the effect of seasonal fluctuations in temperature on the rate of development of all stages and, assuming that the food supply is not limiting, I examine the length of the life-cycle under field conditions.

The data in Table 4.14 have shown that the temperature records at 1" below the surface on Yudnapinna station give an approximate, though exaggerated, estimate of the effective temperature experienced by ticks in the field. The site where the temperatures were recorded was not shaded by trees, as wallows are, and so the mean maximum summer temperatures will be higher than those experienced by ticks in shaded wallows. This bias can partially be counteracted by assuming, for the purpose of calculating day-degrees, that the mean maximum does not rise

Table 4.17

The Effect of Pre-conditioning in High and Low Humidity on Subsequent Oogenesis

	Low R.H.	High R.H.
Mean Pre-oviposition Period (Days)	11.3	11.8
S.D.	3.5	5.1
Sample Size	53	57

above 37.5°C. This assumption is justified in a biological sense because development proceeds normally at 35°C, but is inhibited at 40°C. Thus these temperature records were considered to give an approximate, but slightly exaggerated estimate of the average temperature conditions experienced by ticks in wallows in these desert areas, throughout the year. Thus, calculations based on the Yudnapinna temperature records should give a useful estimate of the amount of development that occurs in each season.

The equations below were used to calculate the average number of day-degrees experienced during each month by each developmental stage, using the Yudnapinna meteorological data (Table 5.12). These equations assume that:-

(i) temperatures fluctuate in a regular way, and

(ii) development continues at a maximum rate at temperatures $\geq 37.5^{\circ}C$.

The results of the calculations are shown in Table 4.18.

T_{max} = maximum temperature (°C)

T_{min} = minimum temperature (°C)

D° = developmental zero

$\bar{T}$ = mean temperature $(\frac{T_{max} + T_{min}}{2})$

n = number of days in a month

Table 4.18

The Number of Day-degrees Experienced by each Instar during each Month of the Year Calculations are based on the meteorological data from Yudnapinna Station

Month	Oogenesis	Incubation	Larvae	Engorged 1NN	2NN	3NN	4NN
January	447	432	528	496	441	405	342
February	404	362	418	396	370	307	280
March	377	331	393	359	343	262	264
April	221	186	230	207	194	146	117
May	88	31	108	82	68	37	23
June	8	6	19	11	7	0.1	-
July	8	6	19	11	7	0.1	-
August	29	19	70	50	42	18	10
September	170	138	179	155	145	92	67
October	246	216	270	245	231	150	152
November	358	313	384	340	325	273	248
December	455	408	470	436	417	346	326

If $T_{min} > D^{o}$

Then number of day-degrees per month = $(\bar{T} - D^{o}).n$

Except when $T_{max} > 37.5$. $\therefore$ Take $T_{max} = 37.5$

No. day-degrees per month

$$= \frac{1}{2}n(\bar{T} + \frac{T_{max} - \bar{T}}{2} - D^{o}) + \frac{1}{2}n(\bar{T} - \frac{\bar{T} - T_{min}}{2} - D^{o})$$

If $T_{min} \leq D^{o}$ and $\bar{T} > D^{o}$, then

$$\text{No. day-degrees per month} = \frac{n}{2}(\bar{T} + \frac{T_{max} - \bar{T}}{2} - D^{o}) + \frac{n}{4}(\bar{T} - D^{o})$$

But, if $\bar{T} < D^{o}$, then

$$\text{No. day-degrees per month} = \frac{n}{4}(\frac{T_{max} - D^{o}}{T_{max} - \bar{T}})(T_{max} - D^{o})$$

If the number of day-degrees is divided by the thermal constant for that instar, the resultant factor will be the number of times that stage could be completed within a month, i.e. it is an index of the relative rate of progress through an instar (or the rate of development of that instar) during that month. The results are shown in Figure 4.11 and 4.12.

Because the developmental zero is lower in the early nymphal instars than in the later ones, the early instars develop during the colder months of the year while the later instars are quiescent

Figure 4.11 The Amount of Development which could occur in Engorged Larvae, 1NN, 2NN, 3NN and 4NN during different Seasons of an Average Year.

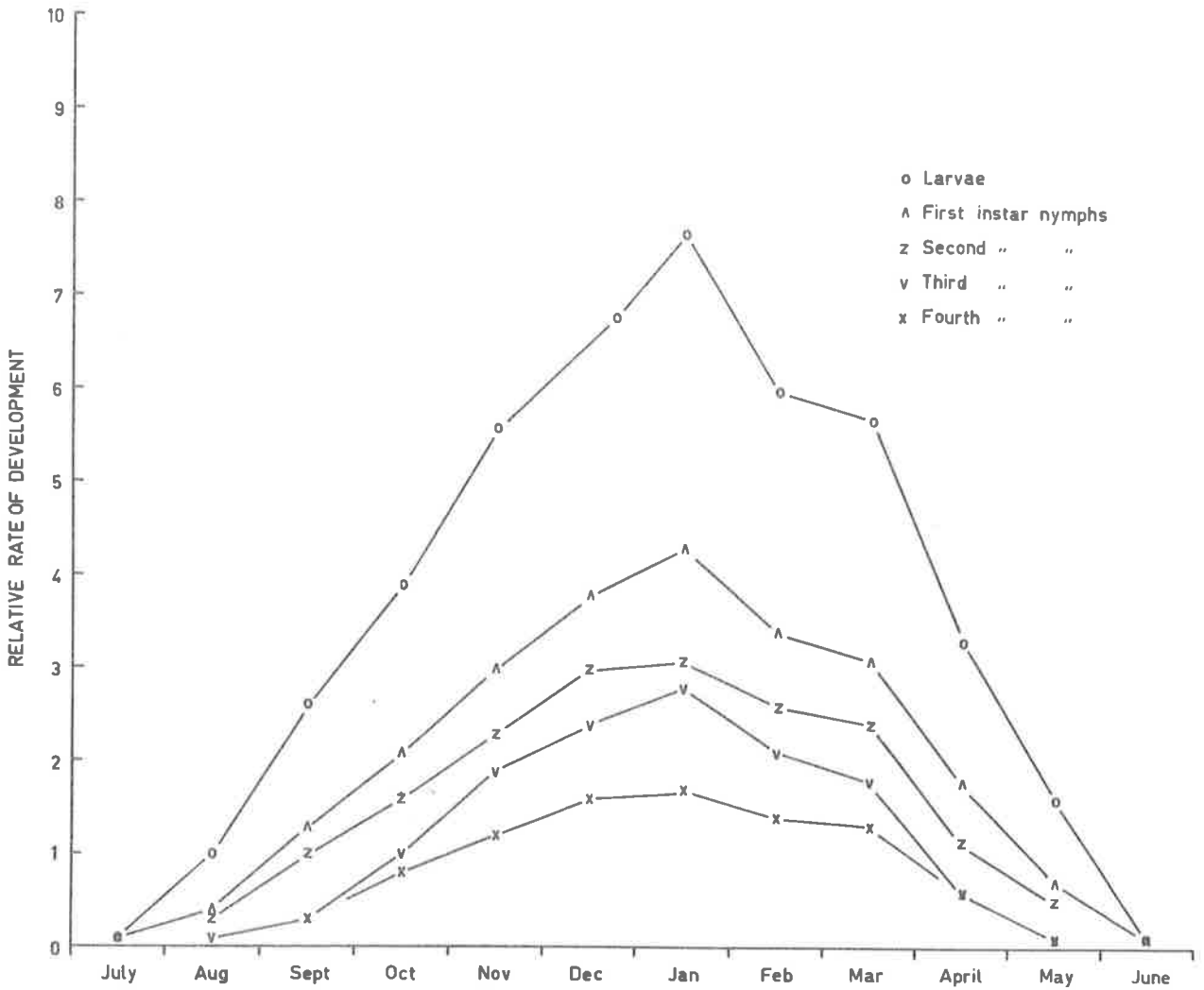
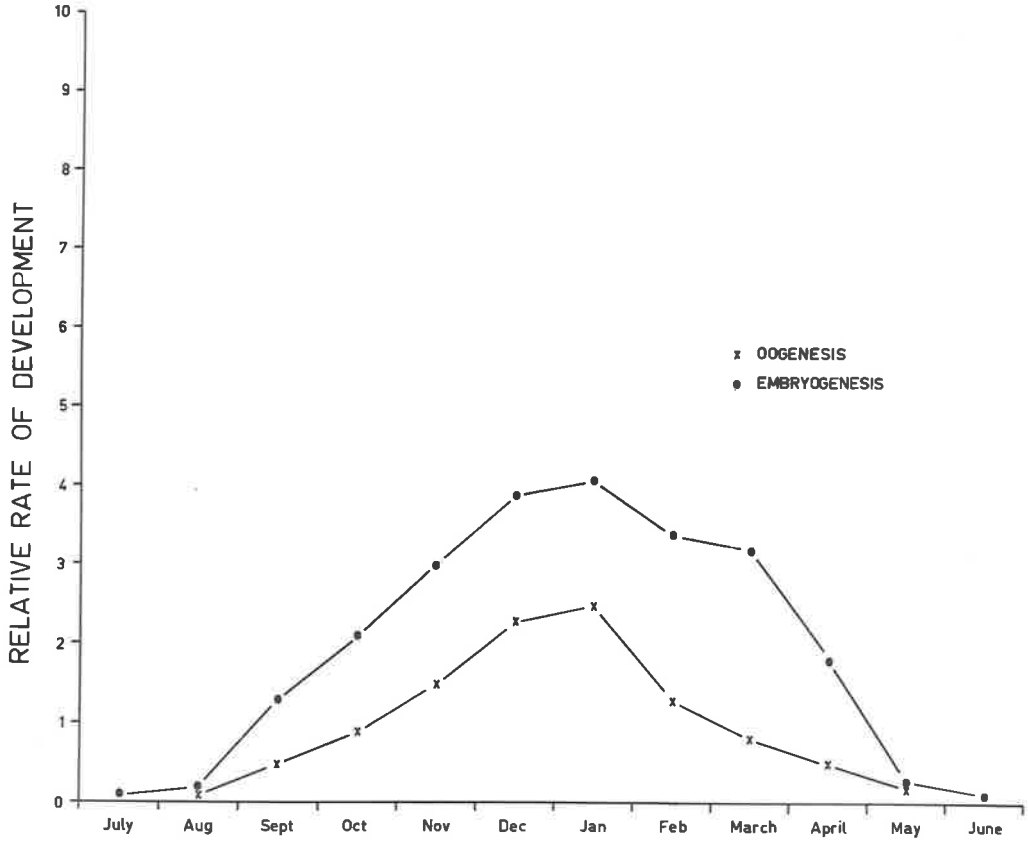

RELATIVE RATE OF DEVELOPMENT
10
9
8
7
6
5
4
3
2
1
0
July
Aug
Sept
Oct
Nov
Dec
Jan
Feb
Mar
April
May
June
o Larvae
∧ First instar nymphs
z Second ,, ,,
v Third ,, ,,
x Fourth ,, ,,

Figure 4.12 The Amount of Development which could Occur in Engorged Females and in Eggs during the different Seasons of an Average Year.


RELATIVE RATE OF DEVELOPMENT
10
9
8
7
6
5
4
3
2
1
0
July
Aug
Sept
Oct
Nov
Dec
Jan
Feb
March
April
May
June
OOGENESIS
EMBRYOGENESIS

(Table 4.13). Furthermore the rate of oogenesis at a constant temperature, varies depending on the season (Figure 4.10). Thus the duration of the life-cycle will vary depending on when during the year the life-cycle begins.

For the following calculations I have taken the engorged female as the beginning of the life-cycle and the newly-moulted female as the end. Allowing five days for 'hardening', and taking into account the time a tick spends on the host while feeding, I have calculated the mean duration of the life-cycle of the tick beginning on November 1, February 1, May 1 and September 1 (Table 4.19).

Beginning of Life-cycle	Siderial Duration of Life-cycle	Date of Completion of Life-cycle
November 1	4.0 months	February 27
February 1	10.3 months	November 9
May 1	8.7 months	January 22
September 1	5.2 months	February 5

If food is not limiting, the differential response to temperature of the early instars will have a synchronising effect on the population, causing the proportion of late instar nymphs in the population to increase as autumn and winter progress.

A life-cycle involves between five and seven meals (Chapter 2) and it will be shown later (Chapter 7) that wallows are visited by kangaroos so infrequently that one life-cycle probably takes between

Table 4.19

The Development of the Tick at Different Times of the Year

Female engorges	September 1	November 1	February 1	May 1
Female oviposits	October 17	November 20	February 22	October 5
eggs hatch	November 1	November 30	March 2	October 20
larvae harden	November 6	December 5	March 7	October 25
larvae detach	November 11	December 10	March 12	October 30
larvae moult	November 17	December 15	March 18	November 5
1NN harden	November 22	December 20	March 23	November 10
1NN moult	December 4	December 27	March 31	November 19
2NN harden	December 9	January 1	April 5	November 24
2NN moult	December 22	January 11	April 25	December 6
3NN harden	December 27	January 16	April 30	December 11
3NN moult	January 8	January 28	September 30	December 24
4NN harden	January 13	February 2	October 5	December 29
4NN moult	January 31	February 22	November 4	January 17
Females harden	February 5	February 27	November 9	January 22

two and five years. Thus this synchronising effect is unlikely to contribute greatly to the annual changes in the structure of the population.

Because the wallows are visited infrequently, the survival of the tick during the intervals between visits will be crucial to the maintenance of the population. The following chapter examines this aspect of the biology of the tick.

CHAPTER 5

Longevity of *O. gurneyi*

5.1 Summary

This chapter is concerned with the question 'how long does each stage persist without a meal'?

Larvae, nymphs and adults were exposed to desiccating conditions and their longevity was noted. The ability of nymphs, larvae and adults to absorb water from unsaturated air and the influence of this capacity on longevity was studied. The aridity of field conditions is discussed and the longevity of ticks in the field was examined.

Eggs and larvae proved very susceptible to desiccation. The rate of desiccation is determined by saturation deficit and not by relative humidity. Larvae survive for only a few days at low relative humidity. In the absence of any stress due to desiccation the larvae starve to death; the longevity is inversely proportional to metabolic rate.

On the other hand nymphs and adults are extremely resistant to desiccation and can survive at least for several months in dry conditions (s.d. = 30 mm Hg). Periodic rehydration permits the ticks to persist for many more months without a meal. Rehydration occurs relatively slowly (3% of body weight per day), and to complete rehydration may take ten days or more.

Because of intermittent showers of rain and relatively low temperatures, winter, spring and autumn pose little threat to the survival of nymphs and adults. Summer, however, can be hot and dry for protracted periods. If the ticks fail to feed during summer neither late instar nymphs nor adults are likely to die from desiccation or starvation but a proportion of the first instar nymphs may perish. However during summer kangaroos use the wallows most frequently and so the chance of feeding (and hence rehydrating) is relatively high.

The three aspects of the water relations of the tick which enable it to persist in these arid climes are:

(1) The ability to resist desiccation. Late instar nymphs have survived hundreds of days at 10% R.H. at 30°C.

(ii) The ability to tolerate the loss of a high proportion of its body water. Females can rehydrate and survive after losing two thirds of their body water.

(iii) The ability to rehydrate by absorbing water from unsaturated air. Rehydration at 98% R.H. can take 10 to 20 days.

5.2 Literature Review

Desert-dwelling animals have evolved two principal mechanisms which reduce the rate at which they desiccate and so allow them to persist in their arid habitat. They are:-

(i) behavioural mechanisms which restrict animals to the least arid areas within the desert: for example, woodlice (Edney, 1957), or permits them to venture into the more arid areas only during the least arid time of the day: for example, the tick Hyalomma asciaticum (Balashov, 1960) or the scorpion Hadrurus (Hadley, 1970).

(ii) physiological mechanisms which permit them to tolerate desiccation.

A few species of tick are noted for their mobility. For instance Hyalomma asciaticum is able to cover distances of several metres in search of a suitable habitat (Balashov, 1959). However, most species are relatively sedentary when not attached to their hosts, and so the places where they live are determined to a large extent by where they detach from their hosts; in the desert these places are likely to be arid. The physiological mechanisms which allow animals to persist in these arid areas are of four types:-

(i) the ability to resist desiccation,

(ii) the ability to tolerate desiccation,

(iii) the ability to rehydrate by extracting water from unsaturated air,

(iv) the ability to produce water from oxidation.

(i) The Ability to Resist Desiccation

Water may be lost by cuticular and respiratory transpiration and by defecation and excretion. In ticks, very little information is available concerning the quantitative aspects of excretion in relation to water balance. However the blood-sucking insects Rhodnius (Wigglesworth, 1931), Glossina (Lester and Lloyd, 1928; Jack, 1939; Bursell, 1960) and Cimex (Mellanby, 1932a) have been studied in some detail. In the blood-sucking insects and in ticks, a liberal excretion of fluid takes place towards the end and soon after a blood meal. This 'primary' excretion (Jack, 1939) is usually completed within the first hour or so of detaching. Subsequent excretary products contain some water but the amount, at least in Glossina, depends upon the state of hydration of the fly and is to a large extent independent of the saturation deficit of the air. In ticks there is usually obvious excretion immediately after the moult but the amount of subsequent excretion is minute. Thus it appears that excretion, except immediately after feeding and moulting, contributes only slightly to the total water loss.

Most previous studies of water loss in Arthropods have stressed either changes in cuticular permeability with increased temperature and-or saturation deficit (Wigglesworth, 1945; Mead-Briggs, 1956; Beament, 1959; Beament et al., 1964), or spiracular control of respiratory evaporation (Mellanby, 1932; Browning, 1954; Bursell, 1957), so that

the relative contribution of the two pathways over an animal's normal temperature range remains speculative. Recently Loveridge (1968a, b) investigated both pathways and found water loss and ventilation to be interdependent: similarly Hadley (1970) demonstrated a close relationship between oxygen consumption and transpiration in Hadrurus arizonensis. Both ventilation rate and oxygen consumption were a function of temperature. Thus at low temperatures cuticular water loss makes up a large proportion of the total water loss but as temperature approaches the lethal limit (40°C for Hadrurus) there is a rapid increase in respiratory water loss and this becomes the major pathway for water loss.

The rate at which a whole animal loses water by transpiration varies with the physiological condition of the animal, but for a particular animal it is a function of the saturation deficit (s.d.) and not of the relative humidity (R.H.). As the s.d. increases, the rate of water loss increases steeply to a plateau, and then the transpiration rate remains constant despite some further increases in s.d. As the drying power of the air is further increased a second rise in transpiration rate occurs. These effects are presumed to reflect an active regulation of water loss by the spiracles (Bursell, 1964).

The phenomenon of transpiration decreasing with time at the

same s.d. has been demonstrated in Agriotes and Aphodius larvae (Wigglesworth, 1945), in Pieris pupae, Rhodnius nymphs, and young Tenebrio pupae (Beament, 1959) and in the African migratory locust, Locusta sp. (Loveridge, 1968a). In all cases the decrease was observed at high s.d. This is possibly due to the decreasing water content of the animal.

(ii) The Ability to Withstand Desiccation

The water content of arthropods is usually taken as the difference between fresh weight and the dry weight and is often expressed as a percentage of the wet weight. Although the difference in weight may represent in part, the volatile constituents of the body other than water, it is probably reasonable to take this loss in weight as representing the water content of the animal at death.

The water content of a newly-emerged tsetse fly was between 75 and 77% (Jackson, 1937; Jack, 1939; Bursell, 1959). This is also the level to which the water content adjusted after the fly had taken a blood meal (Bursell, 1960), and may reasonably be taken to represent the water content of the fully hydrated insect. Similar values have been given for newly-emerged specimens of the garden chafer, Phyllopthera (Laughlin, 1956) and of Musca (Pearnicott, 1960). The firebrat, a heat adapted animal, contained 66% water (Noble-Nesbitt, 1969). These values are quite normal for most arthropods (Bursell,

1964; Rapoport and Tschapek, 1967).

This parameter has also been assessed for some ticks. Belozerov (1967a) has shown that the water content of fully hydrated Ixodes ricinus was 53% before feeding and 59% after feeding; rapid hydration to 48% water proved lethal to one third of them. The water content of "hungry" Dermacentor marginatus was 56% in males and 58% in females; this rose to 62% after feeding. Hafez et al. (1970) gave the rate of weight loss and the longevity of various developmental stages of Hyalomma dromadarii and Ornithodoros savignyi at a range of relative humidities. From these data I extrapolated to the weight of the tick at the time of death. (The rate of weight loss remained relatively constant after moulting.) It appeared that a tick died when it had been reduced to between 45 and 25% of its original weight. This loss includes metabolic loss which could be considerable in long-lived individuals. Nevertheless it suggests a degree of toleration of desiccation rarely found in insects. There is, however, one notable exception. The larva of the chironomid Polypedilum is extremely tolerant to desiccation and will rehydrate and metamorphose after it has been subjected to more-or-less complete dehydration (Hinton, 1960).

The extent to which the water content may be reduced before death supervenes has been discussed by Wigglesworth (1950) and Bursell (1964), and it would seem that in insects, decreases from 75 to 60% can usually be tolerated. In the tsetse fly the critical water content

is between 61 and 64% depending on the species (Bursell, 1959) (% expressed as % water in the animal at the time of death). Thermobia can lose up to 40% of its body weight (i.e. water content 50%) and yet rehydrate. However the two studies of ticks in which this aspect of their water physiology has been examined suggest that the water content of ticks may be lower than that of insects, and furthermore that ticks may be more able than insects to tolerate desiccation (Belozerov, 1967; Hafez et al., 1970).

(iii) The Ability to Rehydrate by Absorption of Water from Unsaturated Air

The phenomenon of absorbing water from unsaturated air occurs in a wide range of arthropods. Amongst insects the following possess the ability:- the mealworm, Tenebrio molitor (Buxton, 1930; Mellanby, 1932b; Locke, 1964), the grasshopper, Chortophaga viridifasciata (Ludwig, 1937), the pre-pupae of the flea, Xenopsylla cheopis (Edney, 1947; Knulle, 1967) and its larvae (Knulle, 1967), the firebrat, Thermobia domesticus (Beament et al., 1964; Noble-Nesbitt, 1969, 1970), the nymphs and adults of the cockroach, Arinevaga sp. (Edney, 1966) and the psocopteran, Liposcelis (Knulle and Spadaforda, 1969). The phenomenon is also widespread in the Acarina. Lees (1946) demonstrated it in five species of tick, including Ornithodoros moubata. Browning (1954) expanded the study of O. moubata; other cases include

Alectorobius (= Ornithodoros) tholozani (Belozerov and Seravin, 1960), the rabbit tick, Haemaphysalis leporispulustris (Camin, 1963), some Argasids (Balashov and Filipova, 1964), the larvae of three species of Dermacentor (Knulle, 1966), Hyalomma asciaticum (Balashov, 1960), and Ornithodoros savignyi and Hyalomma dromadarii (Hafez et al., 1970; Hefnawi, 1970). Amongst other Acarina water vapour uptake has been reported in the grain mite, Acarus siro (Knulle, 1962; and others) and in the spiny rat mite, Ecinolaelaps echidninus (Wharton and Kanugo, 1962; and others).

This process has several properties and these appear not to differ from the insects to the acari. They are:-

(i) The humidity at which the animal neither gains nor loses water, the critical equilibrium humidity (CEH) usually lies between 80 and 95% relative humidity (R.H.) although there are instances where the C.E.H. is as low as 45% R.H.

(ii) The humidity at which the process begins to operate is a function of relative humidity and not of saturation deficit. (Nevertheless at humidities below the C.E.H. the rate of water loss is a function of saturation deficit.)

(iii) The process is an active one and so energy is expended in absorbing water. The amount of energy spent, however, is only a small fraction of that used for normal metabolic processes.

(iv) The rate at which water is absorbed varies with the species, with the R.H., and the temperature, and is faster at higher R.H.s and temperatures.

(v) The site of absorption varies with the species but the cuticle, the anus and the spiracles have all been implicated.

(vi) As an animal becomes senile, its capacity to absorb water decreases and its C.E.H. increases until it can maintain body weight only in a saturated atmosphere. The process is also inhibited temporarily during moulting and during feeding in blood-sucking animals.

(vii) When a healthy animal rehydrates, it absorbs water until it reaches a water content characteristic of fully hydrated animals.

Lees (1946) found that the C.E.H. of unfed females of Ixodes ricinus, I. canisuga, I. hexagonus, Amblyomma cajennense, A. maculatum, Dermacentor andersoni, D. reticulatus, Rhipicephalus sanguineus and Ornithodoros moubata ranged from 82 to 96%. The C.E.H. for Hyalomma asciaticum is about 80% R.H. (Balashov, 1960) and for larval Dermacentor andersoni, D. variabilis and Amblyomma cajennense it varies from 80 to 85% R.H. (Knulle, 1966). Hafez et al. (1970) state that the C.E.H. of each developmental stage of Hyalomma dromedarii and Ornithodoros savignyi was about 75% R.H. and 75-84% R.H. respectively. Furthermore they found that the C.E.H. was lower in the early instars than in the late instars and adults.

By sealing the spiracles and the anus of dehydrated ticks and then exposing them to high humidity Lees (1946) showed the cuticle to be the site of uptake of water in three Ixodid species. However this technique has given equivocal results in several cases [Ornithodoros moubata (Lees, 1946), Ornithodoros savignyi and Hyalomma dromadarii (Hafez et al., 1970)]. Although these species failed to absorb water when their spiracles were blocked, the authors concluded that the spiracles were unlikely sites for the absorption of water vapour. Belozerov and Seravin (1960) obtained similar results with Ornithodoros (Alectorobius) tholozani; but they concluded that the respiratory system itself was the site of water uptake.

The longevity of ticks in the absence of suitable hosts depends primarily on the relative humidity of the atmosphere. Knulle (1966) pointed out that above the C.E.H. ticks can survive for months, or even years, while below this humidity they die in a comparatively short time. For example, unfed female Ixodes ricinus kept at 78% R.H. at 25°C usually survive for three months or more, whereas at 70% R.H. they die in 4-6 days (Lees, 1946); but they may survive as long as 27 months under favourable conditions (Nuttal and Warburton, 1911). Larvae of Dermacentor variabilis survive without food for 400 to 500 days under favourable conditions (Smith, Cole and Gouch, 1946). Feldman-Musham (1947) also reported considerable longevity at humidities close to saturation for larvae and nymphs of Hyalomma savignyi and (1951) for

adults of the same species. Larval Ixodes hexagonus soon die when exposed to dry conditions but when kept at humidities close to saturation they survive for up to 15 months (Arthur, 1951). Larval Boophilus microplus survive 240 days at 90% R.H., 22.2°C but only 12 days at 70% (Hitchcock, 1955).

The successful hatching of eggs of Dermacentor variabilis and Amblyomma americanum is similarly dependent upon humidity (Sonenshine and Tigner, 1969). Almost all the eggs in undisturbed egg masses of D. variabilis hatched when the R.H. was between 95 and 65% at 27°C (s.d. = 9.3 mm Hg) but few hatched from egg masses stored at lower humidities. Eggs of A. americanum were even less resistant and only a small proportion hatched at 65% R.H. and none hatched at 55% R.H. (s.d. = 11.9 mm Hg). These data were used to estimate the proportion which should hatch in different types of vegetation where records of the R.H. and temperature for these areas were available.

The longevity of Argasids is even more outstanding. The data of Pavlovsky and Skrynnik (1960) show that individuals of Ornithodoros papillipes survive without a blood meal for 11 years, Alveonasus lahorensis for 10 years and the smaller Ornithodoros nereensis, O. alactagalis and O. tartakovskyi for $1\frac{1}{2}$ to 4 years. Adults of Ornithodoros p. porcinus (= moubata) held at 85% R.H. and 22°C have a 50% survival rate after 5 years of starvation (Walton, 1964). Clifford

(1921) studied the longevity of different developmental stages of O. moubata at temperatures between 22° and 27°C and found the mean survival period to range from 11 weeks to 2 years for fed imagos, and from 7 weeks to 1 year for nymphs, depending upon temperature.

The larvae of Argasids, however, are less hardy than the nymphs and adults and may survive for a few weeks (O. tarkakovskyi) to several months (O. lahorensis, O.papillipes, Argas persicus) (Pavlovsky and Skrynnik, 1960). Brett (1939) studied the survival of the eggs, larvae and first instar nymphs of O. moubata at 25°C between 6 and 85% R.H. Humidities down to 50% allowed a high proportion to survive; at lower humidities a lower proportion survived but Brett believed that a proportion of eggs was able to develop to the first nymphal instar at any "low humidity normally met with in nature". Thus high humidities favour survival but sometimes ticks survive at low humidities. This finding appears contrary to that of Cuncliffe (1921) who found that very high humidities (close to saturation) were deleterious. Hafez et al. (1970) found that the earlier stages of O. savignyi and Hyalomma dromedarii (two species of desert-dwelling tick) were more susceptible to desiccation than were the later instars. It is noteworthy that in this comparison the Argasid was significantly more long-lived than the Ixodid (at 96% R.H. the first nymphal instar of O. savignyi lived, on the average, for 243 days whereas that of H. dromararii lived only 52 days).

Two further points of note are:-

(i) The rate of water absorption in Argasids is much slower than in Ixodids. For instance, the Ixodids cited by Lees (1946) absorb water at a rate of between 3 and 20% of their original weight per diem whereas Ornithodoros moubata's rate was only 1.5% per diem. Similar results were obtained by Hafez et al. (1970). Ornithodoros savignyi took 10 to 20 days to rehydrate fully whereas Hyalomma dromedarii was fully hydrated within 7 days.

(ii) The absorption of water from unsaturated air does not operate during feeding and moulting.

(iv) Water from Oxidation

Another factor which might contribute to survival in arid areas is water from oxidation (metabolic water). Bursell (1959a) states that metabolic water in the tsetse fly contributed about 10% of the total water reserves of starving flies in dry air. On the other hand Edney (1966) claims that the desert cockroach Arenivaga derived only a small fraction of the net water gained from oxidative water. Long-lived species with considerable food reserves should gain an appreciable quantity of water from such a source.

Rate of Weight Loss and Longevity

The ability of different species of tick to resist desiccation shows considerable variation. Lees (1946) showed that of the eight

species he examined at 0% R.H. and 25°C, "Ornithodoros, Dermacentor and Rhipicephalus were the most resistant, losing only 1-3% of their original weight per diem and surviving 35, 27 and 17 days respectively". Ixodes ricinus, however, died in 1-2 days under these conditions. Other ticks have been known to live for many months under similar conditions (Hafez et al., 1970).

The three main points that emerge from this discussion are:-

(i) At R.H. ≥ C.E.H. ticks can survive for a long time, but below the C.E.H. the longevity is drastically shortened.

(ii) The eggs, larvae, early instar nymphs and adults are progressively less susceptible to desiccation.

(iii) Argasids are longer lived and more resistant to desiccation than are Ixodids.

5.3 Introduction

This chapter is concerned with the manner in which ticks gain and lose water and with the physiology of these exchanges. From a knowledge of the water relations under experimental conditions and the conditions of moisture and humidity normally encountered by the kangaroo tick in nature, we can perceive something of the significance of these phenomena under natural conditions.

The deserts of inland Australia, like the hot deserts of other continents, are very arid during much of the year. Kangaroo ticks live

buried in the sand of wallows underneath shade trees in some of these areas. The sand underneath the trees is shielded from direct sunlight during some of the day. Furthermore the soil stays damp for some time after rain and so the ticks in the sand are protected, temporarily, from the desiccating effect of the atmosphere. Thus the ticks are not exposed to the extremes of temperature and dryness that surface-dwelling animals experience; nevertheless the environment of the tick is frequently hot and dry.

The principal host of the tick is the red kangaroo. However kangaroos are semi-nomadic animals and so, because there are many shade trees and relatively few kangaroos in these desert areas, the chances of any one wallow being visited frequently are low. Furthermore during the winter, kangaroos use the wallows infrequently (Section 7.1). Thus the ability to survive extended periods without a meal must be one of the most crucial adaptations of the tick to its life in the desert.

The aim of this chapter is therefore, to answer the question 'how long can each stage persist without a meal?'. This period will depend on the temperature and humidity experienced and the stage exposed. Temperature will determine the metabolic rate and hence the rate at which energy reserves are depleted, and humidity (or saturation deficit) will determine the rate at which the animal desiccates.

Four aspects of the survival of the tick were studied in the laboratory, namely:-

(i) The resistance of each stage to the lethal effect of prolonged exposure to low relative humidity.

(ii) The effect of temperature on the longevity of larvae when they are not stressed by desiccation.

(iii) The degree of desiccation which can be tolerated before the tick dies.

(iv) The question of rehydration by absorption of water from unsaturated air.

The validity of predictions made by extrapolating from laboratory results to the field situation was tested by examining the longevity of ticks exposed to field conditions.

5.4 Survival: Starvation, Humidity and Temperature

In most species of ticks studied, the eggs and larvae are the stages which are most vulnerable to death by desiccation (or starvation). The proportion of eggs which hatch and the longevity of the larvae in relation to the availability of the host will have a profound effect on the population dynamics of the species. Thus the effects of a range of temperatures and humidities on the survival of eggs (i.e. successful hatching) and on the longevity of larvae were studied.

TABLE 5.1

The percentage of eggs failing to hatch after exposure to the conditions described in the Table (n = 100 per treatment)

Temperature °C	20	25	30	35
Humidity				
100%	4	3	7	9
95%	9	9	10	10
75%	8	6	12	12
0%	100	99	99	100

TABLE 5.2

The effect of aridity on the pre-collapse period
(A range of temperatures at 0% R.H.)
n = 100 per treatment

Temperature °C	Saturation deficit	Mean Pre-collapse Period	S.D.
20	17.5 mm Hg	17.4 days	8.8
25	23.8 " "	7.4 "	3.2
30	31.8 " "	5.3 "	0.8
35	42.2 " "	< 3 "	-

5.41 Survival of Eggs

The experiment in Section 4.5 examined the effect of temperature and humidity on the incubation period of eggs. At low humidities most eggs shrivelled up and the embryo died in various stages of development, depending upon the temperature and humidity to which they had been exposed. Even at high humidities there was a proportion which did not hatch although they did not collapse until long after the other eggs in the treatment had hatched (Table 5.1). Very few of these eggs had reached a stage of development at which embryos could be recognised under x40 magnification. Thus I concluded that 3-9% was the proportion of eggs which were unable to develop (perhaps due to infertility). Other observations have given a similar proportion which hatch successfully (97% hatch, at 30°C and 76% R.H.; n = 400).

It is also noteworthy that at 0% R.H. the eggs shrivelled and the embryo died much more quickly at high than at low temperatures. The time taken for each egg to collapse had been noted and so the mean of that period (the pre-collapse period) for the eggs in each treatment was calculated and taken as an index of susceptibility to the treatment. The results are shown in Table 5.2.

From these data it is clear that s.d., and not the R.H. determines the rate at which eggs desiccate. The relationship between

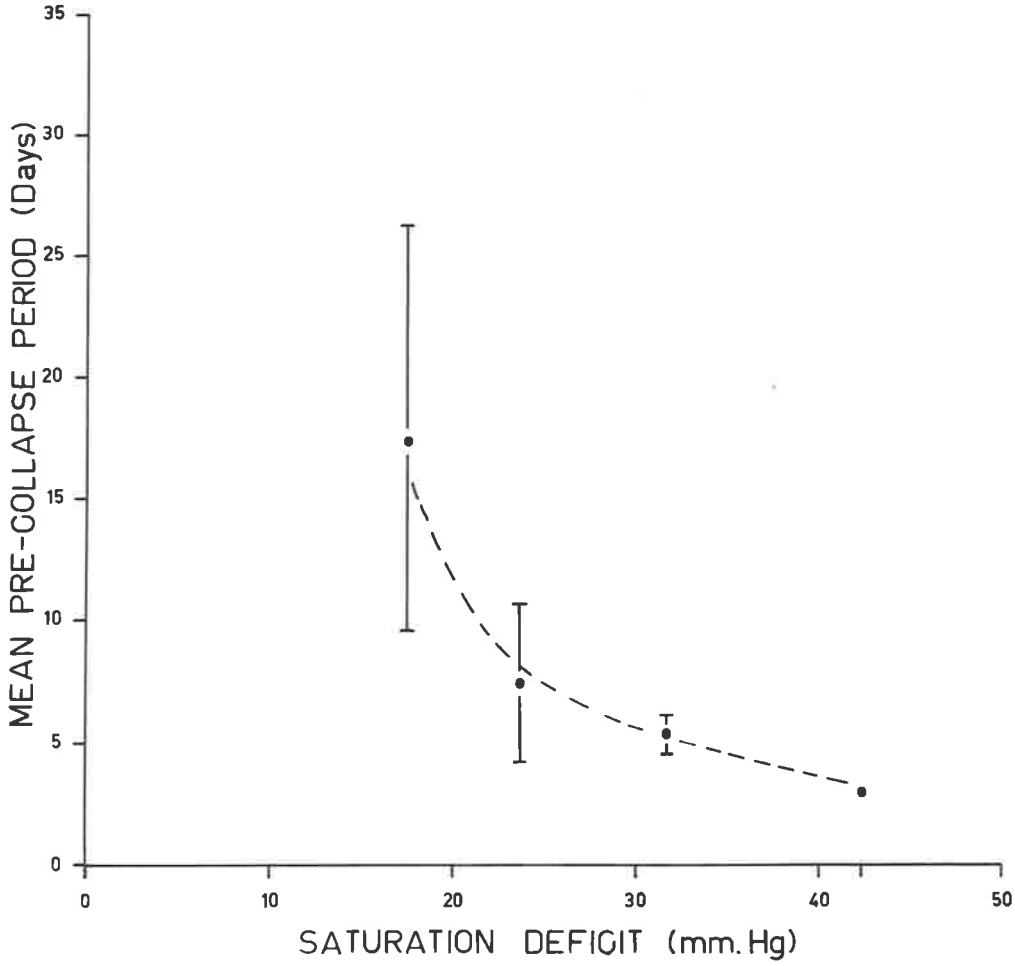
s.d. and the mean pre-collapse period is plotted in Figure 5.1.

Many of the embryos at 20°C, 0% R.H. (s.d. = 17.5 mm Hg) were well developed before they died. Thus a s.d. of 17.5 mm Hg almost permits successful development and hatching. At 35°C and 75% R.H. (s.d. = 10.1 mm Hg) a high proportion of the eggs hatch successfully. Thus the critical s.d. below which most eggs survive and hatch, and above which most eggs perish must lie within the range 10 to 17 mm Hg. As mentioned before (Section 5.2), a similar situation was found in both Dermacentor variabilis and Amblyomma americanum, where the critical s.d. was about 10 mm Hg.

These data, however, apply to solitary eggs which were resting on a wire mesh. The eggs of O. gurneyi are laid in clumps of 200-800; the female sits on and 'broods' the egg-mass until she is disturbed or until the eggs hatch. The chances of an egg surviving might be quite different for an egg within an egg-mass which was brooded by a female while buried in sand.

To test this possibility, engorged females were placed in shallow sand and examined daily. When a batch of eggs had been laid the tube containing the female was filled with sand to a depth of 2 cm and transferred to a desiccator at 0% R.H.at either 30° or 35°C. After 15 or 20 days the tubes were examined and the proportion of eggs which had failed to hatch was noted.

Figure 5.1 The Influence of Saturation Deficit on the Survival of Eggs of *O. gurneyi*.


MEAN PRE-COLLAPSE PERIOD (Days)
35
30
25
20
15
10
5
0
0
10
20
30
40
50
SATURATION DEFICIT (mm. Hg)

It is clear from a comparison of the results in Table 5.3 with those in Table 5.1 that eggs in clumps hatched successfully in atmospheric conditions which were so dry that solitary eggs would die from desiccation in less than three days. Furthermore it appears that the larvae were also protected from desiccation because, when brooded, they lived for many days more than solitary larvae would, under the same conditions (Table 5.4). Thus the habit of brooding the egg-mass (and the subsequent larvae) protects the eggs and the larvae from the desiccating effect of the atmosphere.

5.42 The Longevity of Larvae

The longevity of larvae hatched at 30°C and 75% R.H. was examined by placing them at a range of temperatures and humidities. Plastic petri dishes (9 cm in diameter) were used for the humidity chambers. Two sheets of filter paper were put in the bottom of each dish and wetted with the appropriate saturated salt solution. Crystals of the salt were scattered over the wet paper. At intervals throughout each experiment the salt solution was inspected to make sure that the surface was still wet and that crystals of salt were still present. The filter paper was included to prevent the solution splashing up onto the base of the cage when the chamber was being handled at inspection times. The lid of the petri dish was sealed with silicone grease.

TABLE 5.3

The survival of eggs and larvae in egg masses

Treatment	Percentage Hatching	Percentage of Larvae Alive	Number of Eggs Laid
30°C 0% R.H.			
15 days after oviposition			
Tick No. 1	78	100	419
No. 2	89	99	708
20 days after oviposition			
Tick No. 3	79	98	30
No. 4	78	95	272
No. 5	80	97	164
35°C 0% R.H.			
15 days after oviposition			
Tick No. 1	23	59	178
No. 2	29	62	228
20 days after oviposition			
Tick No. 3	7	7	368
No. 4	3	0	330
No. 5	12	0	113

The tick cage (Figure 5.2) was a disc of perspex (8.5 cm in diameter; 3 mm thick), drilled with 20 holes (6 mm diameter). The bottom of each hole was closed with 200 mesh phosphor bronze gauze (ca. 6,000 holes per cm); a coverslip was laid on the top (a recess was drilled to a depth of 0.5 mm to accommodate the coverslip and prevent it slipping sideways). Perspex legs kept the disc 6 mm above the humidity solution. One tick was put in each hole. There were 20 larvae per treatment. A larva was considered to be dead when it could not be induced to move (even a leg) when the coverslip capping its cage was tapped with a glass rod. This was considered to be a good index of death for such ticks were never observed to recover.

About one week after the larvae had hatched they were allocated to their cages and these were placed over saturated salt solutions to control R.H. (Winston and Bates, 1960) in petri dishes and then placed in constant temperature cabinets. Four temperatures (20°, 25°, 30° and 35°C) and three humidities (95%, 75% and 10%) were examined.

There is a possibility that the larvae may be able to rehydrate if exposed to humid air, as has been demonstrated in the larvae of other species of tick (Section 5.2). Thus another series of treatments was set up at 30°C in which the larvae were exposed every seven days to two days of high humidity (2 days per week). For this part of the experiment a fourth humidity, 44% R.H., was studied. The results are expressed as mean longevity and it is clear from the data in Table 5.4 that both R.H. and temperature had a profound effect on longevity. For example, longevity varies from 3.0 days at 35°C, 10% R.H., to 127.5 days at

TABLE 5.4

The influence of temperature and humidity on the longevity of larvae

Mean survival time (days) standard deviation in brackets

Temperature °C	20	25	30	35
R.H.				
95%	127.5 (56.3)	107.4 (29.6)	33.3 (18.7)	26.3 (12.3)
75%	39.1 (21.8)	40.0 (19.0)	16.9 (3.7)	15.0 (5.9)
10%	9.0 (2.8)	4.5 (1.8)	3.9 (1.0)	3.0 (0.6)

Figure 5.2 Apparatus Used to Control Relative Humidity.

20°C, 95% R.H.

Starvation is likely to be the factor primarily responsible for mortality at high relative humidities, but as R.H. decreases the relative importance of desiccation should increase. The relative contribution of these two factors will depend upon the metabolic rate and the energy reserves on one hand, and the ability of the larvae to resist desiccation on the other.

The metabolic rate (and hence the rate at which energy reserves are consumed) will be a function of temperature. The rate of embryogenesis, being dependent upon metabolic processes, should bear a similar relation to temperature. Thus the way in which metabolic rate varies with temperature can be estimated (even if only approximately) by examining the way in which the rate of embryogenesis varies with temperature. Similar information may be derived from studying the effect of temperature on morphogenesis in the engorged larvae.

If 20°C is taken as a 'base-line' we can see that the rate of embryogenesis (as measured by the pre-hatch period) is 5.1 times faster at 35°C than at 20°C (5.9 days at 35°C and 30.7 days at 20°C; Table 4.5). Similarly morphogenesis in engorged larvae is 5.5 times faster at 35°C than at 20°C (4.9 days at 35°C and 17.1 days at 20°C; Table 4.1). Thus one might predict that the metabolic rate in unengorged larvae is about 5 times faster at 35°C than at 20°C. If

the larvae at 95% R.H. are dying from starvation alone, one would expect them to live about five times as long at 35°C as they live at 20°C. In fact they live 4.8 times as long (26.3 days at 35°C and 127.5 days at 20°C; Table 5.4).

Similar calculations comparing the metabolic rates at 25°C and 30°C with that at 20°C (in order to estimate the rate at which energy reserves will decay at 25° and 30°C) are set out in Table 5.5. Longevity of the larvae at these temperatures relative to that at 20°C is also presented. This information suggests that at 95% R.H., mortality amongst the larvae is principally due to starvation. Thus at high humidities the longevity of larvae was limited principally by the food reserves and the rate at which they were used up.

At low humidities there are two components, starvation and desiccation, which contribute to mortality. The metabolic rate is unlikely to be greatly affected by the R.H. (Section 4.5) and so the decrease in longevity due to desiccation will be the difference between the longevity at 95% R.H. and that observed. These have been tabulated in Table 5.6. From these data one can calculate the relative contribution of starvation and desiccation to mortality (which are complementary). The relative contribution of desiccation is set out in Table 5.7A.

The relationship between s.d. and the percentage of mortality due to desiccation at each s.d. is plotted in Figure 5.3A. It is

TABLE 5.5

The relationship between relative metabolic rates (as measured by the ratio of the mean incubation period at 20°C to the mean incubation period at t°C) and the relative longevity of larvae (as measured by the ratio of the mean longevity at t°C to that at 20°C)

Temperature (°C)	35	30	25	20
egg development				
mean incubation at 20°C / mean incubaction at t°C	5.1	3.9	1.9	1.0
larvae (engorged)				
mean pre-moult at 20°C / mean pre-moult at t°C	5.5	3.5	2.5	1.0
larvae (unengorged)				
longevity expressed as a fraction of potential longevity at 20°C	$\frac{1}{4.8}$	$\frac{1}{3.8}$	$\frac{1}{1.2}$	$\frac{1}{1.0}$

TABLE 5.6

Decrease in longevity of larvae due to desiccation (days)
(i.e. the difference between potential and
observed longevity)

Temperature (°C) % R.H.	20	25	30	35
10%	23.3	29.4	102.9	118.5
75%	11.3	16.4	67.4	88.4

TABLE 5.7A

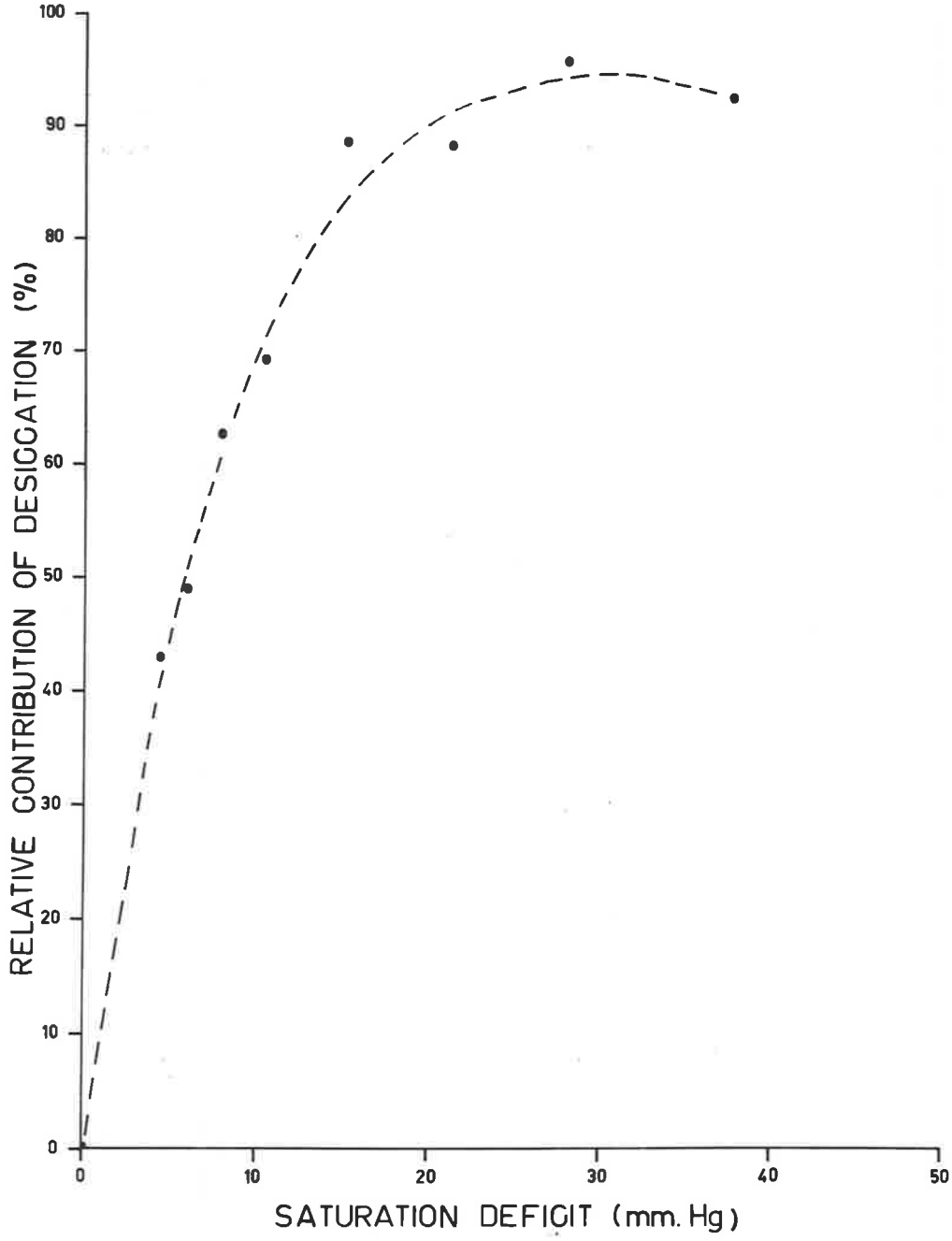
Relative contribution of desiccation to mortality of larvae

*Percentage of mortality due to desiccation

Temperature (°C) / % R.H.	20	25	30	35
10%	88.6	88.3	95.8	92.6
75%	43.0	49.2	62.8	69.1
95%	0	0	0	0

$$\text{* Percentage} = \frac{\text{decrease in longevity of larvae due to desiccation (Table 5.6)}}{\text{potential longevity (= longevity at 95\% R.H.)}}$$

Figure 5.2A The Relationship between the Saturation Deficit and the Relative Contribution of Desiccation to Mortality at that Saturation Deficit. (Curve fitted by eye)


RELATIVE CONTRIBUTION OF DESICCATION (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
SATURATION DEFICIT (mm. Hg)

clear from this that the rate of desiccation is regulated by s.d. and not by R.H. Furthermore there appears to be an upper limit to the rate of water loss, because further increases in s.d. do not increase the rate. Similar behaviour has been observed in other species of Arthropods (Section 5.2).

In the other part to this experiment larvae were exposed to two days of high humidity once a week, and the effect on longevity was noted. It is clear from the data in Table 5.7Bthat exposure to high humidity did not increase the longevity of the larvae. Nevertheless at high humidity the ticks appeared to rehydrate because before exposure they were flat but after exposure they were swollen. This is an unexpected result and throws some doubt on the survival value of rehydration in other species of tick as well as O. gurneyi, especially since the increased longevity was merely inferred from the increase in weight of the larvae and so was not measured (Knulle, 1966).

5.5 Regulation of the Water Content of Nymphs and Adults, and Survival under Dry Conditions

The period for which a tick can survive in the field will be determined by the interaction between its ability to resist desiccation and the rate at which its energy reserves are depleted. In this section I shall show that nymphs are well able to survive long periods without feeding because they have a well developed ability to resist and

TABLE 5.7B

The effect of periodic exposure to high R.H. on the longevity of larvae at 30°C
(2 days in every 7 at 100% R.H.)
Mean survival period (days) (S.D. in brackets)

% R.H.	95	75	44	10
Treatment				
Constant R.H.	33.3 (18.7)	16.9 (3.7)	5.2 (1.5)	3.9 (1.0)
Periodic rehydration	23.9 (12.9)	18.1 (4.7)	10.8 (2.3)	3.7 (0.8)

tolerate desiccation, and when desiccated, are able to rehydrate by extracting water from air which is not saturated with water vapour. These abilities combined with substantial energy reserves should enable the tick to survive extended periods in the field without feeding. Field experiments have substantiated this prediction.

5.51 How Dry is Too Dry?

The tick's ability to survive without feeding is enhanced by an ability to tolerate desiccation to a remarkable degree; beyond that which most animals can. Desiccated ticks which weigh only 35% of their post-moult weight have rehydrated and survived. This poses the problem, 'How much water can a tick lose before it is moribund?'.

Initially, however, it was necessary to determine the water content of hydrated ticks. Thus ten females which had been kept at 30°C and 10-20% R.H. for three to four months after moulting (but still appeared very healthy) were weighed, allowed to rehydrate at 100% R.H. for 15 days and then weighed again. The ticks were then placed in an oven at 55°C where they died and dried out during the next 10 days. The dry shrivelled corpses were weighed and the percentage water content before and after hydration was calculated. Before rehydration the mean water content of the ticks was 56.6% (S.D. = 5.2) of the wet weight, and after hydration was 70.0% (S.D. = 2.5).

The water content of ticks which appeared very desiccated was next determined. A group of such females, all of which would move if breathed upon, were treated in the same way as the first group (weighed, hydrated, weighed, dried, reweighed). Six of the 18 females examined failed to rehydrate and their mean water content was 53% wet weight. However, the mean water content of the 12 females which rehydrated successfully was 48.1% (S.D. = 6.0) before hydrating and 71.7% (S.D. = 6.3) after hydrating. It is noteworthy that four of these females rehydrated when their water content had dropped to 43% of the wet weight.

In summary, the water content of fully hydrated healthy ticks was between 70 and 72% of the wet weight. Ticks are active and appear healthy when this drops to 57%. It appears that most ticks cannot survive if their water content falls much below 50%; but there are cases of ticks rehydrating and surviving when their water content fell to 43%. In such cases the ticks have tolerated a loss of about two-thirds of their total body water.

5.52 The Rate of Weight Loss at Different Humidities

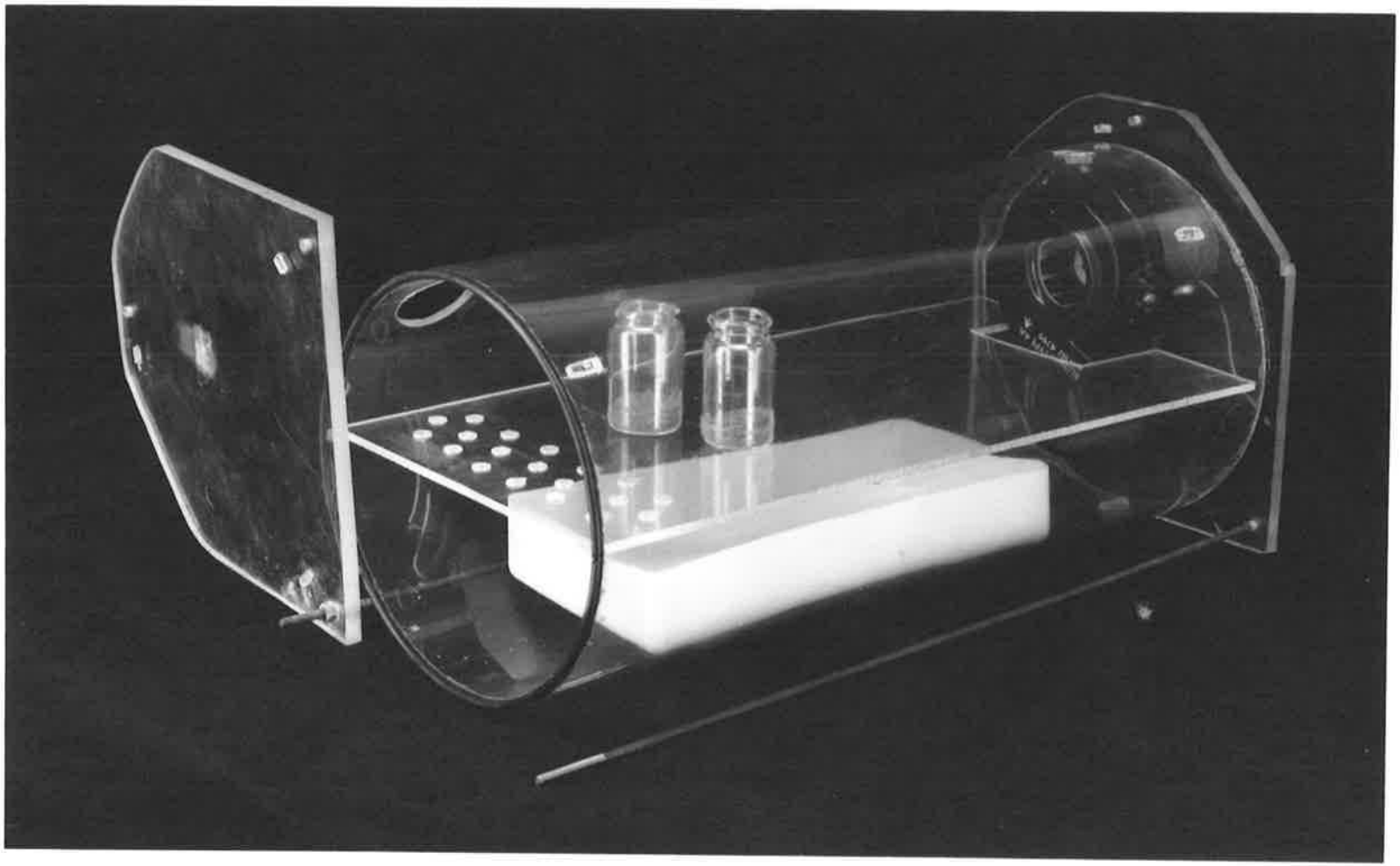
The rate at which engorged nymphs (and the ticks subsequent to moulting) lose weight was examined at 12%, 45% and 75% R.H. at 30°C. There are several ways one might express the change in weight of these ticks but I have chosen to use relative rather than absolute units so that I can compare more easily the relative abilities of the different

instars to resist desiccation. The unit is the weight of the tick expressed as a percentage of the weight of the ticks shortly (about one day) after moulting. This stage was chosen as the standard because the energy reserves of a tick are maximal immediately after moulting and, furthermore such a tick should be fully hydrated. Thus the standard tick should have the maximum potential longevity.

The rate of weight loss of one engorged first instar nymph was studied at 12% R.H. at 30°C. Similar observations were made on 6 engorged second instar nymphs at 12% and 44% R.H. and on 8 engorged fourth instar nymphs at 12% and 75% R.H. After feeding, the engorged ticks were allowed to burrow into $\frac{1}{2}$ cm of dry sand in plastic vials which were placed in constant humidity chambers similar to those described by Doanne and Allan (1968) (Figure 5.3B). The humidities within the chambers were maintained by using concentrated salt solutions as described by Winston and Bates (1960). The ticks were weighed on a Mettler balance which was sensitive to 0.10 mg.

The rates of change of weight of one first, two second and two fourth instar nymphs are plotted in Figures 5.4, 5.5 and 5.6. The individuals whose rate of weight loss is plotted were chosen because their rates of weight loss fell in the middle of the observed range. From these it is clear that the rates of weight loss change considerably with time. At intervals after feeding the ticks which survived were given an opportunity to rehydrate. There appear to be six character-

Figure 5.3B Apparatus Used to Control Relative Humidity.



istic phases in the plot of weight loss against time. They are:-

(i) The initial feeding in which the weight of the nymph increases about four-fold.

(ii) A relatively rapid rate of water loss immediately after feeding; leading up to

(iii) Moulting, during which, and immediately after which, there is a very rapid rate of weight loss which is partly due to the newly-moulted tick voiding excrement.

(iv) The rate of weight loss then slows to a very low level which may be interrupted by,

(v) Rehydration.

(vi) About the time of death there is a relatively rapid rate of water loss as the moribund animal loses control of its water-regulating mechanism and as the corpse dries out.

The rate of change in weight for each of these six phases (expressed as the percentage of standard weight lost per day) is set out in Table 5.9 for each of the 25 ticks examined and a comparison of the means for each treatment is set out in Table 5.8. Apart from the absolute rate of weight loss there are two points which emerge from these observations. Firstly, the rate of weight loss subsequent to rehydration is about the same as that before rehydration. Secondly, the rate at which the moulted fourth nymphs lose weight was little

Figure 5.4 The Rate of Change in Weight of an Engorged 1NN at 10% R.H. at 30°C.

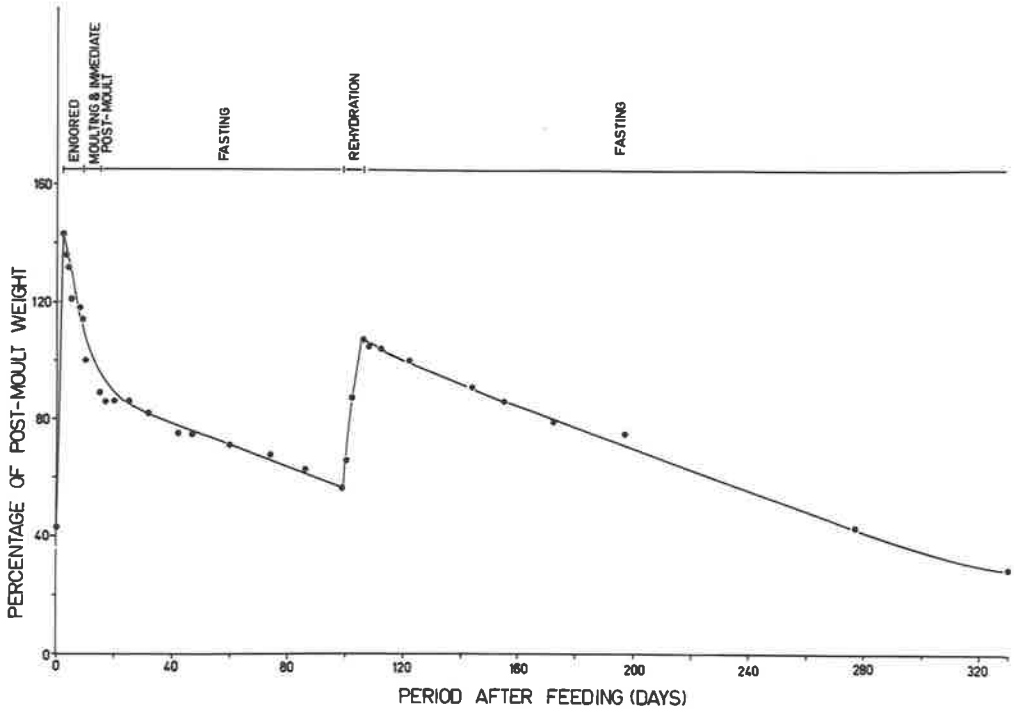

ENGORED
MOULTING & IMMEDIATE POST-MOULT
FASTING
REHYDRATION
FASTING
PERCENTAGE OF POST-MOULT WEIGHT
160
120
80
40
0
0
40
80
120
160
200
240
280
320
PERIOD AFTER FEEDING (DAYS)

Figure 5.5 The Rate of Change in Weight of two Engorged 2NN at 10% R.H. at 30°C.

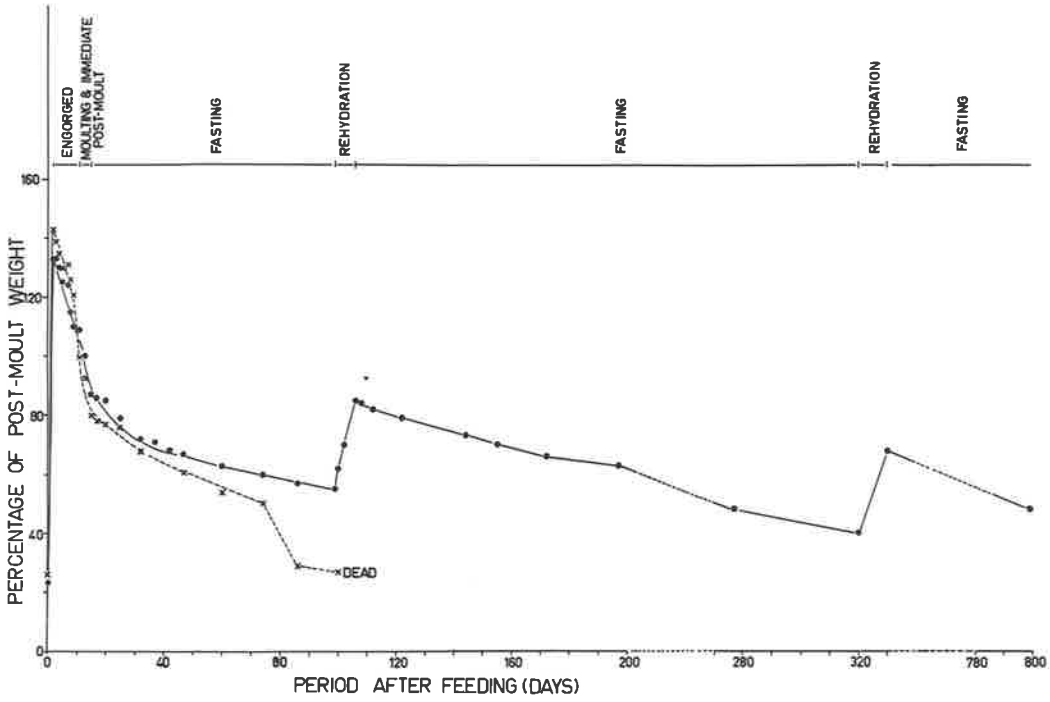

ENGORGED
MOULTING & IMMEDIATE POST-MOULT
FASTING
REHYDRATION
FASTING
REHYDRATION
FASTING
PERCENTAGE OF POST-MOULT WEIGHT
160
120
80
40
0
DEAD
0
40
80
120
160
200
280
320
780
800
PERIOD AFTER FEEDING (DAYS)

Figure 5.6 The Rate of Change in Weight of two Engorged 4NN at 10% R.H. and 30° C.

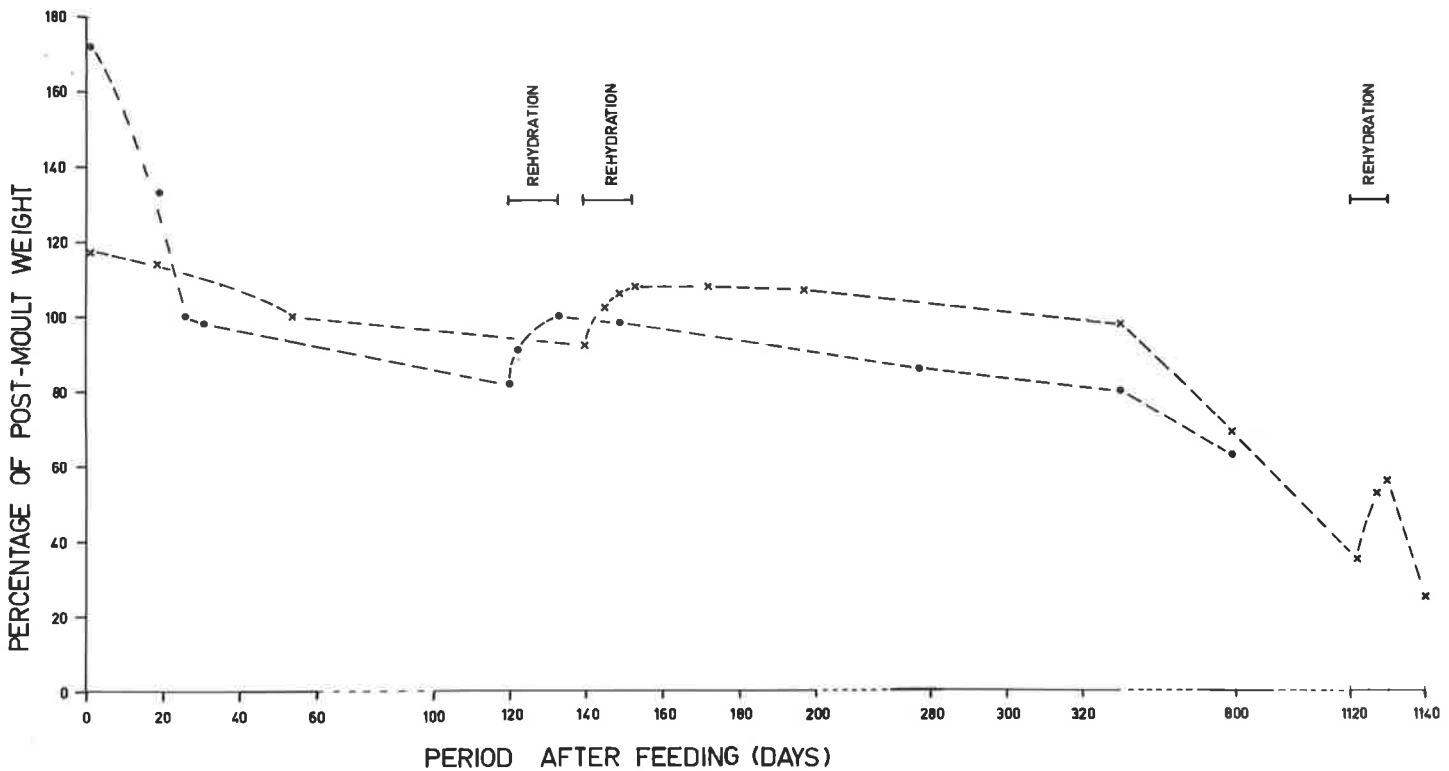

PERCENTAGE OF POST-MOULT WEIGHT
180
160
140
120
100
80
60
40
20
0
REHYDRATION
REHYDRATION
REHYDRATION
0
20
40
60
100
120
140
160
180
200
280
300
320
800
1120
1140
PERIOD AFTER FEEDING (DAYS)

TABLE 5.8

Mean rate of change in weight
(percent of post-moult weight per diem)

Treatment	Engorged	Moult and immediately post-moult	Fasting	Rehydrate	Fasting	Fasting	S.D.
1NN at 12%	4.2	4.2	0.38	8.3	0.37		
2NN at 12%	3.3	4.8	0.39	4.95	0.24	3.12	
2NN at 44%	2.10	6.08	0.11	2.57	0.07		
4NN at 12%	1.5	1.7	0.15	2.1	0.68	0.10	
4NN at 75%	1.4	4.5	0.17	4.0	0.085	0.11	0.07

TABLE 5.9

The rate of change in weight of ticks in different humidity conditions

2NN at 45% R.H. at 30°C

Interval (days)	0-11	12-15	16-126	127-139	139-330
Tick No.	*Engorged	Moult and immediately post-moult	Fasting	Rehydrate	Fasting
1	2.7	2.6	0.18	- **	- **
2	2.1	9.8	0.07	3.0	0.10
3	0.7	2.6	0.10	0.5	0.15
4	2.5	4.3	0.12	3.0	0.04
5	0.9	8.0	0.12	3.5	0.06
6	2.9	10.0	0.03	2.5	0.07
7	2.7	5.3	0.09	3.5	0.03
8	2.3	6.0	0.13	2.0	0.05

1NN and 2NN at 12% R.H. at 30°C

Interval (days)	0-10	11-15	16-99	99-106	106-320	321-330
Tick No.	Engorged	Moult and immediately post-moult	Fasting	Rehydrate	Fasting	Rehydrate
1. 1NN						
1. 2NN	4.2	4.2	0.38	8.3	0.37	- **
2. 2NN	3.7	6.5	0.38	5.0	0.21	2.0
3. "	3.1	4.0	0.50	- **	- **	- **
4. "	1.4	2.5	0.44	4.2	0.21	3.3
5. "	5.3	7.0	0.33	5.8	0.30	- **
6. "	2.9	4.0	0.29	4.8	0.23	4.2

* See Figures 5.4 and 5.5 for explanation of headings.

** - dead.

TABLE 5.9 (continued)

4NN at 12% at 30°C

Interval (days)	0-25	26-50	51-138	139-172	173-330	331-799	800-1122	1123-1127
Tick No.	Engorged	Moult and immediately post-moult	Fasting	Rehydrate	Fasting	Fasting	Fasting	Rehydrate
1	2.3	4.2	0.16	3.0	-	-	-	
2	1.0	0.6	0.25	2.6	-	-	-	
3	1.0	2.1	0.14	2.3	0.09	0.18	-	
4	0.3	0.4	0.10	0.8	0.07	0.06	0.11	3.6% 53(18)
5	2.2	1.5	0.17	2.8	0.06	0.06	-	
6	2.2	1.1	0.07	1.0	0.05	-	-	

4NN at 75% at 30°C

Interval (days)	0-25	26-50	51-120	121-133	134-277	278-330	331-799
Tick No.	Engorged	Moult and immediately post-moult	Fasting	Rehydrate	Fasting	Fasting	Fasting
1	1.2	4.9	0.20	4.5	0.09	0.12	0.04
2	1.6	4.4	0.12	3.0	0.09	0.06	0.12
3	1.7	4.4	0.11	5.0	0.09	0.19	-
4	1.2	4.4	0.25	3.5	0.07	0.08	0.04
5	2.3	14.7	0.14	14.4	0.37	-	

affected by humidity. Furthermore it is noteworthy that when ticks remain hydrated, or are periodically rehydrated, they are capable of surviving several years without feeding.

Thus it is clear that nymphs have a considerable capacity to resist desiccation and so it seems unlikely, except during extended droughts, that desiccation would be a very significant cause of mortality in nature. Starvation, on the other hand, could cause considerable mortality if the wallows were visited very infrequently.

The above-mentioned observations were carried out at the beginning of the study when only the cave variety of the tick (Appendix III) was available. Since the remainder of the thesis is concerned with the plains variety it is necessary to compare the resistance of the two varieties to desiccation. This was done by hydrating fourth instar nymphs of both varieties, and then placing them at 12% R.H., 30°C for 200 days. The rate of weight loss was expressed as a percentage of the hydrated weight lost per day. The mean rate of weight loss for the plains variety was 0.16% (S.D. = 0.08) per diem (n = 9) and that of the cave variety was 0.19% (S.D. = 0.06) per diem (n = 15). Thus there was no significant difference between the rates of weight loss of the two varieties under these conditions.

5.53 The Site of Absorption of Water Vapour

The site at which water is absorbed from unsaturated air varies from species to species. The cuticle appears to be that site

in most species of tick examined (Section 5.2) but in Argasids the rôle of the cuticle has not been clearly demonstrated.

Thirty desiccated fourth instar nymphs were divided into three groups of 10. The spiracles of one group and the anuses of another were occluded with paint. The three groups were then placed at 98% R.H. and 30°C where they rehydrated. They were weighed after one and seven days' exposure. In Table 5.11 the change in weight is expressed as a percentage of the desiccated weight of the group.

Similar results have been obtained for desiccated females. Thus it is clear that neither the anus nor the spiracles is the site of water absorption, and the cuticle was implicated by default.

5.6 The Survival of Ticks in the Field

There are three aspects of field conditions which must be determined before it is possible to predict survival in the field. They are:-

(i) One must know how dry are the rainless periods. This will determine the rate at which ticks desiccate and hence the duration of the dry period which can be survived.

(ii) One must know the maximum duration of the rainless periods at different times of the year. This will determine the proportion, if any, of the nymphs which die from desiccation at different times of the year.

TABLE 5.11

The effect of occluding the anus or spiracles on the absorption of water from humid air

Percentage of desiccated weight

Days at 98% R.H.	1 day	7 days
Control	100.5	117.8
Spiracles occluded	100.7	120.5
Anus occluded	100.0	118.3

(iii) One must know how much rain is needed to raise the R.H. of the soil air-spaces above the C.E.H. (Critical Equilibrium Humidity) of the tick and how long the R.H. remains at or above that level. This will determine the amount of rehydration which can occur after a shower before the soil dries out agains.

There are two ways one can gain this information. Firstly, by measuring the amount of moisture in the soil, and secondly by consulting meteorological records and the scanty literature on moisture in inland soils.

5.61 How Dry are the Rainless Periods?

The measure of dryness which is relevant to the tick is saturation deficit because the rate at which ticks desiccate (when the R.H. is less than C.E.H.) is proportional to s.d. The s.d. of the soil air-spaces depends on the temperature and the absolute water content of those spaces.

The micro-climate of soil-dwelling arthropods has been estimated on a number of occasions. Williams (1923, 1924a, 1924b) found that the atmospheric R.H. rose to a peak every morning even in the dry season and situations could always be found - in caves, for instances - where evaporation was low and consequently where the R.H. was relatively high. From his figures of percentage water content of the desert sands (1924a) it is probable that the R.H. of the sand air-spaces was

relatively high (e.g. 0.6% water in sand gave 90% R.H.). Kasharov and Kurbatov (1930) obtained similar results in the Kara Kum desert in central Turkestan. Buxton (1932b) found that, in Palestine, the relative and absolute humidity in cracks in walls and in holes drilled in walls (sandstone) were appreciably higher than outside, even at the driest time of the year. The same author (1936), when studying the soils in which two species of Glossina pupate (in Nigeria), found that even under extreme conditions of heat and drought, the atmosphere in the soil air-spaces may be nearly saturated, even when the soil seems powder dry.

On a number of occasions during field trips I estimated the R.H. of the soil air-spaces in wallows at different times of the year. This was done by two-thirds filling a glass tube with soil from the wallow and then sealing the soil in the tube with a plug of cotton wool. A strip of cobalt thiocyanate paper was then introduced and the air-tight cap was screwed on. In the laboratory the tube was placed at 25°C for one week to allow the water vapour to equilibrate throughout the tube. The R.H. of the air was then estimated from the cobalt thicyanate paper (Solomon, 1957). This reading gave an estimate of the absolute water content of the air in the soil air-spaces.

The extremes of dryness in the study area would occur during the hot rainless periods of summer. On three occasions I estimated

the R.H. of the soil air-spaces during a series of hot, dry summer days. On each there had been no rain during the three previous weeks. The daily maximum air temperatures were between 90° and 110°F. Soil samples were taken from the surface, from 1" and from 2" below the surface. Samples were taken at intervals throughout the day. The temperature of the soil at 1" was measured on each occasion, and was up to 5°C above air temperature depending on whether or not the soil had been shielded from direct sunlight by the foliage of the tree over the wallow.

At 25°C, the R.H. of all the soil samples was between 10 and 20%. Thus the absolute water content of the soil did not vary greatly; but as the temperature changes so will the s.d. Thus there was a diurnal fluctuation in s.d. from about 40 mm Hg during the hottest part of the day to about 10 mm Hg in the early morning. It was also noteworthy that, during the periods measured, the diurnal variation in the s.d. of the air was between 5 and 35 mm Hg. These observations demonstrate that the air spaces in the sand in the wallows of the study area were about as arid as the atmosphere, and at times even dryer. The data from the different desert areas of the world and the observations on my study area suggest that, after a period without rain the humidity of the soil air-spaces is about the same as, or higher than, that in the atmosphere.

The more general idea of the aridity of the soils at different times of the year can be extracted from the climatological data of Yudnapinna station (Table 5.12). The temperatures at 1" below the soil surface were measured for eight years.

A combination of the records of atmospheric humidity at Yudnapinna and the mean maximum temperature at 1" below the surface at Yudnapinna (Table 5.12) should give an estimate of the mean maximum s.d. experienced in the different months of the year. Hence the mean maximum s.d. for January is 33.9 mm Hg (116°F at 45% R.H.) with a diurnal fluctuation of 33.9 to 8.8 mm Hg, while for June the mean maximum s.d. is 3.1 mm Hg (67°F at 78% R.H.) with a diurnal fluctuation of 3.1 to 1.0 mm Hg. These figures are only means, and so naturally the extremes in the field will be greater. Nevertheless they give an indication of the average conditions likely to be encountered. Furthermore the soil in which the ticks live is shaded for part of the day and so the average maximum temperatures (and hence the s.d.) experienced will probably be less than those calculated for the bare soil at Yudnapinna. Thus it appears that the dryest conditions likely to be experienced by ticks buried in wallows in the study area are about 40 mm Hg (with diurnal fluctuations between 10 and 40 mm Hg).

TABLE 5.12

Soil temperatures (°F) at 1 inch and air temperatures (°F) at Yudnapinna

Means of 8 years, 1946-1953

Month	1 inch		Air temperature		
	Maximum	Minimum	Maximum	Minimum	Mean
January	115.7	66.4	92.8	62.9	77.8
February	110.1	65.3	89.1	62.9	76.0
March	105.1	60.6	85.6	58.2	71.9
April	88.7	52.6	75.4	50.1	62.7
May	77.3	47.1	69.0	45.2	57.1
June	66.7	41.6	62.4	39.6	51.0
July	67.2	40.7	62.2	37.8	50.0
August	73.3	44.2	66.3	39.6	52.9
September	84.3	50.0	73.2	44.1	58.6
October	92.8	55.6	76.8	49.4	63.1
November	102.4	61.1	83.5	55.3	69.4
December	114.7	65.3	89.5	60.3	74.9
Mean	91.5	54.2	77.2	50.4	63.8

Climatological data for Yudnapinna

Means of 15 years, 1939-53

Month	Rainfall inches	Evaporation inches	Relative humidity per cent
January	0.72	14.60	45
February	1.21	11.58	50
March	0.54	10.46	49
April	0.36	6.73	60
May	0.91	4.32	68
June	0.98	2.58	78
July	0.68	2.95	76
August	0.52	4.10	68
September	0.42	6.50	54
October	0.82	8.92	48
Movember	0.72	11.09	44
December	0.84	13.19	41
Total	8.74	97.02	Mean 57

Data were taken from meteorological records at the Waite Agricultural Research Institute.

5.62 How Long do the Dry Periods Last?

The occurrence of a dry period is only relevant to the tick if that period is so long and so dry that some ticks die from desiccation. We have already seen that in hot, dry periods the s.d. fluctuated between 10 and 40 mm Hg. Thus laboratory experiments with a continuous s.d. of 30 mm Hg (30°C at 10% R.H.) should allow me to predict, roughly, the maximum period that one might expect a tick to survive in the field during summer, without rehydration.

Larvae are much more susceptible to desiccation than are nymphs. Individual larvae at 30°C and 0% R.H. (s.d. 33 mm Hg) survive only three to five days; however, when they are brooded by their dam they are likely to survive for a week or two (Section 5.41). Nevertheless hot, dry periods of one month or more are likely to be lethal for most larvae if they do not find a host.

On the other hand, nymphs do not begin to die from desiccation until they have spent at least several months in dry conditions (s.d. 30 mm Hg), others may live for a year or more under the same conditions (Section 5.52).

The rainfall on Moralana is low and erratic, with slightly more fain falling during the winter than at other times of the year. Yudnapinna station has a similar climate. Jackson (1958) studies the changing seasonal pattern of precipitation on Yudnapinna; Table 5.13

TABLE 5.13

Daily readings are available for the 17 years' records collected by the Waite Institute, and from those it was possible to calculate the frequency of rains of various magnitudes. Six arbitrary classes were set up as follows:

(i) Very heavy rains: more than 2 in. of rain over a 2-day period.

(ii) Heavy rains: 0.91-2.00 in. recorded over a 2-day period.

(iii) Moderate rains: 0.51-0.90 in. recorded over a 2-day period.

(iv) Significant rains: 0.31-0.50 in. recorded in a single day and not followed by rain on the following day.

(v) Light rains: 0.11-0.30 in. in a single day and not followed by a fall of sufficient magnitude to qualify for the moderate class.

(vi) Very light rains: 0.10 in. or less in a single day and not followed by a fall of sufficient magnitude to qualify for the moderate class.

TABLE 5.13

Yudnapinna Rainfall Data*

Mean annual and monthly rainfall based on years 1885-1955; all other data based on years 1939-1955

	January	February	March	April	May	June	July	August	September	October	November	December	Year
Mean rainfall (in.)	0.57	0.65	0.47	0.54	0.79	1.04	0.71	0.77	0.65	0.69	0.58	0.54	8.00
Mean No. of rain days	2	3	2	4	6	8	9	8	4	5	5	4	57
Total No. of falls in 17 years - very heavy**	0	2	1	0	0	0	0	0	0	1	0	1	-
Heavy**	6	4	3	3	5	6	1	0	1	1	2	3	-
Moderate**	1	2	2	2	2	3	1	5	1	4	6	3	-
Significant**	3	2	2	3	4	3	3	3	2	5	1	3	-
Light**	7	7	8	12	21	19	26	14	14	22	17	13	-
Very light**	17	26	20	42	60	105	116	96	54	48	43	31	-

* Derived from records of the Commonwealth Bureau of Meteorology and data supplied by the Waite Agricultural Research Institute.

** See facing page for definition of these terms.

is taken from his paper and illustrates the pattern. The most obvious feature of this pattern is the prevalence of light and very light falls during winter. For instance, of the average of nine rainy days in July, seven produced less than 10 points. There is also some tendency for heavy and very heavy falls to occur during the summer months. Moderate and significant falls are distributed fairly evenly throughout the year. While it is clear that mean rainfall does not vary greatly from month to month, the number of occasions on which rain falls during summer is less than at other times of the year. For example, during the 18 years for which Jackson presented data, rain fell 34 times during January, 81 times during October and 147 times during July. Thus, on the average, the longest periods without rain occur during summer, but even in January there is an average of one fall of rain during the month.

The rainfall records for Hawker were also examined for the past 40 years and the number of one, two, three and four-month periods, 15 two-month periods and one four-month period; it is noteworthy that none of the two-month periods occurred during winter.

In conclusion, it seems unlikely that the dry periods will be so long that they cause considerable mortality in the nymphal stage. On the other hand, the larvae are very vulnerable to desiccation and so during summer larvae are likely to lead a very precarious existence.

Ticks have been shown to rehydrate at a relatively slow rate; about three percent of their body weight per day, and so it may take ten days or more for a tick to become fully hydrated. Therefore, the rate at which soil dries out will greatly influence the degree to which a tick can rehydrate after a shower of rain.

5.63 How Quickly Does the Soil Dry Out?

Very little is known about the water content of soils in arid areas of Australia, and how it varies with time and rainfall. There are a number of factors which will affect the R.H. of the soil air-spaces and the rate at which the soil dries out. They are:-

(i) The period since rain has fallen.

(ii) The size of the shower.

(iii) The state of hydration of the soil prior to the shower.

(iv) The variability of water impinging on small areas. For example, the tree may act as an umbrella and so shield the ground from rain, or the trunk may act as a catchment area and channel the water to the base of the tree.

(v) The proportion of the incident rainfall which is absorbed into the soil.

(vi) The climatic conditions after the rainfall.

(vii) The water-holding characteristics of the soil.

(viii) The mulching effect of leaf litter.

(ix) The dehydrating effect of the vegetation (transpiration).

The complex interaction of these factors creates a highly variable field situation which renders accurate prediction almost impossible because there is very little data available on the above factors.

Measurements of the R.H. of the air-spaces from wallows on Moralana have shown that after a shower of 10 points the soil is damp and the R.H. of the first 2 cm is 100%. How long this high humidity persists should vary greatly, depending on the above factors, but on one occasion in May and another in October the R.H. of the soil air-spaces was still between 90 and 100% at a depth of one inch, three days after rain.

In order to get some idea of the rate at which the soil from wallows dries out, I examined the rate at which half a cubic foot of such soil dries out in the laboratory under known conditions. A box, 1' square and 6" deep, was filled with sand from a wallow on Moralana, allowed to stand at 15% R.H. for ten days, and then sprinkled with 0.42 pints of water (the equivalent of 10 points of rain). The sand was then placed in a room at 30°C and 15% R.H. The R.H. in and above the sand was monitored by a series of strips of cobalt thiocyanate which were encased in blotting paper and buried vertically. The water disappeared immediately after application and wet the first 2" within a few minutes. Two hours later the wetness had moved no deeper but the R.H. of the soil air-spaces was 100%. One day later

the soil had dried out to 15% R.H., the same as that of the room. Thus it seems unlikely that, in the field, small showers after prolonged dry periods will permit complete rehydration of desiccated ticks.

In summary, it appears that there will be little chance of nymphs or adults dying from desiccation during the winter because of the low s.d., due to the low temperatures and relatively frequent showers. Spring and autumn are more arid but summer is the most arid season. However, it seems that even during summer, the dry periods are rarely long enough to result in serious mortality due to desiccation. The larvae, on the other hand, are very vulnerable to desiccation and it seems unlikely that any would survive as long as one month of hot dry summer weather.

5.64 The Longevity of Ticks under Field Conditions

Engorged larvae and first- and fourth-instar nymphs were placed in field cages (described in Section 4.46) and these were buried in the study area on 16 March, 1970. Ticks were buried in two types of place; in sand in the wallows under bushes, and in the sand of sand dunes about 10 feet from the wallow in each case. This comparison gave an estimate of the relative aridity of the two sites. The ticks were examined at intervals of about two months over the next year, and the resulting data are expressed as the cumulative percentage dead (Table 5.14).

TABLE 5.14

Cumulative percentage mortality

Treatments		Buried in Wallow			Buried in Sand Dunes		
Instar		Engorged larvae	Engorged 1NN	Engorged 4NN	Engorged larvae	Engorged 1NN	Engorged 4NN
Period	Period of exposure (weeks)						
March - May	9	8.5%	0.5%	0%	23%	1%	0%
Autumn May - August	20	13.0%	0.5%	0%	37%	4%	0%
Winter August - October	30	22.0%	6.0%	1%	44%	5%	0%
Spring October - December	42	31.5%	7.0%	1%	85%	52%	0%
Summer December - March	52	41.0%	15.5%	6%	100%	98%	27%
Sample size		200	200	200	100	100	100

It is clear from these results that the wallows are far less arid than the sand dunes. The relative aridity of the different periods throughout the year can be estimated by examining the rate of mortality (expressed as percent mortality per week) for each period. The results are shown in Table 5.15. From these it is clear that winter and spring were the least arid times of the year. This is in accord with what was expected in the light of the changes in weather conditions throughout the period (Tables 5.12 and 5.16).

However there is a possibility that the deaths during summer are due to starvation rather than to desiccation. This appears unlikely in the light of the remarkable longevity of the ticks under favourable conditions. Nevertheless I examined this possibility by placing some engorged larvae and first instar nymphs in wallows in the field in August 1970 and noting the mortality in December. Thirty-eight percent of the engorged larvae (which had moulted to first instar nymphs) and 4% of the engorged first instar nymphs (which had moulted to second instar nymphs) died during this 22-week period. These proportions are similar to those of the ticks which had been exposed since March (42 weeks), namely 32% of the engorged larvae and 7% of the first instar nymphs). Thus desiccation rather than starvation was responsible for the mortality observed.

One further point of note is the remarkable hardiness of the

TABLE 5.15

The rate at which ticks died at different times of the year (% death per week)

Season	In wallows			In sand dunes		
	larvae	1NN	4NN	larvae	1NN	4NN
Autumn (March - May)	0.96	0.06	0	2.44	0.1	0
Winter (May - August)	0.42	0	0	1.35	0.3	0
Winter-Spring (August - October)	0.88	0.6	0.1	0.67	0.1	0
Spring-Summer (October - December)	0.79	0.1	0	4.10	3.9	0
Summer (December - March)	0.97	0.85	0.5	1.5	4.6	2.7

TABLE 5.16

Rainfall at Hawker (points)

Except where otherwise indicated there was general rainfall throughout the north of South Australia whenever rainfall was recorded at Hawker. (* local thunderstorm.)

Days of month	1969 March	April	May	June	July	August	September	October	November	December	1970 January	February	March
				-							18	1*	
1				31		-	16			-	5		
2							23						
3						3				3			
4					66		5		-				
5					12		2		38				2
6							1						
7						12							
8													
9				49									
10											6		
11			7		24			1*					
12	6		18		53								
13							5	-					
14	25	21						-					
15	15	2						3					
16	2							3					
17													
18				28									
19		20	...	49	-		-						2
20				20									
21			33		35		3						
22	-				31		40						
23	23		60		8								
24	36		2										
25					2								
26								9					
27													
28								45					
29			19										
30			11			11							
31			9										

ticks which moulted from the engorged fourth instar nymphs; 94% of those in the wallows were still alive at the end of one year of exposure to the arid conditions of the inland desert.

The studies in this chapter have shown that larvae are very vulnerable to hot, dry conditions. Imaginal diapause becomes manifest in mid-summer and so most larvae are produced in spring and early summer (when conditions favour larval survival) and few are produced during late summer(the hottest and dryest season of the year). The next chapter discusses the regulation of imaginal diapause.

CHAPTER 6

The Regulation of Diapause

6.1 Summary

A seasonal cycle in the incidence of imaginal diapause has been found, in which most females are in diapause between mid-summer (December) and mid-winter (July). Diapause is induced in early summer and diapause development proceeds during autumn and winter. Figure 6.3 summarises the results of a series of field experiments on the regulation of diapause.

Diapause is induced by high temperature. Figure 6.4 summarises the results of laboratory studies on the effect of temperature on the incidence of diapause.

Humidity only slightly affected the incidence of diapause.

There was a 'spontaneous flux' in and out of diapause at constant temperature, which tended to increase the incidence of diapause at high and low temperatures but to decrease it at moderate temperatures ($30^{\circ}C$).

Diapause development is promoted by low temperatures.

Short photoperiod induces diapause. This response is irrelevant to the ecology of the tick and is dealt with in Appendix I.

6.2 Literature Review

Diapause occurs as a noteworthy adaptation in many species of arthropod enabling them to persist in regions where they might otherwise be killed by extremes of climate, and enabling them to synchronize their development with the favourable seasons of the year. Diapause usually occurs in a stage of the life-cycle which is also adapted in other ways to resist the rigours of the climate. In the kangaroo tick there is a reproductive diapause in the adult female.

The extensive literature on the biology of ticks contains little information on their adaptations to seasonal changes in the environment. It was long believed that the length of the life-cycle was determined by host availability, and that the host-searching behaviour and the development of engorged ticks was directly dependent upon temperature (Belozerov, 1968). However, photoperiod was found to have an important rôle in regulating the host-seeking behaviour of the larvae and nymphs of Dermacentor variabilis (Smith and Cole, 1941). Two important steps in the study of seasonal adaptations were the investigations of Alfeev (1948, 1952, 1954), and those of Campbell (1952) who demonstrated the decisive rôle played by diapause in organising the seasonal development of ticks. Alfeev described four types of diapause: estival interruption of host-seeking activity; interruption of feeding of females on the host; retardation of oogenesis in replete females; and finally, retardation of development

in engorged larvae and nymphs.

Following Alfeev's papers it was demonstrated in many Ixodids that diapause was controlled by a photoperiodic reaction which was sometimes modified by temperature. Thus, in Ixodes ricinus (Campbell, 1952; Belozerov, 1968; Kemp, 1967; Loew, 1962, 1964), I. persulcatus (Babenko and Platonova, 1956; Babenko, 1966), I. triangulicepS (Bobrovskikh, 1966) and also in Haemaphysalis punctata (Kemp, 1967) the photoperiod could induce diapause in larvae and nymphs. In the females of Dermacentor marginatus (Belozerov, 1963; Belozerov and Kuitko, 1965) and possibly D. pictus (Razumova, 1965) photoperiod determined retardation of oogenesis. Day-length determined not only the morphogenetic processes in replete ticks but also the behavioural reactions of unfed ticks. For example, an interruption of host-seeking activity was found in larvae and nymphs of D. variabilis (Smith and Cole, 1941), I. ricinus nymphs (Belozerov, 1968), the larvae of D. albipictus (Wright, 1968, 1970) and the imagos of D. marginatus (Belozerov, 1967b).

Imaginal diapause is an adaptation commonly found in blood-sucking arthropods. Dissociation of the gonadotrophic processes is the most commonly observed manifestation of imaginal diapause, but the behaviour of the animal may also be modified. Amongst Ixodids imaginal diapause is known for D. marginatus (Alfeev, 1948, 1954; Yashkul, 1959, 1961, Belozerov, 1967b), and D. pictus (Belitser, 1927; Netskii and

Ol'skevskaya, 1950); also for I. triangulicepS (Kuznetsova, 1964), Hyalomma asiaticum and H. punctata (Pomerantsev, 1950). However D. marginatus is the only tick in which photoperiod has been shown to control the delay in oogenesis. This delay, diapause, was entirely determined by the photoperiod and temperature conditions under which the fasting ticks were kept. The diapause-inducing effect of long photoperiod manifests itself at moderate temperatures (17°C) but becomes more pronounced at higher temperatures (25°C), and so the proportion of females entering diapause in long photoperiod increased with increasing temperature. This is similar to the so-called 'short-day response' in insects (Lees, 1968).

The complementary relationship between high temperature and long photoperiod in inducing diapause in insects and other arthropods, and a similar relationship between low temperature and short photoperiod, is characteristic of photoperiodic induction of diapause in ticks as well as in insects. Studies of imaginal diapause make up a very small proportion of the vast literature on diapause in insects. But in all such cases either photoperiod [e.g. Acrydium arenosum (Sabrosky et al., 1933), Tetranichus urticae (Giespits, 1960), Nomadacris septemfasciata (Norris, 1965, Crysopa carnea (MacLeod, 1967; Tauber and Tauber, 1969), Pyrrhororis apterus (Hodek, 1971b)] or photoperiod and temperature [e.g. the Colorado potato beetle, Leptinotarsa decemlineata (De Wilde and De Boer, 1969), Galeruca tanaceti (Siew, 1965a, b and c)]

have been implicated in the induction of diapause. Other factors such as nutrition (Lees, 1968; Saunders, 1970) and humidity (Loew, 1962) have also been found to influence the manifestation of diapause, but their influence was relatively small and only active in certain critical conditions. For example, in the case of the mite, T. urticae, nutrition was only influential when the photoperiodic and temperature conditions were neutral (Lees, 1952).

There is little reference in the literature to the stages which are sensitive to stimuli inducing imaginal diapause. However, Hodek (1970b) has reviewed the available data on insects. It appears that in some species the most sensitive stage is the newly-emerged adult, whereas other species remain sensitive during the whole of the adult life (this is also true for the tick, Dermacentor marginatus (Belozerov, 1967a)). In some species the larval stages are also sensitive to diapause-inducing stimuli, but the contribution of diapause induced during these stages is small relative to that induced during the adult stage.

Diapause development usually requires a period of chilling (Lees, 1955; Danilevskii, 1965). Photoperiodic reactivation is a rare phenomenon. However, photoperiod has been shown to promote development of imaginal and larval diapause, but only at biologically effective temperatures (Danilevskii and Sheldeskova, 1968; Williams, 1969; Tauber and Tauber, 1970; Hodek, 1971b).

In the light of these studies, it appears that either temperature or photoperiod or both might be responsible for the induction and termination of diapause, but it is noteworthy that the inductive stimuli are likely to differ from those which promote diapause development. This chapter is concerned firstly with the incidence of diapause in the field, then with the factors which induce diapause, and finally with diapause development.

6.3 How to Recognise Diapause

The criterion by which diapause was recognised was the failure of the female to oviposit, even though she had engorged, mated and had been placed in conditions favourable for oogenesis. As has been demonstrated in Section 4.6 the oviposition behaviour, even at one temperature, was very variable. However, it is clear from Figures 4.6 and 4.7 that most females which were to oviposit at 30°C had done so within the first 50 days after feeding. Hence at 30°C, those which had not oviposited by 50 days after feeding were considered to be in diapause. The time limit at other temperatures was derived in a similar way.

6.4 Field Observations and Experiments

6.41 The Occurrence of Diapause in Field Populations

During the course of the study, ticks were collected from field populations at intervals of about two months. Most of the females

were collected using 'standard CO_2 traps', but some of the earlier samples were collected by sieving. The two sampling methods trapped both diapausing and non-diapausing females, and so any bias in the type of female caught by the different sampling techniques has been ignored.

All samples collected during 1969, 1970 and 1971 were taken from the study area on Moralana station, but during 1968 samples were taken from many different areas of inland Australia. Between 5 and 20 wallows were sampled on each occasion. Some sites were sampled on successive occasions; others only once. When the ticks had been captured, they were stored under sand in plastic vials until they were brought back to the laboratory, one to three days later, where they were sorted, measured and fed. The incidence of diapause was then assessed at 30°C. Table 6.1 shows that at least 70% of the females collected in the field would engorge when given an opportunity to feed on a rabbit in the laboratory. This occurred irrespective of the time of the year at which the population was sampled. Most of the rest would engorge if given a second opportunity.

It is clear (Figure 6.1, 6.2) that most females are in diapause between mid-summer (January) and mid-winter (July) each year. In some years, however, a small proportion (5-10%) were still not in diapause during late summer and autumn.

One consequence of this behaviour is that there should be an increase in the proportion of engorged (diapausing) females in the

TABLE 6.1

Occurrence of imaginal diapause in field populations

Collection Date	% of sample feeding	Incidence of diapause (% of females which engorged)	No. female ticks captured	Origin of ticks
1968				
March		90	10	Lilydale Station, S.A.
May		100	6	Braemar Station, S.A.
June		100	50	Alice Springs, N.T.
October		20	10	Moralana Station, S.A.
November		10	10	Broken Hill, N.S.W.
December		0	26	Moralana Station, S.A.
1969				
February 12	77	100	13	Moralana Station, S.A.
April 1	75	100	27	
May 4	83	100	6	
August 24	75	30	12	
November 18	70	20	17	
December 4	100	0	3	
1970				
January 4	75	90	26	
March 19	89	85	64	
May 5	83	100	6	
May 20	70	85	35	
July 25	80	25	15	
August 26		31	16	
October 22	70	7	14	
December 23	85	68	47	
1971				
March 30	91	97	64	

Figure 6.1 Seasonal Fluctuations in the Incidence of Imaginal Diapause.

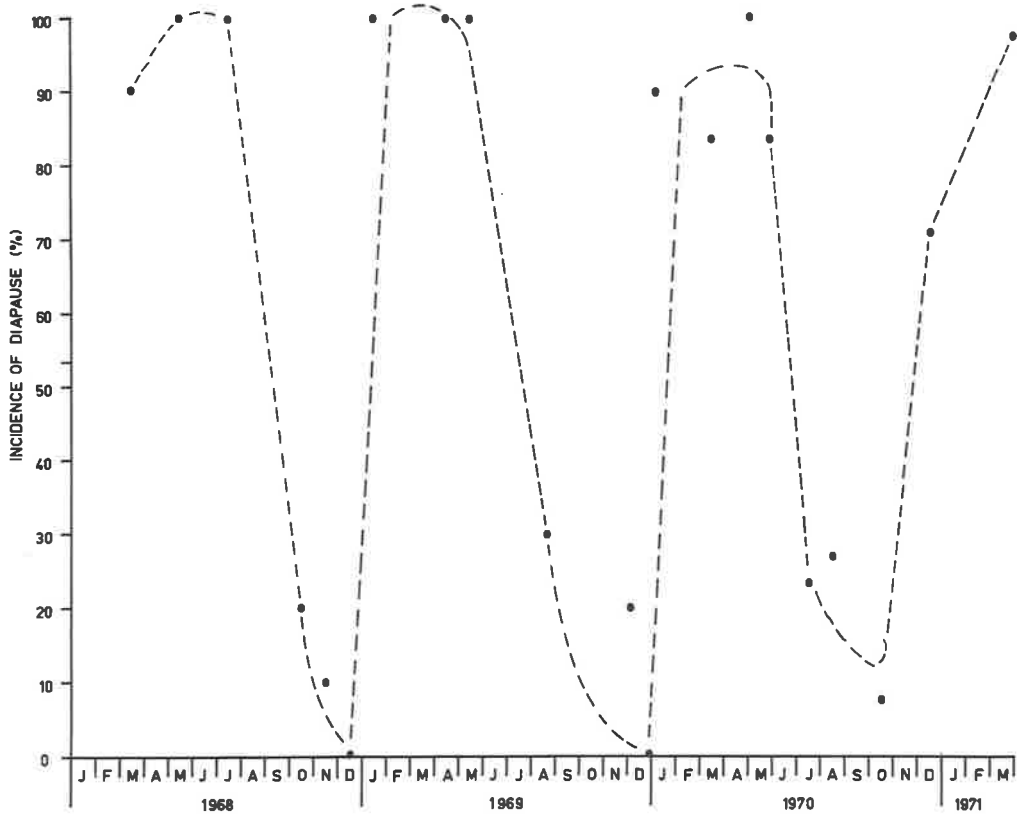
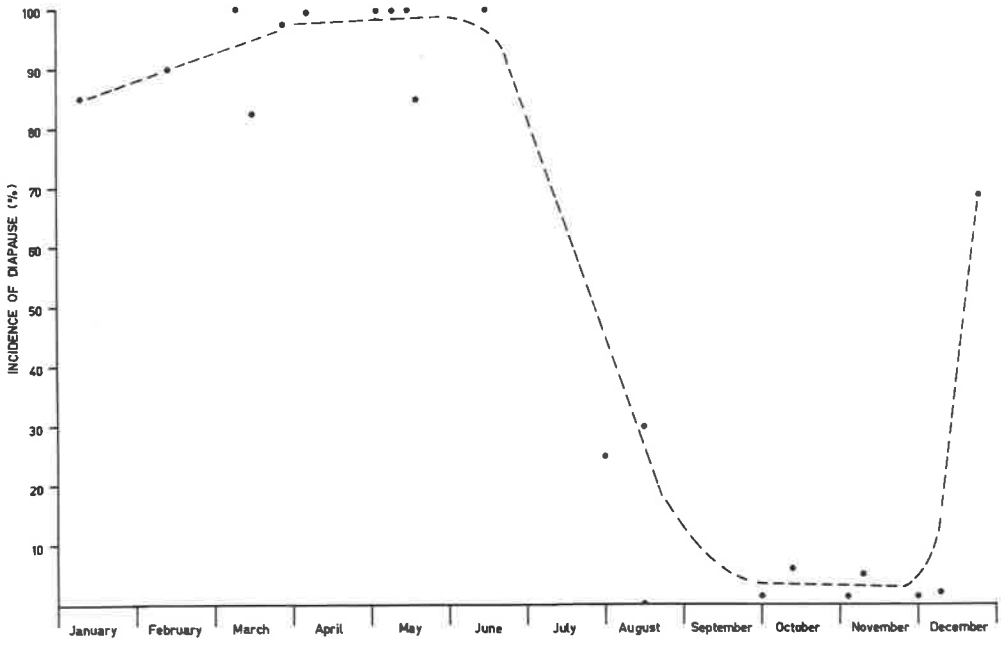

INCIDENCE OF DIAPAUSE (%)
100
90
80
70
60
50
40
30
20
10
0
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M
1968
1969
1970
1971

Figure 6.2 The Way in which the Incidence of Diapause changes throughout the year. (the data for each year have been superimposed on a common axis)


INCIDENCE OF DIAPAUSE (%)
100
90
80
70
60
50
40
30
20
10
January
February
March
April
May
June
July
August
September
October
November
December

population as summer and autumn progress (provided that kangaroos continue to visit wallows). With this in mind, the females from the field sample were weighed and the proportion of engorged females noted. Newly-moulted females from the laboratory culture weigh between 0.3 and 0.7 gm. Thus females weighing more than 0.8 gm were taken as being engorged. Table 6.2 shows the changing proportions of engorged females at different times of the year.

From these results it is clear that the proportion of engorged females in the population, in the field increases during late summer and early autumn and remains high during winter. This pattern is predicted from what is known about the seasonal incidence of diapause in the field and the behaviour of the kangaroo (Section 7.1).

6.42 When is Diapause Induced?

It is clear that diapause becomes manifest in mid-summer, but it is not known when it is induced. The possibility that diapause is obligate can be discounted because females have been known to lay four successive batches of eggs over a period of seven months. However there still remains a possibility that diapause was induced during spring, but lay dormant until it became manifest in mid-summer. This possibility was tested and discounted as follows.

TABLE 6.2

The proportion of females in field samples weighing more than 0.8 gm

Time of year when population sampled	Proportion > 0.8 gm	Sample size
November	12%	25
February	19%	16
March	14%	65
April	25%	40
May	36%	42
May	38%	8
August	35%	17

Non-diapausing females from the field sample taken in November, 1969 were given a series of opportunities to engorge and oviposit in the laboratory, at 30°C, in long photoperiod, during the summer, autumn and winter of 1970. The field samples taken in January 1970, March 1970 and May 1970 were treated in a similar way. If diapause were latent in the sample taken in November 1969 then one would expect them to behave in a way similar to the January, March and May samples, i.e. to be in diapause by mid-summer and during autumn and winter.

It is quite clear from Table 6.3 that those females captured in November 1969 did not enter diapause during the six months subsequent to capture, while those captured during December 1969 were in diapause and remained that way. Hence diapause did not lie latent in those females captured in November 1969, and so diapause must have been induced in early summer, i.e. from November to January.

6.43 The Induction of Diapause in the Field

In mid-summer, when diapause is induced and becomes manifest, there are two main types of female present in the field population. They are:

(i) Females which were recruited into the population during spring and summer of that season.

(ii) Females which had overwintered as adults.

Longevity and survival studies (Chapter 5) have shown that both types

TABLE 6.3

Percentage of engorged females in diapause

	November 1969	January 1970	March 1970	May 1970
November 1969	20%			
January 1970	30%	90%		
March 1970	14%	84%	85%	
May 1970	30%	94%	97%	85%

of female are likely to be present in the field during summer and so the questions arise 'do both types of female enter diapause during summer?' and 'What are the important stimuli inducing diapause in nature?'. These problems were examined by exposing ticks of known physiological condition to field conditions for certain periods of time during different seasons of the year. The ticks were then returned to the laboratory and the incidence of diapause assessed. Any change was attributed to the effect of field conditions.

The above questions were examined in the following two experiments. The experimental design in each was very similar and so I shall describe it only once.

Method: A large group of ticks was placed in the field in field cages (described in Chapter 4) at a particular time of the year. Samples of that group were taken at intervals and the incidence of diapause was assessed in the laboratory at 30°C in long photoperiod. In the first experiment a group of engorged third and fourth instar nymphs were placed in the field in March 1970 and sampled at two-monthly intervals until March 1971, and in the second experiment engorged nymphs and adults were put in the field in October and December 1970 and sampled in December 1970 and in March 1971.

In these two experiments the effects of two variables; hosts and photoperiod, were kept constant, and so any changes in the incidence of diapause must be due to some other variable(s) in the environment.

On the other hand the effect of denying the tick access to hosts and photoperiod can be assessed by comparing the behaviour of those ticks in the field cages with the seasonal pattern already observed. Thus the possible causal agents have been partitioned, photoperiod *et al.* on the one hand and temperature *et al.* on the other. Figure 6.3 gives a schematic summary of Experiments I and II.

Experiment I. The fate of nymphs which engorge during autumn

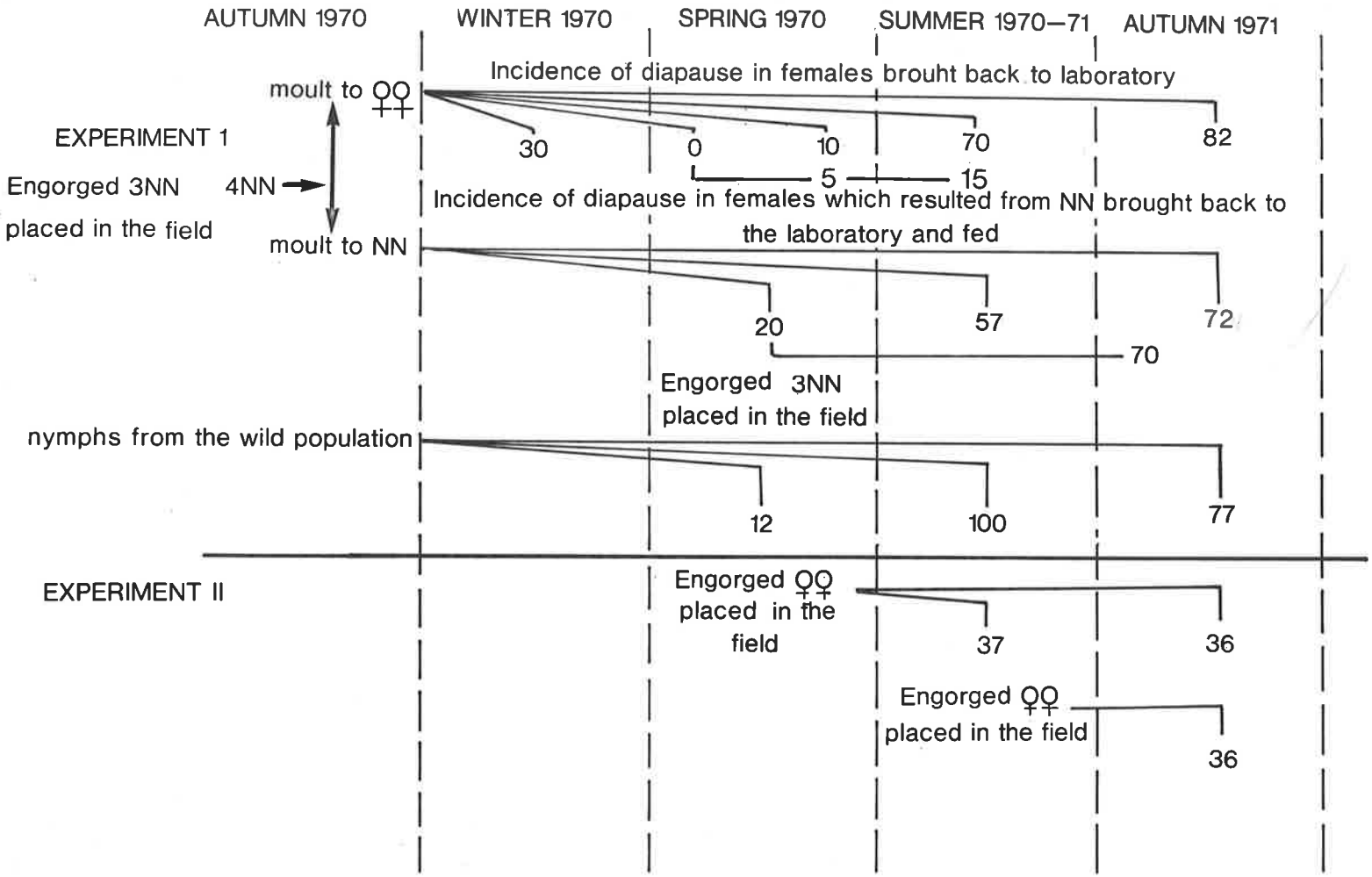
This experiment was carried out to determine when, if at all, diapause became evident in females resulting from nymphs which engorged in autumn. To do this a group of engorged third and fourth instar nymphs was placed in the field (in field cages) in March 1970. The majority of these had moulted to imagos or nymphs by May 1970 (two months later) and thus there were two types of unengorged ticks over-wintering. There were newly-moulted imagos (in which at least 30% of the females present would have been in diapause) and newly-moulted nymphs. These two types will be treated separately. Samples were taken in August, October and December of 1970 and in March 1971.

The Behaviour of the Females

The females in each sample were fed and placed with a male, and the incidence of diapause at 30°C and in long photoperiod was noted. The results are presented in Table 6.4. The incidence of diapause in the wild population on these sampling dates is also presented (transcribed from Table 6.1).

Fig. 6.3

Schematic summary of Field Experiments



x= % diapause in laboratory at 30° C

TABLE 6.4

The incidence of diapause at different times of the year

Sampling Date	Females from Field Experiment I		Females from the Wild population	
	% Diapause	Sample size	% Diapause	Sample size
May 1970	30	20	85	35
August 1970	0	30	31	16
October 1970	10	25	7	14
December 1970	70	20	68	47
March 1971	82	17	97	64

It is clear from Table 6.4 that although at least 30% of the females were in diapause in autumn, there was a very small proportion in diapause in spring. Thus diapause development must have been completed during the winter. Photoperiod could not have affected the females because they were in total darkness. Therefore, the other possible factor, low temperature, was probably responsible for the observed diapause development.

Diapause was almost absent from the spring samples, but 70% of the December sample and 82% of the March sample were in diapause. Thus it is clear that diapause was induced during summer. It is also noteworthy that the incidence of diapause in the ticks in the experiment was closely correlated with the incidence of diapause in the wild population. Hence it is likely that the factors inducing diapause are acting similarly on both groups.

The females in Experiment I were shielded from light for the entire period spent in the field. Nevertheless they entered diapause with the same facility as the wild population, so it is clear that the regulation of diapause in the field is independent of photoperiod. Hence some other factor(s) in the environment of the tick acted to induce diapause while the tick lay buried in the field cage; high temperature, low humidity and starvation may be three such factors. The relative inductive effect of these three factors is examined in laboratory experiments in Section 6.5.

In addition, female ticks from the August sample were given opportunities to feed and oviposit in December 1970 and January 1971. (Table 6.5). Three trends emerge from these observations. Firstly, many females (57%) did not engorge in August even though they were given two opportunities (each on a different rabbit). Nevertheless two months later nearly all engorged at the first opportunity. Thus two months at a moderate temperature (30°C) reactivated the females which had not fed. Laboratory experiments have shown that exposure (5 weeks) to 12°C reduces the proportion of ticks which engorge from 90% to 50%. Thus the exposure to cold during the winter could account for the observed failure of many of the field females to engorge in August. Furthermore, since moderate temperatures reactivate the ticks the warmer weather of spring may have reactivated the field females so that, by October a high proportion would engorge. However, the females from the wild population did not show this trend (Table 6.1).

The second trend occurs in the mean pre-oviposition period. It can be seen in Table 6.5 that the mean pre-oviposition period of the August sample decreased from 27 days in August to 9 days in January 1971, even though all females were kept in 30°C in long photoperiod all the time. This trend is very similar to that found in the field population (Section 4.6). Thirdly, the incidence of diapause increased slightly (from 0% to 15%) during spring and summer.

TABLE 6.5

Feeding and oviposition behaviour of the females from field experiment I in August 1970

Date of feeding	% engorging	% diapause*	Mean pre-oviposition period (days)	S.D.
August 15, 1970	43	0	26.9	9.7
October 13, 1970	95	5	15.9	11.1
January 13, 1971	95	15	8.9	4.1

* % of those which engorged.

The Behaviour of the Nymphs

The other aspect of this experiment was the fate of those ticks which overwintered as nymphs. It is possible that the experience of nymphs might affect the incidence of diapause in the females which moult from the nymphs. Thus at intervals during spring 1970 and summer 1970-71, nymphs were brought back to the laboratory, fed and allowed to moult to imagos at 30°C in long photoperiod. The incidence of diapause in the subsequent females was assessed at 30°C. Nymphs from the wild population were also sampled at the same time and were treated similarly.

It is clear from Table 6.6 that the experience of the nymphs in the field significantly influenced the incidence of diapause in females which moulted from the nymphs. During spring, the females recruited to the population were not in diapause but a high proportion of those recruited during summer were. Thus it is likely that either high temperature, low humidity or both induced diapause in the late-instar nymphs during summer.

However there was a possibility that the increase in the incidence of diapause in both females and nymphs as the seasons progressed was merely a response to the length of time they spent without a meal rather than to any external stimulus. To test this possibility, engorged third-instar nymphs were placed in the field in October 1970 and the subsequent fourth-instar nymphs were removed

to the laboratory in March 1971. They were fed and the incidence of diapause in those which moulted to females was assessed at 30°C. Seventy percent were in diapause. These ticks had spent six months in the field, whereas those in Table 6.6 had spent twelve months in the field. Nevertheless there was no difference in the incidence of diapause. Thus it is clear that the season of the year, but not the duration of exposure, determines the incidence of diapause in ticks in the field.

Experiment II The Fate of Engorged Females

This experiment was designed to examine the effect of field conditions during spring and summer on the induction of diapause in engorged females and on the induction of diapause in females which had oviposited.

Females (from the laboratory culture) which had recently oviposited (and so presumably most were not in diapause) were fed and the engorged females were placed in the field, in field cages, in October and in December 1970. In December 1970 and March 1971 the females were examined and the proportion which had oviposited was noted. Most (82%) of the females in the October sample had oviposited by December, 73% of the December sample had oviposited by March 1971. Hence I concluded that little or no diapause had been induced in October and that diapause had been induced in only 27% of the engorged

TABLE 6.6

The incidence of diapause in females which had moulted from nymphs caught in the field and held in the laboratory

Date when ticks were trapped	Numphs from experiment		Nymphs from wild population	
	% Diapause	n	% Diapause	n
October 1970	20	25	25	25
December 1970	57	30	100	31
March 1971	72	15	77	35

females placed in the field in December 1970. Thus it appears that the engorged female is relatively unreceptive to diapause-inducing stimuli (Laboratory Experiments; Section 6.64).

However, diapause may have been induced in the unengorged female after oviposition. To test this, a sample of the females placed in the field in October 1970 were brought back to the laboratory in December 1970 and March 1971. The incidence of diapause in these females was assessed in the laboratory at 30°C. Similarly the females placed in the field in December 1970 were brought back to the laboratory in March 1971 and the incidence of diapause was assessed. The results are shown in Table 6.7.

Thus, although about one-third of the females in each sample entered diapause (Table 6.7) the incidence of diapause appears to be unaffected by experience of field conditions.

In summary, these field experiments suggest that once a female has engorged, field conditions have little, if any, influence on the incidence of diapause in those ticks. Nevertheless a proportion of non-diapausing females (i.e. those which oviposit) enter diapause. This peculiar behaviour is discussed further in Section 6.64. On the other hand diapause can be induced in females which have not oviposited, and in the nymphal stages.

It has been shown that photoperiod is irrelevant to the

TABLE 6.7

The effect of exposure to field conditions on the incidence of diapause in females which have oviposited

Period of exposure	Percentage diapause	Sample size
October 1970 - December 1970	37	24
October 1970 - March 1971	36	14
December 1970 - March 1971	36	33

regulation of observed diapause and so either high temperature, low humidity or both may have induced the observed diapause. Thus a series of laboratory experiments were designed to investigate these aspects of the regulation of diapause.

Laboratory Experiments

6.51 The Induction of Diapause by Short Photoperiod

The response of the tick to photoperiod is described in Appendix I. The photoperiodic response of the tick proved to be irrelevant to the ecology of the tick because it usually lives in continuous darkness (buried in sand). An experiment in which ticks were kept in complete darkness in the field illustrated that females entered diapause in mid-summer, irrespective of the incident photoperiod (Section 6.43). Furthermore the tick enters diapause in response to short photoperiod. But in nature, diapause is induced in mid-summer, a time of long photoperiod. Thus photoperiodism has no obvious rôle to play in regulating the annual diapause cycle in the field. Nevertheless the phenomenon is interesting because it is the first reported case of photoperiodism and diapause in an Argasid tick. For this reason I examined the response (Appendix I).

6.6 The Induction of Diapause by Temperature-Sensitivity of Adults

The females in the population in early summer can be classified into three groups:-

(i) those which moulted to the adult stage during early spring,

(ii) those which overwintered as females,

(iii) those which moulted to adults in early summer.

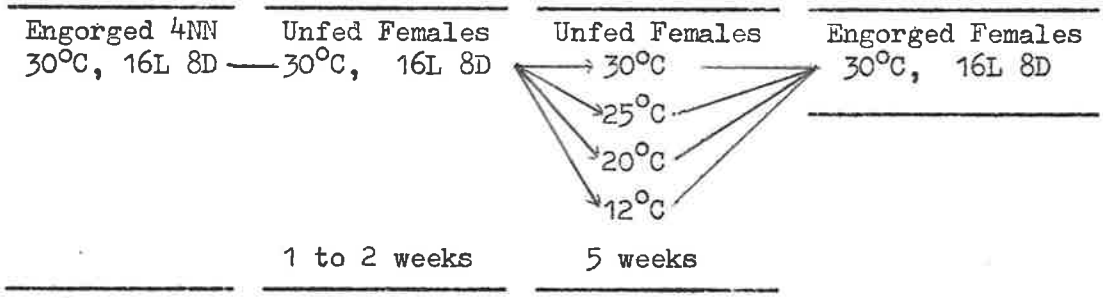
The sensitivity to temperature of each of these three groups of females was examined. The ticks in group (i) would have developed through their later nymphal instars during spring when the temperatures were moderate. Thus females with 'temperature histories' similar to the females in group (i) were those which had been reared in the laboratory at 30°C. The reaction of such ticks to temperature is examined in Sections 6.61, 6.62 and 6.63. Females in group (ii) have completed diapause development. The reaction of such ticks to temperature is examined in Section 6.9. Females in group (iii) had experienced the hot, dry conditions of summer as nymphs. The effect of rearing nymphs in hot, dry conditions on the incidence of diapause in the subsequent female is examined in Section 6.8. These laboratory experiments are summarised in Figure 6.4

6.61 The Sensitivity to Temperature of Unfed Females

To test the effect of temperature on the incidence of diapause,

Figure 6.4 Schematic Summary of the Laboratory Experiments on the Induction of Diapause.

groups of newly-moulted females (reared at 30°C) were exposed to 30°, 25°, 20° and 12°C for five weeks, whereupon they were fed and the incidence of diapause was assessed at 30°C, in long photoperiod. During the period of exposure the females were buried in 2 to 3 cm of sand. The results are presented in Table 6.8.



The influence of temperature on the proportion which enter diapause was analysed using a 2x4 contingency table; effect of temperature was significant ($X^2_3 = 9.6$, $0.05 > P > 0.02$). On closer examination it appears that low incidence of diapause in treatment 3 (20°C) and the high incidence of diapause in treatment 4 may be partly responsible for this significant difference. Thus the difference between treatments 3 and 4 was compared and proved highly significant ($X^2_1 = 9.3$, $0.01 > P > 0.001$). Only one other comparison within the Table was significant; treatment 2 with treatment 4 ($X^2_1 = 5.5$, $P < 0.05$). Thus exposure to cold appears to induce diapause.

Furthermore exposure to cold (12°C) significantly decreased

TABLE 6.8

The influence of five weeks' exposure to a range of temperatures on the incidence of diapause

Treatment No.	1	2	3	4
Temperature to which the ticks were exposed	30°C	25°C	20°C	12°C
Percent diapause	38	34	23	58
Sample size	29	41	43	31
Mean pre-oviposition period (days)	15.5	13.4	11.4	16.0
S.D.	8.3	6.7	4.9	9.4
Percentage of females which fed	91	95	92	68

the proportion of females which will engorge when given an opportunity ($X^2_1 = 15.8$, $P < 0.001$). It is likely that the females placed at 12°C were fully developed, i.e. the physiological processes leading up to readiness to feed (gut clearance, 'hardening') had been completed before the experiment began because at 30°C most females will engorge 3 to 5 days after moulting, but this experiment was not begun until 1 to 2 weeks after moulting. Thus, in conclusion, it appears that exposure to continuous 12°C decreases the proportion of females which engorge and increases the incidence of diapause amongst those that do engorge (relative to 20° and 25°C). Of those temperatures examined 20°C appeared to be the most favourable for diapause-free development.

6.62 The Sensitivity to High Temperature of Unfed Females

The previous experiment examined temperatures up to 30°C but temperatures in the field during summer are frequently in excess of 30°C. It is therefore appropriate to examine the effect of temperatures in excess of 30°C on the incidence of diapause.

Newly-moulted females which had been reared at 30°C in long photoperiod were allocated to temperatures of 35°, 40° and 45°C* for one, two and four weeks; a control group was kept at 30°C. The periods of exposure were begun so that all treatments finished the

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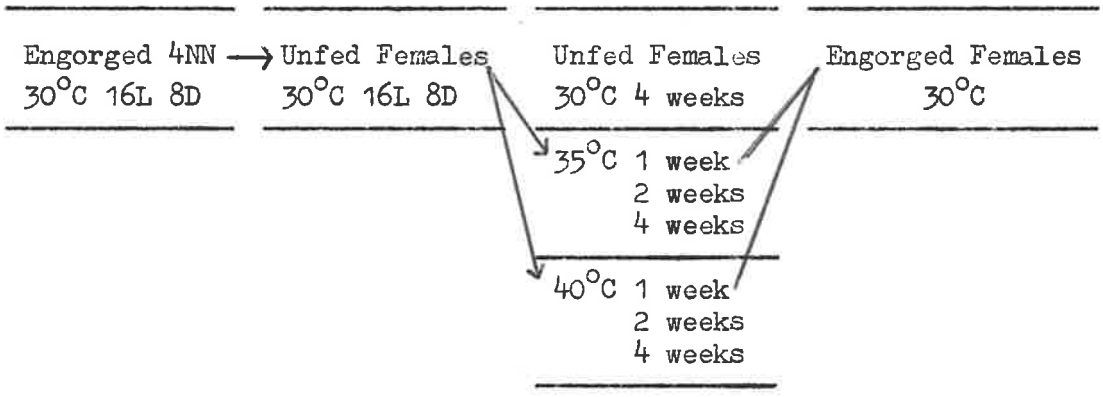
* Exposure to 45°C for one week was lethal.

period of exposure on the same day. All ticks were then given an opportunity to feed. Those which engorged successfully were placed at 30°C in long photoperiod. The proportion in diapause is shown in Table 6.9.



It is clear from Table 6.9 that the proportion of females which engorged and oviposited decreased as the duration of exposure to 40°C increased (X^2_2 = 11.7, P***). This trend, however, is principally due to the inhibition of feeding by exposure to high temperature. There is an apparent trend of increasing incidence of diapause with increasing duration of exposure to 40°C but this trend is not statistically significant, possibly due to the small sample size. The duration of exposure to 30° and 35°C did not affect the proportion which feed and oviposit.

The condition of those ticks which did not feed was studied by placing them at 30°C in long photoperiod for three weeks before offering them another meal. Since most of them (85%) engorged successfully, it

TABLE 6.9

The effect of exposing unfed females to high temperature on the incidence of diapause

Temperature	30°C		35°C		40°C		
Duration of exposure	4 weeks	1 week	2 weeks	4 weeks	1 week	2 weeks	4 weeks
percent diapause	13	13	19	0	14	25	38
percent feeding	95	94	89	80	100	80	57
percent feeding and ovipositing	82	82	72	79	86	60	33
sample size	22	17	18	19	23	20	21

was concluded that high temperature induced a temporary disinclination to feed which did not persist if the females were allowed to recover at a lower temperature.

Exposure to high temperature (40°C) induced a temporary inhibition of the ability to feed but this ability was regained after a period at 30°C and so it was thought that periodic short exposure to high temperature might induce diapause without inducing the inability to feed. Furthermore diurnal temperature fluctuations are more representative of field conditions.

Unengorged females of three types (newly-moulted females, females which had oviposited and females which had just finished diapause development) were allocated to two treatments; the first was five days at 40°C; the second was ten periods of twelve hours at 40°C interspersed with ten periods at 30°C. Thus each group was exposed to 120 hours at 40°C. The incidence of diapause was then assessed at 30°C. The results are shown in Table 6.10.

From the results in Table 6.10 it is clear that diapause was induced only in those females which had recently moulted, and not in the other two groups. This result agrees with that obtained in the field studies (Section 6.43); i.e., that only those females which had not fed were sensitive to diapause-inducing stimuli.

TABLE 6.10

The effect of diurnal temperature fluctuations on the incidence of diapause in unengorged females

Type of female:	Newly-moulted		Having oviposited		Having completed diapause development	
Treatment	% Diapause	n	% Diapause	n	% Diapause	n
5 days at 40°C	6	29	11	28	13	16
10 periods of 12 hours	26	66	13	57	10	36
X^2_1	4.5	P*	N.S.		N.S.	

6.63 The Sensitivity to Temperature of Females during and after Oogenesis and Oviposition

The two previous experiments have shown that the temperature ($\geqslant 20^{\circ}C$) experienced by unengorged female did not significantly influence the incidence of diapause. It is possible, however, that although the females oviposited, the conditions experienced before and during oviposition may have induced diapause which would become manifest after the next meal.

To test this possibility engorged females were subjected to temperatures of 40°, 35°, 30° and $25^{\circ}C$ (all at long photoperiod) prior to and during oviposition, and those which had oviposited after 32 days were removed, fed and placed at $30^{\circ}C$ in long photoperiod where the proportion in diapause was noted. There is also a possibility that prolonged exposure after oviposition may influence the incidence of diapause and so another group was kept at $30^{\circ}C$ for 80 days after feeding before being fed again. The results are presented in Table 6.11.

From these results it is clear that the temperature experienced by the ticks which oviposit, affects neither the subsequent incidence of diapause nor the feeding behaviour of the ticks. Furthermore exposure of unengorged (post-oviposition) females to these temperatures did not affect the incidence of diapause. This supports the findings in Section 6.61 and 6.62; that the incidence

TABLE 6.11

The effect of temperature during oogenesis and oviposition on the incidence of diapause

Temperature experienced during and after oviposition	Percent diapause after second feed (sample size in brackets)	Percent feeding
40°C for 32 days	20 (5)	100
35°C for 32 days	26 (31)	97
30°C for 32 days	34 (59)	91
30°C for 80 days	38 (31)	97
25°C for 32 days	28 (57)	95

of diapause is little affected by the temperature (if it is $\geq 20^{\circ}C$) experienced by unengorged females.

Unfed Females	Engorged Females	Unengorged Females	Engorged Females
$30^{\circ}C$ 16L 8D	$40^{\circ}C$ for 32 days	$40^{\circ}C$ for 32 days	$30^{\circ}C$
	$35^{\circ}C$ for 32 days	$35^{\circ}C$ for 32 days	deep sand
	$30^{\circ}C$ for 32 days	$30^{\circ}C$ for 32 days	
	$25^{\circ}C$ for 32 days	$25^{\circ}C$ for 32 days	
	$30^{\circ}C$ for 80 days	$30^{\circ}C$ for 80 days	

6.64 The Sensitivity to Temperature of Engorged Females

The reaction of engorged females to field conditions has been touched upon in Section 6.43, the results of which suggest that the incidence of diapause in newly-moulted females differs from that in females which have already oviposited. Thus the reaction of the two types of females was examined; both experimental groups had been reared at $30^{\circ}C$ in long photoperiod.

Engorged females were placed in deep sand and were then allocated to constant temperatures within the range $15^{\circ}C$ to $45^{\circ}C$: $45^{\circ}C$ proved lethal. The proportion of females which did not oviposit is reported in Table 6.12.

At high and low temperatures, only a small proportion of the females oviposited. This failure to oviposit may have been due to the induction of diapause or the failure of normal metabolic processes

TABLE 6.12

The influence of temperature on the oviposition behaviour of engorged females: The percentage which did not oviposit (sample size in brackets)

Temperature (°C)	GROUP I Newly-moulted females	GROUP II Females which had already oviposited
40	100 (40)	95 (39)
35	93 (42)	42 (70)
30	33 (380)	13 (70)
27.5	..	30 (52)
25	43 (60)	29 (70)
22.5		65 (50)
20	100 (40)	90 (50)
15	100 (45)	100 (50)

at the extremes of temperature experienced. In order to differentiate between the two possibilities the females in Group II which remained engorged after 50 days at their respective temperatures, were returned to 30°C and any further oviposition was noted. Those which oviposited were considered not to have been in diapause, but rather in a temporary condition of inhibited oogenesis induced by the extremes of temperature. But those which failed to oviposit were considered to be in diapause. The results are set out in Table 6.13.

From these results it is clear that 50 days' exposure to high temperature induced diapause. However the failure to oviposit after prolonged exposure to high temperature could be due to sub-lethal damage to the metabolism of the tick which prevented oogenesis (analogous to the high temperature inhibition of moulting in nymphs) rather than to the temporary (but healthy) dissociation of the gonadotrophic process which is characteristic of diapause. This possibility was examined in the section which examines diapause development (Section 6.9). It was discounted because, after chilling, the females laid eggs and so had not been damaged by high temperatures.

The most favourable temperature for diapause-free oogenesis appears to be about 30°C, but it is noteworthy that even at that temperature there was a significant proportion of females which were

TABLE 6.13

Oviposition behaviour at 30°C after 50 days exposure to the treatments in Table 6.9 (Group II)

The first 50 days		Subsequent period at 30°C		
Temperature (°C)	% which oviposited	% which oviposited	% Diapause	Sample size (n)
40	5	0	95	40
35	58	6	42	70
30	87	2	10	70
27.5	70	8	30	50
25	71	10	22	70
22.5	35	30	35	50
20	10	69	21	50
15	0	71	29	50

still in diapause. Occasionally in my laboratory studies there have been treatments in which all the females oviposited but such instances have been rare. Thus under most conditions studied there appeared to be a proportion of females which did not oviposit (and so were in diapause) even though they had oviposited previously, and conversely, some of the females in diapause would oviposit if fed again.

This flux in and out of diapause in constant conditions was examined in more detail. Females were fed and placed at 25°, 30° and 35°C. Fifty days later the ticks were sorted into those which had oviposited and those which had not, and were given an opportunity to feed again. They were then returned to the conditions from which they had come and the proportion which oviposited was noted (Table 6.14).

Thus there appears to be a spontaneous flux into and out of diapause. At 25° and 35°C the flux into diapause (44%) was greater than that out of diapause (10% and 9%) whereas the trend was reversed at 30°C (14% into and 29% out of diapause). Thus this mechanism would act to increase the incidence of diapause in cool weather, decrease it in warm weather, and increase it again in hot weather. And so this mechanism may serve to induce diapause in summer but not in spring.

In summary, it appears that prolonged exposure to high temperature increases the incidence of diapause in engorged females; this contrasts with the response in unengorged females (Section 6.62).

TABLE 6.14

The flux into and out of diapause

Unfed females 30°C	⟶	Engorged females	Percent not ovipositing after second feed (sample size in brackets)
		35°C	
		Diapause	90 (20)
		Non Diapause	44 (50)
		30°C	
		Diapause	71 (31)
		Non Diapause	14 (51)
		25°C	
		Diapause	91 (48)
		Non Diapause	44 (76)

But there is a complicating factor, in that high temperature also induces a temporary inhibition of oogenesis. This phenomenon differs from diapause because, in contrast to it, oogenesis resumes immediately the animal is returned to moderate temperatures. Temperatures of about 30°C were the most favourable for diapause-free development, but even at this temperature there was a flux into and out of diapause which did not appear to be determined by the prevailing conditions because it occurred in apparently constant conditions. Nevertheless the magnitude and direction of this flux was determined by the temperature.

Continuous high temperatures are not representative of field conditions and so the next experiment was designed to examine the way in which short periods of exposure to high temperature affected the incidence of diapause.

6.65 The Effect of Short Periods of Exposure to High Temperature

Females which had oviposited at 30°C in long photoperiod were fed again and placed at 40°C. One control group was left at 30°C. After 1, 8, 14 and 21 days' exposure to 40°C groups of females were removed to 30°C and the oviposition behaviour and the incidence of diapause was noted. The results are presented in Table 6.15. No females oviposited at 40°C.

It is clear from these results that exposure to 40°C inhibited

TABLE 6.15

The effect of short periods of exposure to 40°C on the oviposition behaviour and the incidence of diapause in engorged females

Days at 40°C	0	1	8	14	21
Percent diapause	7	25	34	27	24
Mean pre-oviposition period (days)	11.2	13.2	23.8	28.5	41.3
Duration at 30°C (days)	11.2	12.2	15.8	14.5	19.3
S.D.	7.0	8.8	9.3	6.3	10.2
Sample size	30	43	44	44	42

oviposition for the period during which the females were kept at 40°C. Furthermore when females were returned to 30°C oviposition continued normally except for a relatively slight protraction of the pre-oviposition period. There are two aspects of this experiment on which I shall comment. The first concerns the oviposition behaviour and the second, the incidence of diapause.

If the pre-oviposition period is considered as that period between being placed at 30°C and oviposition (because 40°C inhibits oogenesis), then it is clear that the pre-oviposition period increases as the period of exposure to 40°C increases. For example, the pre-oviposition period increases from 12.2 days after one day's exposure to 19.3 days after 21 days' exposure. Thus it is clear that not only was oogenesis inhibited at 40°C, but the high temperature experienced retarded subsequent oogenesis at 30°C. A similar process has been observed in Rhodnius prolixus (Okasha, 1970) and it is analogous to the high temperature inhibition of nymphal development observed in O. gurneyi (Section 4.42).

From Table 6.15 it is clear that diapause is induced by high temperature. There appears to be an initial response to one day's exposure to 40°C because the incidence of diapause increases from 7% with no exposure to 25% with one day's exposure ($\chi^2_1 = 4.3$, P*). However, further exposure, up to 21 days to continuous 40°C does not increase the incidence of diapause. This suggests that the initial

sharp increase in temperature may have induced diapause, and so there is a possibility that fluctuating temperatures with a maximum of about 40°C might induce diapause in a high proportion of females. However, this possibility was not studied further because of lack of time.

6.7 How Humidity had but a Slight Influence on the Incidence of Diapause in Adults

Humidity has been shown to affect the rate of development of some insects (Alifanov, 1964; Howe, 1967) and is said to affect diapause in others (Loew, 1962). Kangaroos urinate and defecate in their wallows. They also congregate on areas of fresh grass which grows after thunderstorms (Frith and Calaby, 1969) and so might be available to the ticks after periods of hydration. Thus it was considered relevant to examine the effect of humidity on the manifestation of diapause.

6.71 The Effect of Relative Humidity on Engorged Females

In order to assess the effect of relative humidity on the oviposition behaviour and the incidence of diapause in the kangaroo tick, a group of females was fed and divided into sub-groups which were placed at each of four relative humidities at 30°C. The percentage in diapause was recorded (Table 6.16).

As can be seen from Table 6.16, relative humidities between

TABLE 6.16

The effects of relative humidity on the incidence of diapause in engorged females

Relative Humidity (%)	10	32.5	75	95
% Diapause	16	18	21	3
Sample size	37	33	33	31

10 and 75% have little effect on the incidence of diapause, but at 95% R.H. the incidence of diapause decreases significantly ($X^2_1 = 4.35$, P*) relative to the other humidities tested. A similar trend has been shown in the mean pre-oviposition periods of the four treatments (Section 4.62). Thus it appears that high R.H.'s favour more rapid oviposition and a lower incidence of diapause, than do low R.H.'s.

There was a possibility that diapause may also have been affected by the R.H. experienced during and after oviposition. Thus from this experiment the females which had oviposited were fed again and the incidence of diapause was assessed at 30°C in 95% R.H. Those from 10 and 32% R.H. were pooled and compared with those from 75 and 95% R.H. (Table 6.17).

As can be seen from the Table, there was a significant effect of humidity. Those females from high humidity showed a higher incidence of diapause (33%) than those which had experienced lower humidity (17%) ($X^2 = 3.87$, P*). The mean pre-oviposition periods were not significantly different.

6.72 The Reaction of Engorged Diapausing Females

A second aspect of the reaction of females to humidity was the reaction of engorged diapausing females to rapid changes in humidity. Groups of females at 30°C in long photoperiod at 10-20% R.H. which were engorged and yet had not oviposited within 40 days of feeding were placed at 32.5, 95 and 100% R.H. and oviposition during

TABLE 6.17

Effect of experience of R.H. before oviposition

Relative Humidity	Low (10% & 32%)	High (75% & 95%)
% Diapause	17	33
Sample size	53	57
2x2 X^2_1 test	$X^2_1 = 3.87$	P**

the following 60 days was recorded.

Table 6.18 shows that some ticks in all treatments oviposited and proportionally more females laid eggs at high humidities than at low humidities. Furthermore the eggs were laid more rapidly at high than at low humidities.

In summary, it appears that when engorged females experience high humidities, a higher proportion oviposit than if they experience low humidities. In addition, eggs are laid more rapidly at high than at low humidities. However when engorged females oviposit at high humidities they have a greater tendency to enter diapause than do those which oviposit at a lower R.H. Nevertheless in all cases the effect of R.H. is not dramatic and it effects only a relatively small change in the incidence of diapause. Thus humidity is not likely to be a major factor in regulating diapause in the field.

6.8 The Induction of Imaginal Diapause in the Nymphal Stage

Field studies on the life-cycle of the tick and on the behaviour of its host, the red kangaroo, show that late instar nymphs present during spring are likely to feed and moult to imagos which will be ready to feed during summer and early autumn. Field sampling has shown that most females captured at this time of year were in diapause (Section 6.41) and field experiments have shown that the experience of the nymphal stages influences the incidence of diapause

TABLE 6.18

Effect of rapid changes in humidity on engorged diapausing females (at 30°C, 16L 8D)

Relative humidity (%)	32	95	100
% ovipositing	6.3	13.0	15.5
$\bar{x}$ (days)	19.5	17.1	16.4
S.D.	18.3	11.1	6.2
Sample size (n)	64	54	58

in the subsequent adults (Section 6.43).

From a knowledge of the rate of development (Chapter 4) and the temperatures which prevail in the field during spring and early summer (Table 5.12), it is clear that the ticks which moult to diapausing females during summer must have been derived from third- and fourth-instar nymphs, and so the reaction of only these stages was examined. The sensitivity of both engorged and unengorged nymphs to a range of temperatures was tested. All experiments were carried out with the ticks buried in deep sand.

6.8 Sensitivity of Nymphs

6.81 The Sensitivity of Engorged Third-, and Unengorged Fourth-Instar Nymphs

This experiment was designed to determine the incidence of imaginal diapause when engorged third-instar nymphs were exposed to a range of temperatures.

Engorged third-instar nymphs were placed at 24°, 30° and 35°C in deep sand. Five weeks later the resulting fourth-instar nymphs were fed and placed at 30°C, where they moulted to imagos. The females which emerged from these fourth-instar nymphs had thus experienced the conditions shown in the diagram below. The females from these treatments were fed and placed at 30°C in long photoperiod where the proportion of females in diapause was noted (Table 6.19).

TABLE 6.19

The effect of nymphal experience of temperature on the incidence of imaginal diapause

Temperature experienced as engorged 3NN and unfed 4NN	35°C	30°C	24°C
Percentage of females in diapause	48	34	25
Mean pre-oviposition period (days)	24.1	18.9	22.2
S.D.	10.8	9.9	5.4
Sample size	39	38	4

Unengorged 3rd instar	Engorged 3rd instar	Unengorged 4th instar	Engorged 4th instar	Unengorged females	Engorged females
30°C	24°C	24°C	30°C	30°C	30°C
	30°C	30°C			
	35°C	35°C			

It is clear from these results that high temperature induces diapause in the nymphal stage and it becomes manifest only when the adult females engorge ($X^2_2 = 52.9$; P**). Diapause could have been induced either during the period spent as an engorged third-instar nymph or during that period spent as an unengorged fourth-instar nymph, or during both stages. The following experiment was carried out to test whether the unengorged nymphs were sensitive.

6.82 The Sensitivity of Unengorged Nymphs

A group of unfed fourth-instar nymphs which had been reared at 30°C were allotted to groups and stored at 30°, 35° and 40°C for seven days. The nymphs were then fed and placed at 30°C where they moulted. The resulting females had experienced high temperature only as unengorged fourth-instar nymphs, as shown below. The proportion of females in diapause was noted and is shown in Table 6.20.

TABLE 6.20

Effect of high temperature on unengorged nymphs

Temperature to which unengorged fourth-instar nymphs were exposed for 7 days	30°C	35°C	40°C
Percent diapause in subsequent females	20	73	87
Sample size	20	11	15

TABLE 6.21

The effect of moulting to imago at high temperature on the incidence of diapause in the subsequent female

Temperature experienced while moulting	30°C	35°C
Percent diapause	28	69
Sample size	44	43

Conditions experienced by the ticks:

Unengorged 4NN	Unengorged 4NN	Engorged 4NN	Unengorged females	Engorged females
30°C	30°C	30°C	30°C	30°C
	35°C			
	40°C			
	(seven days)			

The results in Table 6.20 were analysed using the X^2 test on a 2x3 contingency table and the effect of temperature proved highly significant ($X^2_2 = 32.4$; P***). It is clear that exposure of unengorged fourth-instar nymphs to high temperature induces a latent diapause. The following experiment tests the sensitivity of the engorged nymph to high temperature.

6.83 The Sensitivity of the Engorged Nymph

It has already been demonstrated that the incidence of diapause in newly-moulted females is little affected by the temperature experienced as imagos (Section 6.6). Thus any difference between the incidence of diapause in females which moulted to the imago at different temperatures will be due to the reaction of the engorged nymphs to temperature.

A group of fourth-instar nymphs which had been reared at 30°C were fed. Half was replaced at 30°C and the other half was placed at 35°C. When they had moulted to imagos, the females were removed and

fed, then placed at 30°C where the incidence of diapause was noted. The results are presented in Table 6.21.

It is clear from these results that temperature had a very significant effect (X^2_1 = 19.2, P***) and that high temperature induced a latent diapause in the engorged nymph.

In conclusion it is clear that both the unengorged and engorged nymphs are sensitive to high temperature and respond to it by entering diapause.

6.9 Diapause Development

Two experiments were carried out in an attempt to gain some understanding of the processes involved in diapause development. The first experiment examines the effect of long and short photoperiod at a range of temperatures on diapause development and shows that diapause is independent of photoperiod but encouraged by temperatures between 12° and 20°C. The second experiment was designed to determine the optimum temperature for diapause development, to examine the effect of duration of exposure on diapause development and to examine the relative strength of various inductive stimuli.

Experiment I. The Effect of Temperature and Photoperiod on Diapause Development in Engorged Females

The purpose of this experiment was to decide whether temperature or photoperiod or both, had any effect on the rate of diapause development.

A group of engorged females which had not oviposited during the previous 50 days at 30°C in long photoperiod (and so were in diapause) were divided randomly into eight groups. These were allocated to a range of temperatures (30°, 25°, 20° and 12°C) and to long and short photoperiod. At 6 and 12 weeks after the beginning of the experiment samples were removed and placed, without being fed, at 30°C in long photoperiod, where the proportion which oviposited was noted.

Oviposition without feeding was taken as the indicator that diapause development had been completed. Thus the proportion which oviposited was an index of the effectiveness of the treatment in promoting diapause development.

From the results in Table 6.22 it is clear that photoperiod had little or no effect on the rate of diapause development. The failure of the ticks to respond to photoperiod may have been due to their being buried in the sand (and so being shielded from the incident photoperiod) but they were free to search out the incident photoperiod which lay less than 1 cm above them, if that was their habit. Their failure to respond was taken as an indication that this reaction, if it exists, is unimportant to the biology of the tick.

Temperature, on the other hand, significantly influenced diapause development. Although it appears that diapause development occurs at 25°C it proceeds most rapidly at temperatures of 20°C or

TABLE 6.22

The number ovipositing after treatment
(sample size in brackets)

Period of exposure	6 weeks		12 weeks	
Photoperiod	16L 8D	8L 16D	16L 8D	8L 16D
Temperature (°C)				
30	0 (3)	0 (3)	0 (7)	0 (4)
25	2 (6)	1 (2)	0 (2)	0 (3)
20	1 (5)	1 (3)	2 (4)	2 (3)
12	4 (6)	4 (8)	4 (5)	6 (8)

TABLE 6.23

Percentage of females surviving exposure to 5°C
(sample size in brackets)

	Period of exposure (weeks)		
Origin of tick	6	12	24
Type a	100 (11)	55 (11)	0 (10)
Type b	100 (10)	60 (10)	30 (10)
Type c	100 (11)	46 (11)	8 (12)

less and not at all at 30°C. Thus O. gurneyi is similar to other organisms in that, although the most favourable temperatures for morphological and diapause development occupy different temperature ranges there is a certain area of overlap and so morphological and diapause development can proceed concurrently.

The number of ticks used in each treatment of this experiment was not large enough to give precise information on the rate of diapause development at the different temperatures examined; furthermore the experiment did not examine the depth of diapause which different stimuli might induce. Thus the next experiment examines these two aspects of diapause but only within the temperature range where no obvious morphological development takes place (i.e. 20°C and lower).

Experiment II. The Effect of Temperature on the Rate of Recovery of Reproductive Ability to Engorged Females which have not Oviposited at Constant Temperature

In the title of this experiment I have deliberately referred to 'recovery of reproductive ability' rather than to diapause development because some of the engorged females which did not oviposit (e.g. those at 40°C) and were therefore chilled, possibly were not in diapause but were merely inhibited from ovipositing by the high temperatures they had experienced (Section 6.64). The ticks used in this experiment were engorged females which had not oviposited in

constant temperature during the previous 50 days. They came from three sources:-

a. 40°C and L16 D8

b. 25° or 30°C and L16 D8

c. 30°C and L8 D16

The behaviour of these three groups was examined in order to compare the relative 'strength' of the stimuli which induced the reproductive inhibition.

Ticks from each of the three groups were divided into twelve equal sub-groups and three sub-groups were allocated to each of four temperatures (20°, 15°, 10° and 5°C). After 6, 12 and 24 weeks' exposure, one sub-group of types (a), (b) and (c) was removed from each temperature and placed at 30°C in long photoperiod without being fed. The oviposition behaviour was noted carefully.

The ticks were examined regularly and the day that each tick began to oviposit was noted. These data generated for each group an oviposition curve characteristic of the post-diapause condition at 30°C in long photoperiod. Thus two criteria (the proportion which oviposited without being fed, and mean pre-oviposition period at 30°C) were used as indices of the effectiveness of the treatments in terminating reproductive inhibition.

The results from this experiment will be treated in three parts:

(i) mortality due to chilling;

(ii) the effect of chilling on the pre-oviposition period; and

(iii) the effect of chilling on the percentage of females which oviposit.

(i) Mortality Due to Chilling

All the females at 10°, 15° and 20°C survived the treatments, but it is clear from Table 6.23 that prolonged exposure to 5°C was lethal. It is also noteworthy that mortality did not differ significantly between groups (a), (b) and (c). This was taken as evidence that the extreme conditions (such as 50 days at 40°C) experienced before chilling, failed to harm the tick in any discernible way.

(ii) The Pre-oviposition Period

The means and variances of the pre-oviposition period for each treatment are set out in Table 6.24 and the influence of the different variables has been analysed by means of analysis of variance (Table 6.25).

From this analysis it is clear that the temperature and especially the duration of exposure have a marked influence on the pre-oviposition period.

Thus 20 weeks' exposure reduces the mean pre-oviposition period to 10 days (from 26 days after only 6 weeks' exposure). The data are

TABLE 6.24

Effect of chilling on the mean pre-oviposition period

	Mean Pre-oviposition Period (After Chilling) (days)				
	Temperature (°C)				
	20	15	10	5	Mean
Duration of exposure					
Type a					
6 weeks	37	27	22	-	28
12 "	28	19	19	19	21
24 "	32	6	7	-	12
Mean	33	19	15	19	21
Type b					
6 weeks	22	23	24	26	24
12 "	12	24	21	47	23
24 "	8	10	8	6	8
Mean	13	17	15	26	17
Type c					
6 weeks	24	26	29	33	27
12 "	17	16	26	19	19
24 "	8	17	7	-	9
Mean	19	20	15	25	19

Table of Means

Duration of exposure					
6 weeks	27	25	25	30	26
12 "	17	20	22	26	21
24 "	12	13	7	4	10
Mean	19	19	15	25	19

TABLE 6.25

Effect of chilling on the preoviposition period
(Analysis of Variance)

Source of Variation	D.F.	F.	p^y
Type	2	< 1.0	n.s.
Duration	2	20.09	***
Temperature	3	3.36	*
Type x Duration	4	< 1.0	n.s.
Type x Temperature	6	4.64	*
Temperature x Duration	6	3.03	**
Type x Duration x Temperature	12	< 1.0	n.s.
Total	35		

plotted in Figure 6.5. Differences of this order have been found between groups of ticks which have a low incidence of diapause (mean pre-oviposition period; 6-12 days) and groups which have a high incidence of diapause (mean pre-oviposition period; 20-30 days) (Section 4.6). This suggests that diapause development takes more than 12 weeks at the temperatures tested.

The effect of temperature was significant at the 5% level. From Table 6.24 it appears that 10°C is the most favourable temperature for diapause development. It is noteworthy that there is no significant effect due to the type of female. Thus all three inductive stimuli induced a comparable intensity of diapause.

(iii) The Percentage of Females which Oviposit

Table 6.26 shows the percentage of females in each group which laid eggs after chilling (but without being fed). This table presents a confusing picture because the only significant effects (when the data were analysed by analysis of variance (Table 6.21)) were the differences in the responses of the three types of ticks, and the interaction between duration of chilling and temperature.

Analysis of the data in Table 6.26 shows that the proportion which oviposit after chilling is greater in those ticks which entered diapause at 25° and 30°C (45-50%) than it is in those which entered at 40°C (30%).

A similar trend was found in another experiment in which

Figure 6.5 The Relationship between the duration of chilling and the Pre-oviposition Period.

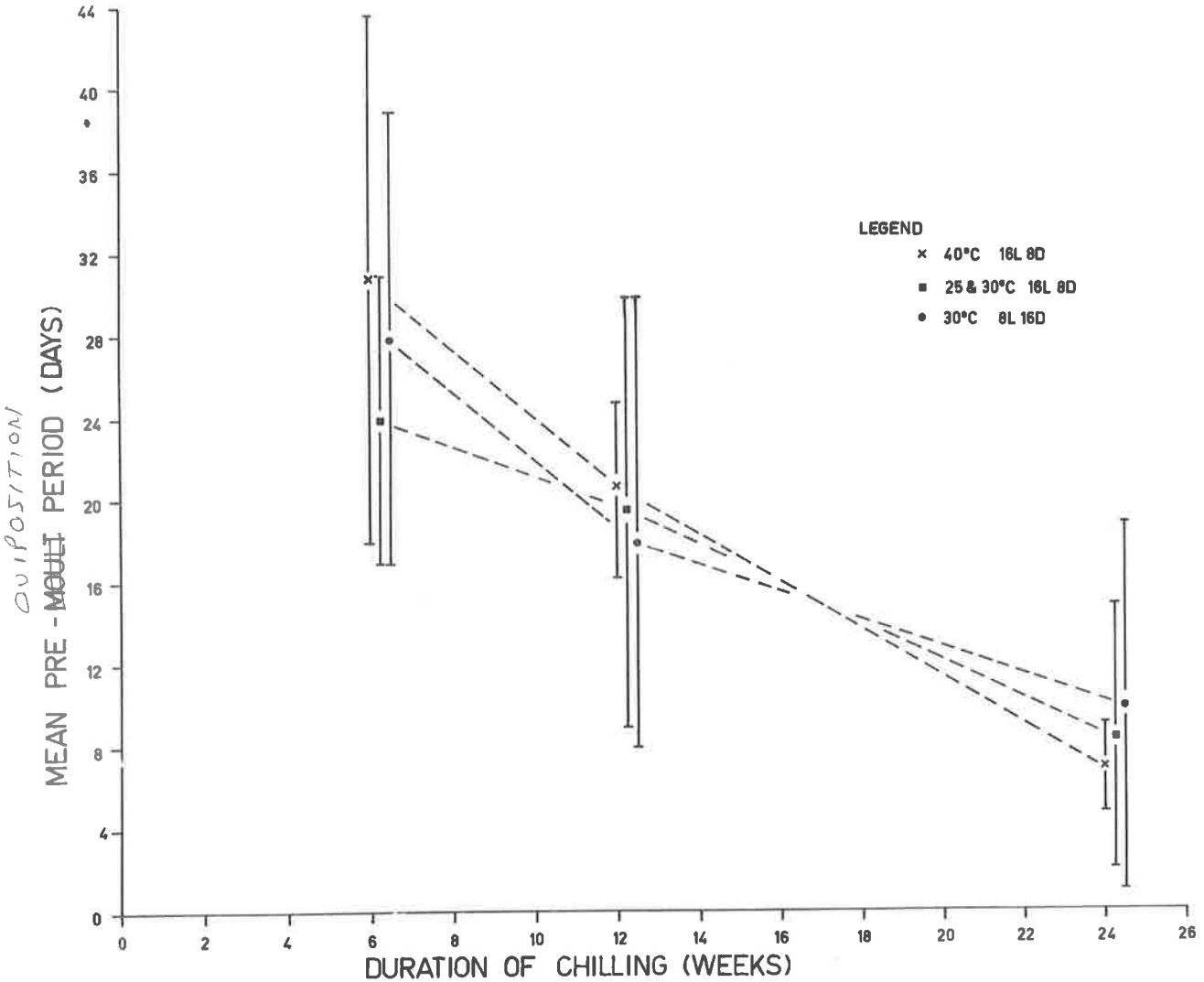

LEGEND
× 40°C 16L 8D
■ 25 & 30°C 16L 8D
● 30°C 8L 16D
MEAN PRE-OVIPOSITION PERIOD (DAYS)
DURATION OF CHILLING (WEEKS)
0
4
8
12
16
20
24
28
32
36
40
44
0
2
4
6
8
10
12
14
16
18
20
22
24
26

TABLE 6.26

Percentage of engorged females which laid eggs after chilling

Duration of exposure	Temperature (°C) 20	15	10	5	Mean
Type a					
6 weeks	40	30	29	9	27
12 "	22	20	30	50	31
24 "	22	20	60	--	34
Mean	28	23	40	30	30
Type b					
6 weeks	40	40	20	40	35
12 "	40	45	45	33	41
24 "	20	72	80	68	60
Mean	33	52	48	47	45
Type c					
6 weeks	75	75	20	54	56
12 "	42	59	33	60	49
24 "	33	67	84	40	46
Mean	50	67	46	38	50

Table of Means

Duration of exposure					
6 weeks	52	48	23	34	39
12 "	35	41	36	48	40
24 "	25	53	75	34	48
Mean	37	48	45	39	42

TABLE 6.27

The effect of chilling on the proportion of females which oviposit
(Analysis of Variance)

Source of Variation	D.F.	F	P[y]
Type	2	5.42	*
Duration	2	<1	n.s.
Temperature	3	1.00	n.s.
Type x Duration	4	1.23	n.s.
Type x Temperature	6	<1	n.s.
Duration x Temperature	6	3.57	*
Type x Duration x Temperature (error)	12		
Total	35		

diapause was induced at 25°, 30°, 35° and 40°C. Fifty days later the diapausing females were placed at 12°C and chilled for 9 weeks, and then the oviposition behaviour at 30°C was observed. The proportion failing to oviposit and the mean pre-oviposition period for each treatment are set out in Table 6.28. When the females which had not oviposited were fed, a high proportion of them oviposited.

Thus it is clear that the higher the temperature at which diapause was induced, the higher was the proportion which failed to oviposit after chilling. There are at least two possible explanations for this behaviour; either 40°C induced a deeper diapause than did 30°C, or 50 days at high temperature depleted the food reserves so that a smaller proportion of females were capable of ovipositing after chilling.

These observations suggest that depletion of food reserves may have a marked influence on whether a tick will oviposit at 30°C, even though diapause development may be complete. To examine this possible explanation the females which had been chilled for 12 weeks (Table 6.26), but which had not oviposited at 30°C, were fed and returned to 30°C where the proportion ovipositing was noted. The total number which had oviposited (with or without feeding) was then expressed as a percentage of the total number of ticks chilled (Table 6.29).

These results strongly suggest that limited food reserves,

TABLE 6.28

The effect of various inductive conditions on oviposition behaviour after chilling

Diapause-inducing conditions	40°C 16L 8D	35°C 16L 8D	30°C 16L 8D	30°C 8L 16D	25°C 16L 8D
Percent failing to oviposit after chilling	80	69	42	32	12
Mean pre-oviposition period (days)	30.4	33.6	31.5	27.4	24.0
S.D.	13.6	28.3	7.8	15.8	8.7
Sample size	35	36	38	41	41

TABLE 6.29

The effect of chilling for 12 weeks on the ability of females to oviposit before and after feeding (numbers in brackets)

Chilling conditions (°C)	20	15	10	5
Percent ovipositing after chilling	35	41	36	48
Percent ovipositing after feeding	58	48	44	16
Total percent which oviposit	93	89	80	64

rather than diapause, prevented the females ovipositing after chilling. Thus it appears that temperatures between 10° and 20°C are equally efficacious in promoting diapause development, and that 12 weeks' exposure is sufficient to complete diapause development in most ticks.

The explanation of the peculiar results for 20° (and 15°C), i.e. decreasing percent oviposition with increasing exposure to low temperature) may be that food reserves had been depleted by metabolic processes and so were no longer available for oogenesis. [At 10° and 5°C, however, metabolic processes are virtually at a standstill and so the proportion which oviposit at those temperatures reflects the rate of diapause development (oviposition will still be modified by the food reserves present at the beginning of the chilling).]

6.10 Discussion

These studies show that there is a seasonal cycle in the incidence of imaginal diapause in the field. The timing of the cycle and the amount of effective temperature experienced during the cycle (Chapter 4) suggest that, while most larvae are produced during late spring and early summer, a small proportion are produced during late summer and early autumn. The seasonal fluctuations in the number of larvae found in the wallow samples corroborates this. The effect of this seasonal recruitment of larvae on the population dynamics is discussed in Chapter 7.

A priori, this behaviour appears inexpedient because kangaroos continue to frequent wallows during summer and early autumn (Chapter 7), and so egg-laying (and consequent recruitment of larvae) virtually ceases long before kangaroos stop visiting wallows for that season. However, January and February are the hottest and dryest months of the year. Data in Chapter 7 show that, on the average, wallows in the study area were visited about once every two months during summer. Chapter 5 has shown that eggs and larvae soon die if exposed to hot, dry conditions, so that during mid-summer desiccation must pose a very serious threat to the survival of larvae; diapause prevents the production of larvae during the season when they are most likely to perish.

High temperature induces diapause in nymphs. Thus females recruited to the population during summer are in diapause. However females recruited during spring (and possibly autumn) are not in diapause. It would be of interest to determine how long the diapause condition, induced during summer, persists in late-instar nymphs which do not feed. Furthermore it would be relevant to know whether diapause development occurs in such nymphs. The low incidence of diapause in females recruited to the population during spring (October, 1970) suggests that diapause development may well occur in nymphs, but this aspect of diapause was not pursued any further.

The induction of diapause in most unengorged females is still

a conundrum. Studies on the longevity of the tick and its ability to tolerate desiccation suggest that females are capable of surviving in the field for several seasons without feeding (Chapter 5). In the laboratory, females have lived and laid eggs over a period of two years. It seems likely that the female population in mid-summer consists of new recruits and of females which have been recruited during the previous years, and so a significant proportion of the females in the population at the beginning of summer is likely to have been adult during the previous season. Thus the problem is, 'Do these females enter diapause in mid-summer and, if so, what are the inductive stimuli?'

The evidence from field experiments on the induction of diapause in females which have not oviposited is supported by laboratory data. Field experiments indicate that diapause is induced in unengorged females during mid-summer, and evidence suggests that high temperature and/or low humidity are the inductive stimuli. Laboratory experiments in Section 6.6 show that high temperature induces diapause in females which have not oviposited, but only in 27% of them. Section 6.7 has shown that low humidity induces little, if any, diapause (Table 6.16). Thus it appears that high temperature (and not low humidity) induces diapause in newly-moulted females in the field.

Both field and laboratory experiments suggest that unengorged

females (once they have oviposited) are insensitive to diapause-inducing stimuli encountered in the field during mid-summer. Nevertheless about one-third of the females which oviposited in the field (Experiment II) were in diapause when fed and placed at 30°C in the laboratory. Similar behaviour was also observed in laboratory populations where a spontaneous flux into and out of diapause was observed (Table 6.14). The magnitude of this flux depends upon their temperature history as unengorged females (Tables 6.10, 6.11). The incidence of diapause may vary from 10% to 60%. Thus if females (which have oviposited) engorge during a hot spell during summer, the high ambient temperatures may induce diapause, so that they would enter winter as engorged diapausing females.

Table 6.2 shows that about 40% of females caught in early autumn and about 10% of those caught in summer were engorged. Figure 6.2 shows that about 90% of the females in the field population at the beginning of February were in diapause. Thus between February and April about one-third (30% of 90%) of the females engorge. This suggests that about one in three females have an opportunity to feed during that period. The probability of a tick finding a meal will be dealt with in greater detail in Chapter 7.

This chapter has dealt with the seasonal occurrence of

diapause. This causes a seasonal flush of larvae. The effect of these larvae on the population dynamics of the tick population will be dealt with in the next chapter.

CHAPTER 7

The Behaviour of Kangaroos and the Annual Breeding Cycle of the Tick

The previous chapters contain information about the life-cycle of the tick, the mechanisms which limit its distribution to wallows, the effect of temperature and humidity on the survival and rate of development and the regulation of diapause. This chapter relates a series of observations on the seasonal changes in the wallowing behaviour of the kangaroos and then presents data on the seasonal changes in the population structure of the ticks. Finally an attempt is made to integrate these various aspects of the biology of the tick.

7.1 Seasonal Changes in the Use of Wallows

The kangaroo, being a nocturnal animal, rests by day and feeds by night. But whether or not it rests in a shaded wallow or on open plains will depend on the temperature of the day.

On cold days kangaroos bask in the sun in areas sheltered from wind (e.g. behind clumps of bushes) whereas on hot days they shelter from the sun under shady trees (Frith and Calaby, 1969). This is thought to be a thermo-regulatory mechanism, and so the quality of shade sought should depend on the temperature of the day. Thus, although kangaroos will lie under all types of shade trees, the frequency of use of any particular tree species will depend on

the quality of shade provided and the temperature of the day. Furthermore the suitability of the wallow may also depend upon the area shaded, whether or not the shaded area is sheltered from the wind and also on the texture of the soil and the amount of litter present.

In order to examine these questions I carried out a series of observations on the study area on Moralana station, where I knew that both ticks and kangaroos were plentiful. The shade trees on an area 1.1 miles by 0.2 miles were mapped and classified, according to the species and the quality of shade provided and the number of wallows* under each tree noted.

The quality of the shade provided by each tree was allotted to one of three classes. These classes are defined in terms of the intensity of light which was transmitted through the foliage on a bright, clear day when the intensity of direct sunlight was about 100,000 lux. Three readings of the light intensity were taken at three randomly chosen places under trees. The average of the three readings was taken as the index of light intensity in the wallow; low light intensity indicated dense shade. The three

---

* Wallows occur under shade-trees and are relatively discrete areas characterised by "hip-hollows" which have been scraped out by kangaroos while lying therein. The number of wallows under each shade tree usually increases as the area shaded increases; if several wallows are present they are usually separated by an area of undisturbed soil or by vegetation. The soil in wallows, due to the movements of the kangaroos, is usually soft and friable and so ticks can burrow into it with ease.

classes were:-

1. Dense shade: almost no direct sunlight reached the ground through the foliage (< 1300 lux).
2. Medium shade: some sunlight came directly through the foliage (1300 < Intensity < 33,000 lux).
3. Sparse shade: the foliage obscured very little of the sunlight (> 33,000 lux).

These trees were examined approximately every two months. The frequency of use of the wallows by kangaroos during this period was estimated by noting the amount and type of dung deposited. Dung was classed (by using its colour and texture) into two groups; old and new - and if more than one class of dung was present in any one wallow then it was recorded as having been visited at least twice. After each tree was examined, all the dung was cleared away.

Data were collected over an 18-month period (August 1969 to March 1971). The proportion of wallows being used varied throughout the year; many were being used in summer and few in winter. When analysing the data, I could not use the simple proportion of wallows occupied as a comparative statistic because the intervals between samples were not equal. Thus in order to compare wallowing activity at different times of the year, I devised a 'relative use index', which included a time factor.

$$\text{Relative Use Index} = \frac{\text{number of times that wallows were visited} \times 10^4}{\text{total number of wallows x interval in days}}$$

e.g. 7/1/70 to 18/3/70 Interval = 70 days

$$\text{Relative Use Index} = \frac{122 \times 10^4}{169 \quad 70}$$

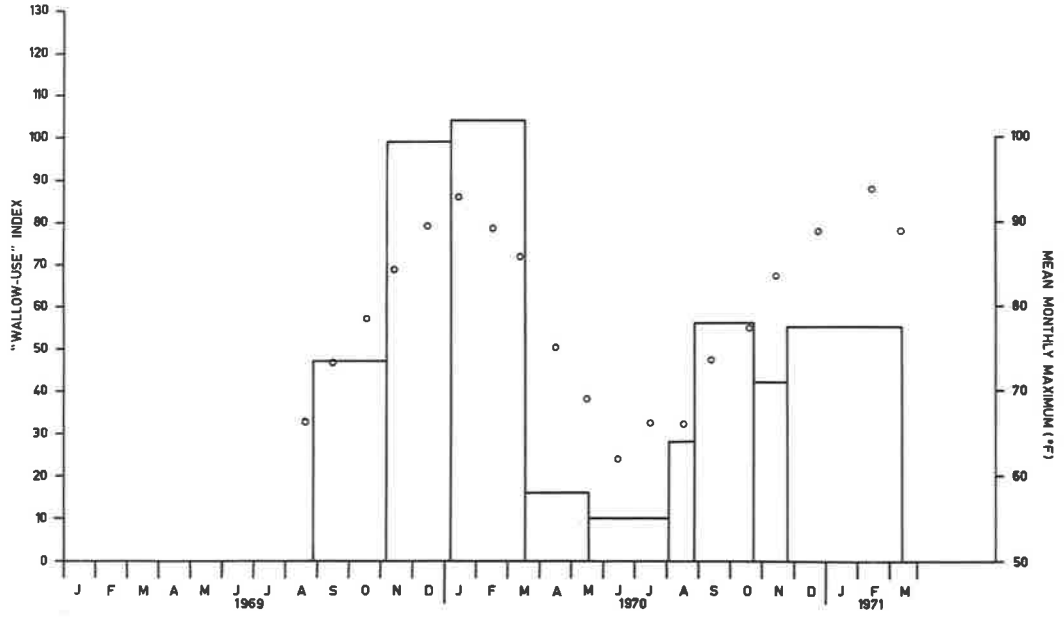
$$= 104$$

The results of these observations are shown in Fig. 7.1. From this graph we can see that during 1969(70)71 wallows were used relatively infrequently from mid-March until August (during autumn and winter), but that relative use increased during spring to a maximum rate which was maintained from November to March.

Also plotted on the graph are the means of the maximum monthly temperature at Hawker (30 m. south of the study area) for each month. There is a high correlation between high temperatures and frequent use of wallows. From this data it appears that kangaroos begin to wallow frequently when the mean monthly maximum is about 70 - 80°F.

Because resting in the shade serves a thermoregulatory function, it is likely that the quality of the shade chosen will vary with temperature and hence with the season of the year. To examine this possibility the above data were reanalysed and the

Figure 7.1 Seasonal Fluctuations in the Frequency with which wallows were visited.


"WALLOW-USE" INDEX
MEAN MONTHLY MAXIMUM (°F)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
100
90
80
70
60
50
J F M A M J J A S O N D J F M A M J J A S O N D J F M
1969
1970
1971

seasonal fluctuations in the relative use of each quality of shade were plotted (Fig. 7.2) (40% of the trees in the study area had either dense or medium quality shade and 20% had sparse shade).

The number of wallows of a particular shade class which had been visited was expressed as a percentage of the total number of wallows which had been visited. This is an index of the class of shade chosen by the kangaroos. As can be seen from Fig. 7.2 there are obvious differences in the quality of shade preferred in summer and winter.

The data in Table 7.1 compare the types of shade preferred in summer and winter (when the data were pooled). It is clear that the proportion of wallows in each shade class used during the cooler months (May to August) was approximately equivalent to the proportion of trees in that class. Thus, during the cooler months of the year the kangaroo showed no preference for any particular shade quality. It is noteworthy that kangaroos seen in the field during winter were sunning themselves while during the hotter months they were resting in the shade. Thus it is to be expected that the behaviour of the kangaroo during winter bears no relation to the quality of shade.

However, during spring kangaroos showed a marked preference for trees which provided medium quality shade. During the summer of 1969-70 kangaroos preferred medium shade to dense shade, but during the summer of 1970-71 medium and dense shade were visited at

Figure 7.2 Seasonal Fluctuations in the Quality of Shade Used by Kangaroos.

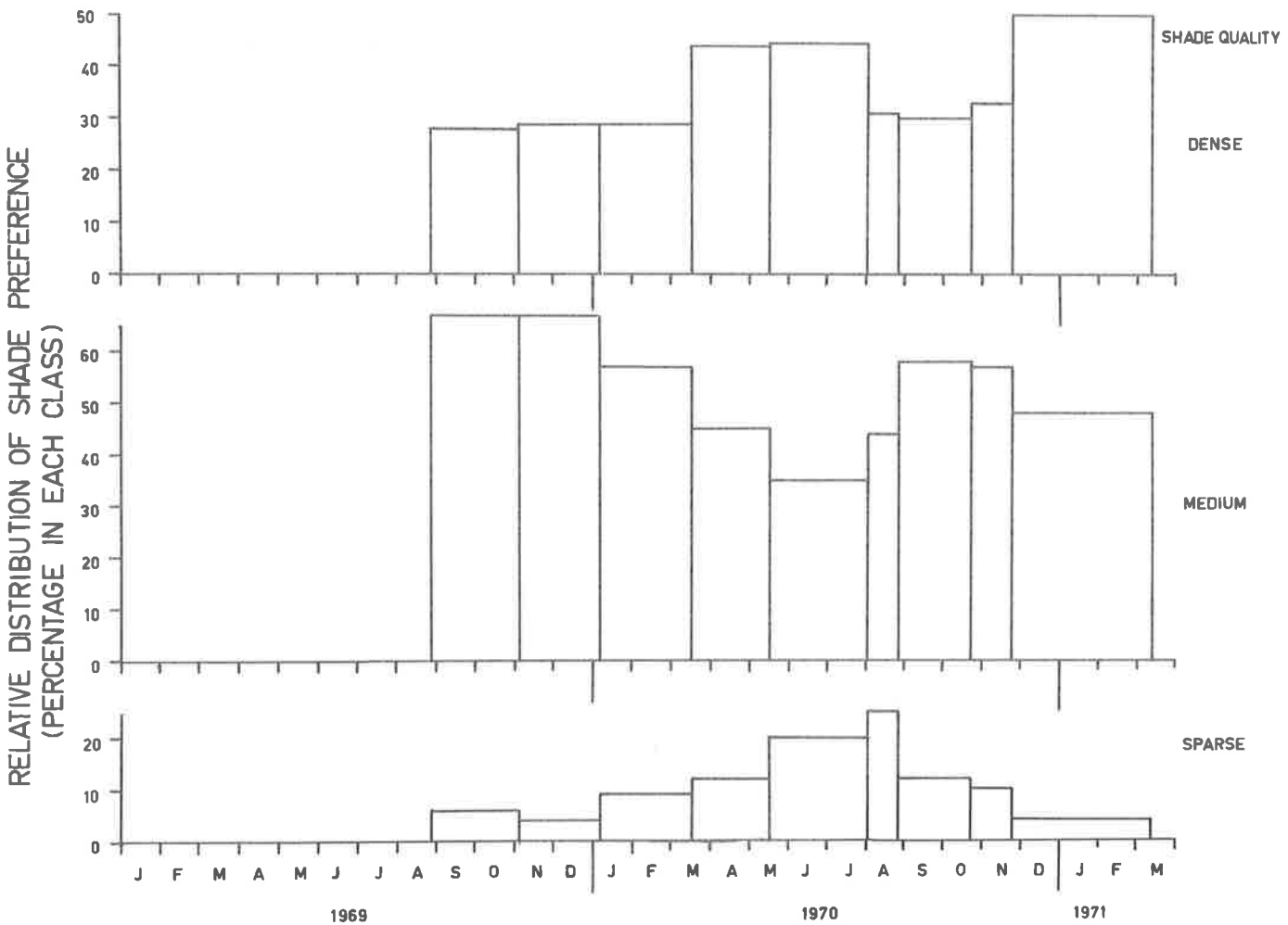

RELATIVE DISTRIBUTION OF SHADE PREFERENCE
(PERCENTAGE IN EACH CLASS)
SHADE QUALITY
DENSE
MEDIUM
SPARSE
50
40
30
20
10
0
60
50
40
30
20
10
0
20
10
0
J F M A M J J A S O N D J F M A M J J A S O N D J F M
1969
1970
1971

TABLE 7.1

Class of shade preferred during different seasons

*Percentage of use attributed to each shade class

Shade Intensity	Dense	Medium	Sparse	Sample Size
Season				
Summer Period (Oct - March)	32.8	58.4	8.6	469
Winter Period (May - August)	40.3	41.3	18.4	80
Percentage of the trees on study area in each class	40	40	20	

* This percentage was calculated by noting the number of times wallows under each class of shade were visited by kangaroos in that season and expressing the total for each class as a percentage of the total number of visits to wallows in that season.

about the same frequency (Fig. 7.2). However in both years, the sparse shade was avoided during spring and summer.

Thus it appears that during the winter the wallowing behaviour of the kangaroo is independent of the quality of shade over the wallow. In contrast, during spring, summer and autumn, the trees providing medium-dense shade are selected as sites in which to wallow.

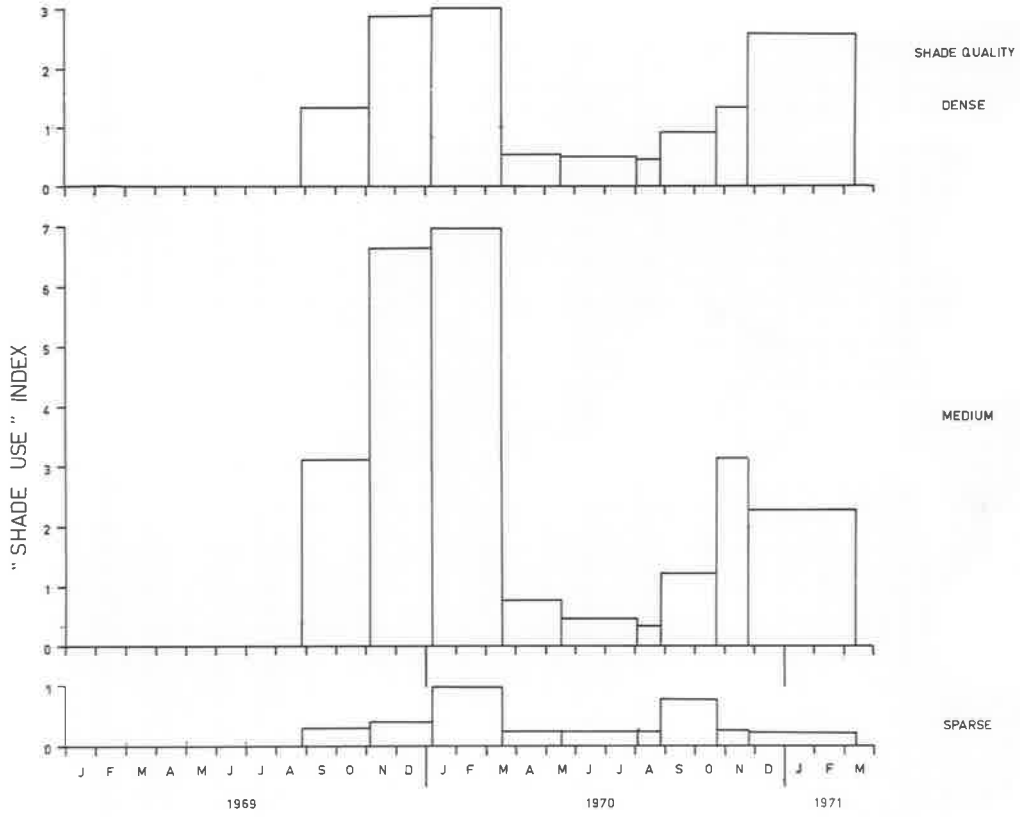
Further insight into the behaviour of the kangaroo can be gained by quantifying the way in which its behaviour changes with the season of the year. By multiplying the relative use index for each period of the year by the percentage use of each quality of shade at that time of the year we can estimate the relative frequency at which each class of shade was used throughout the year (Fig. 7.3). I have called this parameter the 'shade-use index'.

For example: between January 4 and March 18, 1970, the relative use index was 104.

Quality of shade	% Use	Shade Use Index
Dense	29	3020
Medium	61	6340
Sparse	9	940

This index gives the relative chance, for each shade class, of a kangaroo visiting a wallow during any period and so possibly of a tick finding a meal.

Figure 7.3 Seasonal Fluctuations in the Relative Frequency with which each Quality of Shade was used.


"SHADE USE" INDEX
SHADE QUALITY
DENSE
MEDIUM
SPARSE
J F M A M J J A S O N D J F M A M J J A S O N D J F M
1969
1970
1971

7.2 The Relative Density of Ticks in Wallows

Another way in which to examine the behaviour of the kangaroo in relation to the quality of shade is to examine the relative density of ticks in wallows sheltered by shade of differing intensities. A high density of ticks indicates that a wallow was visited frequently. However low numbers do not necessarily indicate that the kangaroo visits the wallow less frequently because there may be some component of the environment, weather for instance, which makes that place unsuitable for ticks in survive in. Nevertheless the distribution and density of ticks is a relevant aspect of the ecology of the tick and may provide information about the behaviour of its host.

The following observations were made on a cloudless day in December 1970 with a maximum shade air temperature of 90°F. The wallows samples were adjacent to the main study area but had never been sampled before. The most common trees in this area were bullock bushes (Heterodendrum spp.) and black oaks (Casuarina spp.) on top of, and on the slopes of, the sand dunes; mulga, Acacia aeneura and small bush Acacia species (plain acacia) on the plains between the sand dunes; and prickly acacia, Acaria victoriae, in the mud pans.

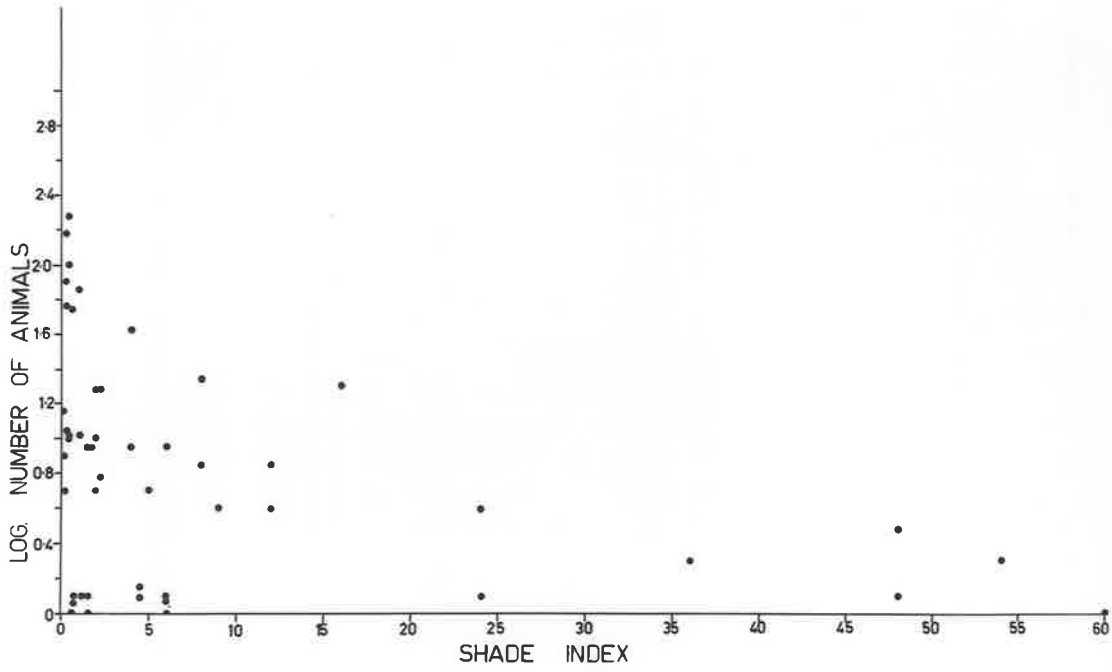
The wallows under ten trees of each of these five types of trees were sampled for ticks using two CO_2 traps per tree. Three

readings of the light intensity were taken at each tree. Three hours later the ticks in the traps were collected. Trapping took place between midday and 4.00 p.m. The log of the number of ticks was plotted against a shade index (the product of the three light intensity readings) (Fig. 7.4). This clearly shows that most ticks were caught under trees which shed the densest shade and that very few ticks were found where the shade was sparse. (The average number of ticks trapped per wallow for each shade class was:- dense, 38.6 ticks per wallow; medium, 14.1 ticks per wallow; and sparse, 4.0 ticks per wallow). Bullock bushes consistently provided the most dense shade whereas black oaks provided the most variable shade quality.

At the same time the area of shade which each tree provided was estimated by pacing the length and breadth of the shaded area and then treating it as a rectangle, e.g. an area one pace wide and two paces long would be taken as 18 sq. ft of shade. An average of 27.7 ticks per tree were trapped under the ten trees with the largest shade-area (an average of 76 sq. ft) while an average of 6.0 ticks per tree were trapped under the trees providing the smallest area of shade (an average of 6.3 sq. ft). Thus it is clear that, on the average, there are many more ticks present where the shaded area is large, than where it is small.

If the density of ticks under a shade-tree is an index of

Figure 7.4 The Relationship Between the Quality of Shade and the Number of Ticks in the Shaded Wallows.


LOG. NUMBER OF ANIMALS
2·8
2·4
2·0
1·6
1·2
0·8
0·4
0
0
5
10
15
20
25
30
35
40
45
50
55
60
SHADE INDEX

the frequency with which the tree is visited by kangaroos then it is apparent that kangaroos prefer wallows under trees which provide a large area of dense shade.

7.3 What is the Density of Kangaroos?

This data can also be used to estimate, even if very approximately, the density of kangaroos on the study area. If we assume that during summer each kangaroo used one wallow per day, then because the study area was 0.2 sq. miles, the density of kangaroos during the summer, 1969-70 was 8.7 kangaroos/sq. mile and during 1970-71 was 4.4 kangaroos/sq. mile. Frith and Calaby (1969) have reviewed the available data and it appears that during the 1960's the kangaroo densities ranged from 1.2 kangaroos/sq. mile in the most arid areas of New South Wales to 8.6 kangaroos/sq. mile in the better grazing country.

Moralana is relatively arid and so the estimates of kangaroo density in the study area appear higher than one might expect. Nevertheless the densities calculated in the studies mentioned by Frith and Calaby (1969) were calculated from samples taken from hundreds of square miles whereas my study area was only 0.2 sq. miles of the most favourable areas on Moralana. Furthermore kangaroos may visit more than one wallow per day and so the estimates of the density of kangaroos on the study area are likely to be an overestimate of the

kangaroo density of Moralana.

7.3.1 How frequently are wallows visited each year?

Knowing the way in which the kangaroo behaviour changes throughout the year, it is now possible to calculate the average number of times a tree in each shade class is visited by kangaroos. For example during January and February of 1970, 72.2% of the wallows were visited at least once. Fig. 7.2 shows how these visits were distributed amongst the three classes of shade. From this we can calculate that, on the average in the study area a wallow covered by sparse shade would be visited 0.22 times, medium shade, 1.2 times, dense shade, 0.8 times during that 70-day period. When this calculation is performed for all the samples and the probabilities added we see that during the study wallows sheltered by sparse, medium and dense shade were visited 0.6, 2.4, and 1.6 times per year respectively.

The data in Section 6.41 show that only about one third of the females in the population engorged between mid-summer and autumn. This is the result of the interaction between the frequency with which kangaroos visit wallows and the ability of the females to find a meal when a wallow is visited.

Although this reflects the number of opportunities a tick has to feed, it does not mean that every or even most of the ticks which are capable of feeding, find a meal every time a kangaroo visits a wallow.

7.3.2 What is the chance of finding a meal?

There are many factors which help determine the probability of a tick feeding, if a kangaroo visits the wallow in which the tick is living. First of all the tick must be capable of sensing and responding to the sensory cues which the presence of a kangaroo provides. Thus the physiological condition of the tick must be such that it can respond (Appendix II), and the ambient temperature must be greater than the temperature threshold for activity (Appendix II). The latter temperature is between 14° and 17°C, but even at temperatures several degrees above that threshold the movements of the tick are sluggish. Thus from the soil temperatures in Table 5.1.2 it seems that during winter, ticks would not feed even if a kangaroo visited the wallows. In spring kangaroos begin to visit wallows more frequently and the mean monthly maximum air temperature is about 25°C; at that temperature ticks are very active. Thus during spring, summer and autumn the probability of a tick feeding will depend upon the ability of the "hungry" tick to locate the kangaroo when it visits a wallow.

In an attempt to determine what proportion of the hungry ticks in a wallow were trapped during one day, CO_2 traps were set in a wallow on three successive days. Ticks were caught on each day and the number of ticks caught on day 3 was more than half that caught on day 1. Thus many ticks may well not find a meal when a

kangaroo visits a wallow. The absolute number of ticks in a wallow is very difficult to determine. CO_2 traps were set at the above mentioned wallow on each of 10 successive sampling trips over a period of two years. There was no evidence (neither dung nor scratchings) that the wallow was visited during that period but ticks were trapped on the first 8 occasions. Altogether about 800 ticks were taken from the wallow. Thus it seems likely that only a relatively small proportion of the ticks in a wallow which are capable of finding a host and feeding, do so each time a kangaroo visits a wallow. Similar behaviour has been observed in O. savignyi in desert areas of South Africa (Neville, pers. comm.).

Thus, although a wallow may be visited as often as three or four times during the hot period of the year, each tick in the wallow is unlikely to feed that many times. Because the tick requires 5 - 7 feeds to complete its life-cycle, it is unlikely that the average life-cycle would be complete in under 2 - 3 years, even under favourable conditions; and it may take 5 - 6 years.

The slow progression through the life-cycle should induce a relatively stable instar distribution in field populations. The next section reports the changes in the age structure of the population over a period of two and a half years.

7.4 The Instar Distribution

Preliminary observations during 1968 suggested that all nymphal instars were present during every season of the year. Furthermore since the life-cycle in the field probably takes between 2 and 5 years it is likely that there will be a relatively stable instar or age distribution. One consequence of reproductive diapause, the relatively low temperatures experienced during spring and the behaviour of the kangaroo, is that the majority of the larvae produced each year are likely to hatch during late spring and early summer. This seasonal flush of larvae should induce an annual perturbation in an otherwise stable instar distribution.

In order to examine this possibility, the population in the study area was sampled at intervals of about two months and the instar distributions were studied for seasonal variation.

7.4.1 Seasonal occurrence of larvae

The presence or absence of larvae in each sampled wallow during 1969, 70 and 71 was noted. It is clear from Table 7.2 that larvae are abundant during summer but relatively rare or absent at other times of the year. This pattern was consistent with that predicted from a knowledge of the incidence of diapause.

7.4.2 Seasonal changes in the nymphal instar distribution

Most samples were taken using CO_2 traps. This method,

TABLE 7.2

The changing incidence of larvae in field samples throughout the year

Date of Sampling	Wallows without larvae	Wallows containing larvae	
	Number	Number	Mean number of larvae per sample
20 March 1971	6	4	72
20 December 1970	0	12	40
28 August 1970	12	0	0
1 August 1970	12	0	0
6 May 1970	9	1	3
17 March 1970	5	3	-
7 January 1970	0	5	-
8 November 1969	1	3	18
24 August 1969	8	1	1
4 May 1969	6	1	-
4 April 1969	3	2	-

although the best available to me, suffers from many of the grave defects common amongst sampling techniques. For example, because the temperature threshold for activity of the early instar ticks is lower than that of the later instars (Appendix II) the catches on winter days may be biased, and might contain proportionally more early instars than would be caught on a warmer day. Furthermore there is no guarantee that, even on a warm day, all instars are equally trappable. In order to examine the latter possibility the relative trappability of each instar was tested in the laboratory.

An artificial wallow 1 - 4" deep and 2 x 1' was infested with 50 first instar nymphs, 50 second instar nymphs, 50 fourth instar nymphs and 50 females. The ticks were left undisturbed overnight. In the morning the "wallow" was placed in sunlight, a standard CO_2 trap was set and the proportion trapped was noted (Table 7.3).

This data suggest that all instars are equally trappable on hot days. However samples may still be biased because the different instars may have differing abilities to search out a host from distances of more than one foot.

On several occasions wallows were observed closely during trapping using CO_2 as bait and it was not uncommon to see ticks, both large and small, crawling towards the trap from distances of up to ten feet away. The ticks invariably crawled up-wind towards the CO_2.

TABLE 7.3

Proportion of ticks trapped in artificial wallow

	% trapped
1NN	68
2NN	60
4NN	70

Ticks of all instars have been trapped from wallows which had not been disturbed since rain fell, several days previously. Since those ticks buried in the soil would have been imprisoned by the setting of the soil, it is reasonable to conclude that those trapped after the rains were either lying under very shallow soil or merely in the litter. Ticks have also been found buried in hard sand up to 8 cm below the surface, where they would have been imprisoned until dug out.

The horizontal distribution of ticks in wallows has been assessed for some wallows by dividing the area under the tree into quadrats and sieving the soil in these quadrats. From this it appears that most of the ticks in a wallow are to be found in that area which is shaded by the canopy of the tree about midday. Nevertheless there are exceptions where ticks have been found in very exposed areas.

When sorting the catches of ticks it was relatively easy to separate out the six-legged larvae and the males and females. However, initially the difference between the nymphal instars was not apparent. The usefulness of several different measurements as a means of sorting the instars was examined. The length of the nymph proved the most satisfactory criterion and the frequency distribution of a field sample is shown in Fig. 7.5. As can be seen, this technique permits the nymphs to be sorted into three distinct groups;

Figure 7.5 The Frequency of Occurrence of nymphs of Different Lengths.

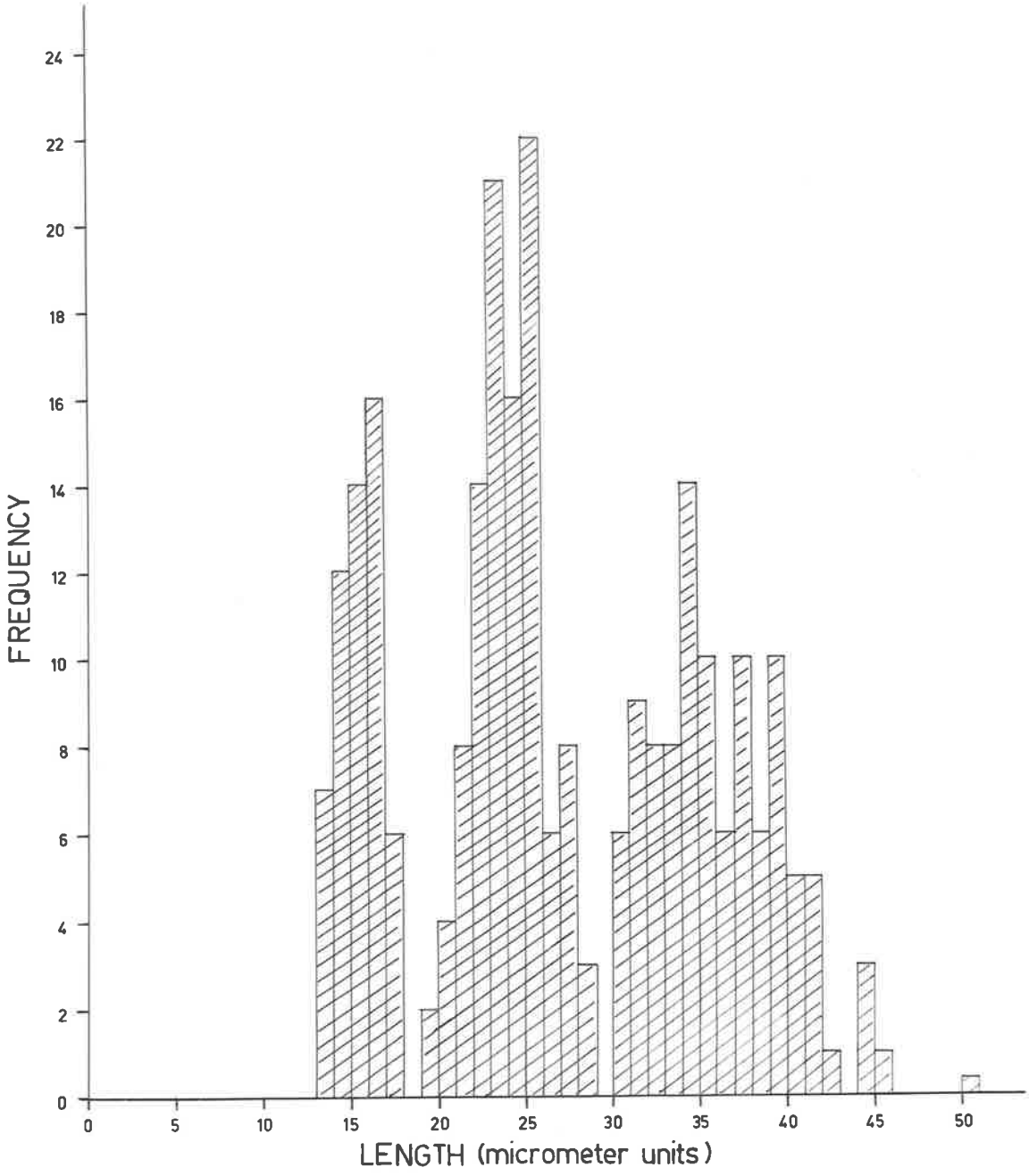

FREQUENCY
0
2
4
6
8
10
12
14
16
18
20
22
24
0
5
10
15
20
25
30
35
40
45
50
LENGTH (micrometer units)

TABLE 7.4

The numbers of ticks trapped at different sites under individual trees

		1NN	2NN	3NN	♂♂	♀♀	Total
Tree 1	Trap 1	1	5	6	6	2	20
	2	1	1	2	0	1	5
	3	1	2	4	6	5	18
Tree 2	Trap 1	2	8	34	12	13	69
	2	2	10	31	21	6	70
Tree 3	Trap 1	16	19	6	4	8	49
	2	20	21	6	3	3	53
Tree 4	Trap 1	0	4	7	4	1	16
	2	1	1	8	5	5	20
Tree 5	Trap 1	16	26	17	7	3	69
	2	33	27	7	1	12	68
Tree 6	Trap 1	158	72	17	5	12	264
	2	252	36	49	35	23	448

first instar nymphs, second instar nymphs and others.

The area shaded by a tree naturally will vary widely according to the nature of the tree. The area shaded (and consequently likely to harbour ticks) varies from a few square feet in the case of small Acacia bushes to several hundred square feet in the case of larger mulgas. Thus it was necessary to determine whether or not the distribution of the ticks over the area underneath the shade-tree was relatively homogeneous (i.e. whether or not samples from different places under the same tree had the same instar distribution).

To do this two standard CO_2 traps were set 5 to 6 feet apart under each of six large shade-trees. The number of each instar caught in each trap is shown in Table 7.4. From this table it is very clear that the number and type of ticks caught under a shade-tree will depend very much upon where the trap is placed because the distribution of each instar was very patchy. In view of this, at least two CO_2 traps were set under each shade-tree that was sampled.

As well as the variation in distribution of ticks in the soil under individual shade trees there is also great variation in the relative proportions of each instar trapped under each shade-tree, even at one time of the year. Table 7.5 sets out the numbers of each instar trapped under seven shade-trees sampled in March 1970. It is clear from this that there is very great variability between them. This is understandable in the light of the feeding and detaching

TABLE 7.5

The number of ticks of each stage caught in CO_2 traps under seven trees in March 1970

Wallow number	Instar	1NN	2NN	3,4,5NN	♂♂	♀♀	Total
1		17	12	11	8	5	53
2		40	127	44	13	9	232
3		2	21	26	18	9	71
4		97	11	4	0	1	113
5		49	53	16	7	4	137
6		440	128	66	40	35	712
7		235	98	13	10	3	359

Percentage of total in each instar

Wallow number	1NN	2NN	3,4,5NN	♂♂	♀♀
1	32	23	20	15	9
2	17	55	19	6	4
3	3	30	36	18	13
4	86	10	4	0	1
5	36	39	18	5	2
6	62	20	6	6	6
7	65	27	4	3	1

behaviour of the early instars.

The instar distribution within any wallow is very variable due to the feeding behaviour of the tick and the behaviour of their host. For example on one day a kangaroo could rest in a wallow and in so doing remove a significant proportion of the larvae and first instar nymphs. These larvae and nymphs are likely to be deposited in relatively large numbers in a small number of wallows over the following few days. Thus the instar distribution of a number of wallows could be dramatically altered in a few days and then remain unaltered for months.

Notwithstanding the above mentioned variability it may be possible to detect annual fluctuations by sampling a number of wallows and posting the data, thus smoothing out some of the intra and inter wallow variability. The data in Table 7.5 illustrate this. For instance in samples from individual wallows the proportion of imagos varied from 1% to about 40% but when the samples from each trip were posted the variability decreased, and the proportion of imagos varied from 12% to 25% of the total catch.

When the number of wallows sampled is small, the contribution of each sample to the instar distribution is relatively large. Thus one atypical sample in a small number of samples can obscure the typical pattern. During 1969-70 between four and six wallows were sampled per trip. On four occasions the sample from one wallow

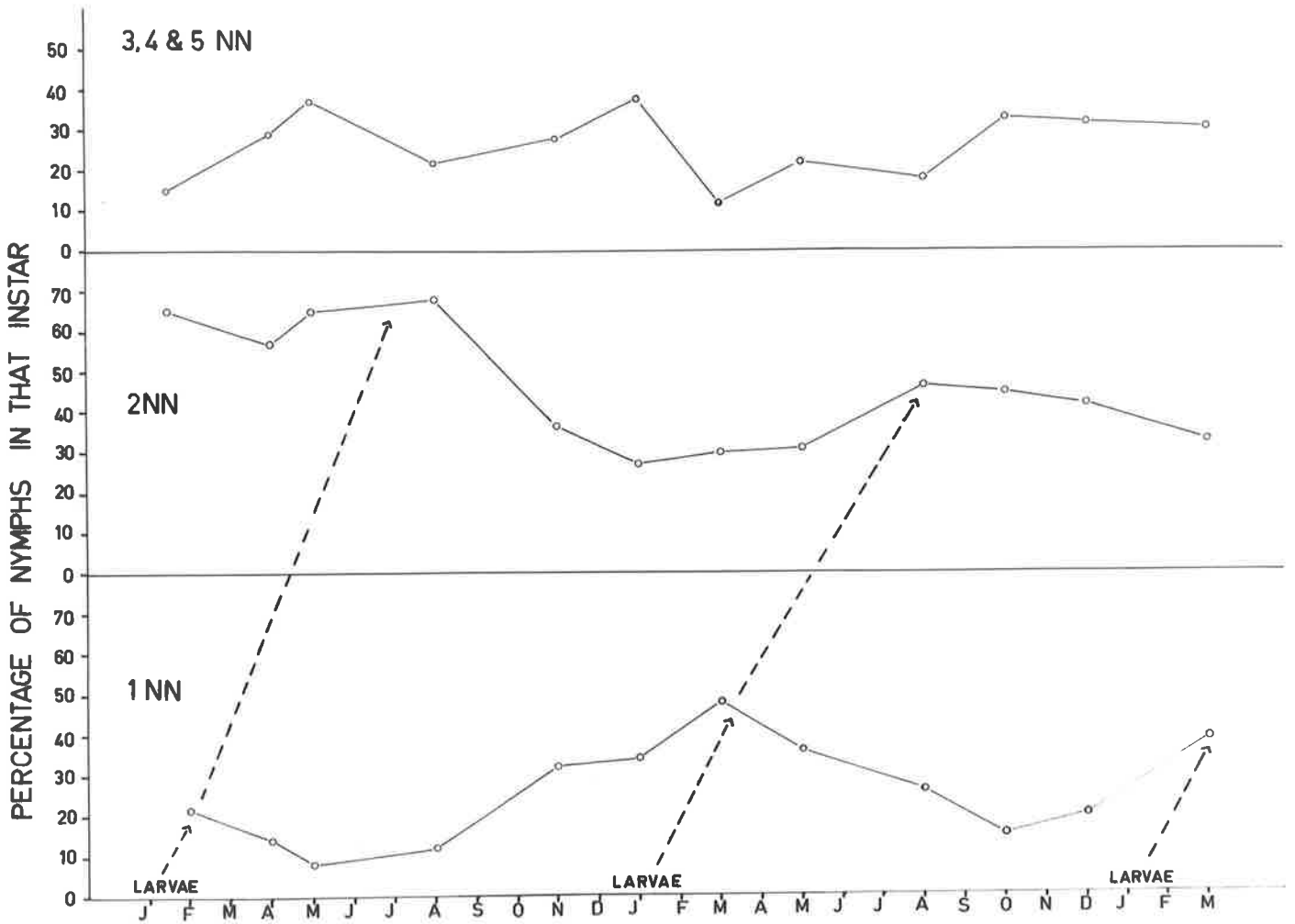
significantly altered the instar distribution pattern for the whole sample. Close examination of the data revealed that when the data from these four wallows was excluded, a seasonal pattern almost identical to that found during 1970-71 emerged. The 1970-71 data were based on samples from at least twelve wallows. Thus the curves described in Fig. 7.6 were derived from 111 out of the 115 samples from wallows and a total of 6,000 ticks.

The fluctuations in the relative proportions of nymphs during 26 months has been plotted in Fig. 7.6. From this it can be seen that the high incidence of larvae in November - January gave rise to a peak in the proportion of first instar nymphs somewhere between January and March and this flush of first instar nymphs probably gave rise to a slight rise in the proportion of second instar nymphs somewhere between March and April.

There appears to be no seasonal trend in the proportion of third, fourth and fifth instar nymphs in the population.

Because kangaroos appear to prefer large well-shaded trees and avoid small, sparsely shaded trees, the relative proportions of the different instars might vary according to the qualities of the shade tree (e.g. there may be differential nymphal mortality in wallows which are visited rarely). To examine the former possibility, the data from the 50 trees sampled in December 1970 were

Figure 7.6 The Fluctuations in the Relative Proportions of 1NN, 2NN and other stages over a 26 month period.


3,4 & 5 NN
2NN
1 NN
PERCENTAGE OF NYMPHS IN THAT INSTAR
LARVAE
LARVAE
LARVAE
J F M A M J J A S O N D J F M A M J J A S O N D J F M

examined. The mean instar distribution of the trees providing the densest shade was compared with that of those providing the sparsest shade. Similarly the instar distributions from groups of trees providing the largest and the smallest areas of shade were compared. The data in Table 7.6 show that there is no obvious change in the instar distribution with the quality of shade nor with the area shade.

In summary, there is a flush of larvae about November to January and some of these larvae appear as a flush of first instar nymphs between January and March. This has a slight influence on the proportion of second instar causing a relatively small flush in March to April. However there was always a proportion of all nymphal instars present in the population. Thus it appears that the tick population, on average, progresses through two or three instars per season. In other words, any tick, on average, has two or three opportunities to feed per season. This estimate agrees with that calculated from a knowledge of the behaviour of the kangaroo.

TABLE 7.6

The instar distribution in wallows

Sample Date - December 1970

50 wallows sampled
(Two CO_2 traps per wallow)

Type of Wallow	Percentage in each class						
	1NN	2NN	3,4,5NN	♂♂	♀♀	Mean No. per wallow	Sample size
Most dense shade (14 trees)	9.6	44.9	27.9	9.2	8.3	37.8	530
Most sparse shade (12 trees)	10.8	41.0	18.1	13.3	16.9	6.8	83
Largest shaded area - 10 trees (av. 76 sq.ft)	14.1	22.4	49.1	11.9	9.7	27.7	277
Intermediate shaded area 26 trees (av. 25 sq.ft)	11.3	44.4	25.2	9.0	10.1	24.4	635
Smallest shaded area - 12 trees (av. 6.3 sq.ft)	22.2	33.3	23.6	11.1	9.7	6.0	72
Total	14.3	38.6	25.9	12.6	8.5	19.9	984

CHAPTER 8
Discussion

'After some time I realized that heterogeneity and instability must not be considered as just a drawback of field data to be neglected ("averaged away" or "seen through by intuition") because they are mere deviations from the "typical" or "representative" case (or even "noise"). On the contrary heterogeneity and/or instability must be recognised as fundamental features of a natural situation. The chance of survival of a population may even be increased, because the variation within the population makes it possible to cope with the variation in space and time of the habitat.

This possibility led me to formulate the concept of "spreading of risk".'

- Den Boer (1968)

The overriding feature of the environment of the kangaroo tick Ornithodoros gurneyi, is its variability and unpredictability, and one important factor which contributes to this is the "wallowing" behaviour of the red kangaroo which changes with the season of the year. This has been dealt with in Chapter 7 but in order to appreciate the host-parasite interaction, one must also have an understanding of the patterns of movement and daily routine of the host.

"Red kangaroos are nomadic and their distribution, at any time, is determined by the availability of green food and perhaps, to

some extent, by shade also. The scale of movement of individuals is related to the severity of drought and the distance that must be travelled to find green food ... when these (green food and shade) are abundant only local movements (several miles) are necessary and larger ones are not undertaken. In times of stress, however, the animals have the ability and the stamina to move considerable distances in search of it (sic)." (Frith and Calaby, 1969; p. 85, p. 96).

When discussing the daily routine of the red kangaroo Frith and Calaby (1969) said "In hot periods they spend the day under shady trees and move out to feed and water at dusk, returning at dawn ... . During the day, the animals camp in dust baths dug under bushes or shrubs and what seem to be the same animals return to the same spot day after day while the mob remains in that general area. If shade, for resting, is available it is greatly favoured by kangaroos".

My observations are slightly at variance with those of Frith and Calaby (1969), in that although trees shedding sparse shade were avoided during summer there is no evidence in my field data suggesting that some wallows were used day after day to the exclusion of others, i.e. the amount of dung under each tree was rarely great and many wallows had a small amount of dung present. This suggests that, in the study area most trees were visited occasionally, but none were visited frequently (even over a short period of time).

On the other hand field data have shown that trees which provide large areas of dense shade are more likely to harbour large tick populations than are smaller trees with less dense shade (Chapter 7). If the relative density of ticks in wallows is an index of the relative frequency with which wallows are visited by kangaroos (rather than an index of the suitability of the wallow for tick survival), then it would appear that there are "preferred" trees that kangaroos will probably visit whenever the mob is in the area. In either case some wallows appear to be more "favourable" for the tick than others.

How frequently trees are visited will depend on the season of the year, the density of kangaroos and the density of suitable shade-trees. If there are few suitable trees then each is likely to be visited frequently and conversely if there are many trees each will be visited only rarely, provided that the density of kangaroos is the same in both cases. Thus the density of the kangaroo population which is necessary to permit a tick population to survive will vary with the density of suitable shade-trees and so the availability of food to the tick could be expressed in terms of the number of visits to a wallow (or a tree) made by kangaroos in a year, but not in terms of kangaroo density.

If this is correct then the distribution and abundance of the tick in Australia will be determined to a large extent, by the

relationship between the density of kangaroos and the density of suitable wallowing sites. Browning (1962) suggests such an explanation for the absence of the tick from heavily wooded country.

A corollary of this concept is that the distribution of the tick may have changed markedly during the two centuries since Australia was settled by Europeans. Before that time the density of trees in the 'back country' was greater than today, the shade was denser and the number of kangaroos fewer. The evidence for this lies in the journals of the early explorers and knowledge of the way in which the pastoral industry has in the past and continues today to ravage the desert vegetation (Frith and Calaby, 1969; pp. 151-154). Two hundred years ago the tick may have been confined to hills and rocky outcrops where it lived exclusively in caves frequented by euros and other cave-dwelling mammals. As the pastoral industry was "developed", the density of trees decreased, the amount of grass (the principal food of the red kangaroo) increased, the predation pressure (aborigines and dingoes) on kangaroos decreased, and O. gurneyi came out of the caves onto the plains.*

In the years leading up to 1959, red kangaroos became increasingly abundant throughout inland Australia, so much so that they were considered, by some people, to be in plague proportions in the 1950's. However during the period 1959-66 the numbers of kangaroos

* This thesis was first proposed by T.G. Browning.

in many areas were greatly decreased by shooting for meat and by drought (Frith and Calaby, 1969; p. 156). The effect of this reduction in kangaroo numbers on the tick population was evident in 1968 when I began the study. In the early 1960's the ticks were abundant and readily caught in many places in inland South Australia (Browning, 1962; Browning, pers. comm.) but in 1968 ticks were virtually absent from the same areas, even though occasional red kangaroos were seen in those areas. Although the tick population had crashed dramatically, there remained a few isolated pockets in which kangaroos and ticks were abundant. For example, on Moralana (due to the management's ban on kangaroo shooting) kangaroos were still abundant and so the tick was still present in relatively large numbers.

If, as time passes, continued grazing and drought steadily denude inland Australia of trees and if the red kangaroo is kept at its present low level by shooting for meat, we may see the distribution of the tick contract to their pristine home, caves in hills and rocky outcrops*. Alternatively, the recent introduction of beef cattle into areas (previously grazed by sheep) in which the tick is found, may provide an alternative host for the tick and so it may survive on the plains to become a pest of cattle, as has O. savignyi in the

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* The idea that O. gurneyi was originally a cave-dweller is supported by the fact that those species which are said to be the kangaroo tick's nearest relatives are also cave-dwellers.

Kalahari Desert of South Africa.

At present, however, one crucial aspect of the ecology of the tick is the frequency with which wallows are visited by kangaroos. In the study area on Moralana there were shade-trees at a density of about 400 per sq. mile (150 per sq. kilometre). There was an average of 2.4 wallows per shade-tree. The unfavourable wallows (small shade area, sparse shade) were visited on average 0.6 times per year whereas "preferred" wallows were visited, on average, two or three times per year (range 0.5 times) (Chapter 7). Dung was often found in all wallows under the same tree, and this suggests that if one wallow is visited at a particular time, then the others under that tree are also visited at that time.

It is clear that two or three visits to a wallow in a year is sufficient to permit the population to thrive (Chapter 7). Ticks were found under nearly every shade-tree and even unfavourable shade-trees have a significant population with the same instar distribution as the "preferred" wallows. This suggests that, at that time, the pattern of immigration and emigration of ticks was similar in both types of wallow.

When the frequency with which kangaroos visit wallows decreases, the distribution of the population should contract to the more favourable wallows. In this case the unfavourable wallows may act as a "store-house" for nymphs and adults but are unlikely to contribute

recruits (larvae) to the population. Thus the distribution of the ticks in the field and their instar distribution should give an idea of the viability of the population. On this point it is noteworthy that in the area where ticks (and kangaroos) were scarce, all the ticks collected were either late instar nymphs or adults and were found only under trees which provided dense shade.

The second important factor which contributes to the variability and unpredictability of the ticks' environment is the influence of climate on the survival of the different stages. The seasonal fluctuations and variability in the weather in the area where the tick was studied are examined briefly in Chapter 5 and it is clear that any time of the year may be hot and dry, but it is most likely that mid-summer will be the hottest and dryest time of year and that the mild, damp periods which occur about that time are rare and of short duration.

All developmental stages of the tick occupy the same habitat and so the relative vulnerability of the different developmental stages should identify the stage(s) in which most mortality is likely to occur. Eggs and larvae are many times more susceptible to desiccation than are nymphs and adults, and so the proportion of larvae which survive to the nymphal stage will largely determine the direction and rate of change of population size. There is a critical proportion of larvae which must survive if the population is to remain stable. If the proportion surviving is greater than the critical level the population will expand;

whereas if it is lower, the population will decline to extinction even though some ticks may be recruited into the population.

The critical proportion will vary with mortality in the nymphal instars and with fecundity. There are two obvious factors which might cause considerable mortality amongst engorged larvae and nymphs; firstly detaching in a place where the probability of finding another meal is very low, and secondly a combination of starvation and desiccation. The latter factor may operate if the wallow is not visited or if the tick fails to feed when a wallow is visited, but if kangaroos visit wallows frequently then neither desiccation nor starvation is likely to be a significant mortality factor for the nymphal instars. The number of ticks which is lost over the plains or killed when the kangaroo scratches itself can only be guessed at. However if one assumes that mortality in the nymphal stages is relatively low, and knowing that each female can produce four to six batches each of about 500 eggs, then it seems likely that only a small proportion (a few out of 2,000 - 3,000) of the larvae produced need to survive to ensure that the population remains stable.

Another point of note is that because the interval between visits is often quite protracted, ticks have almost invariably moulted and are ready to feed again long before the wallows are visited again. And so the effect of ambient temperature on the rate of development rarely, if ever, limits the rate of population growth.

Thus the tick has two major problems, the unpredictable and infrequent arrival of the host and the short survival time of the larvae.

Den Boer (1968) proposed a theory which attempted to explain how phenotypic and developmental variations of populations interact with variations of habitat in space and time in a way which leads to stabilization of animal numbers. He formulated the concept of "spreading of risk" in four ways: by phenotypic variation (SRPV), in space (SPS), in time (SRT)*, and with respect to other species (SROS). The first three ways of spreading the risk can be seen at work when one examines the ecology of the kangaroo tick.

The following is a summary of Den Boer's theory with respect to the first three processes. "Spreading of risk by phenotypic variation: most of the individuals constituting a natural population show some degree of divergence in their tolerance, preference, behaviour, etc., with respect to a number of environmental factors, so that the chance of surviving and reproducing must vary between individuals, even if the environment were spacially and temporally homogeneous - which never occurs in nature.

Since the chance of surviving and reproducing differs in different phenotypes, within a given population, the selective advantage in a fluctuating environment (e.g. unstable weather) will

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* These symbols will be used in the text to indicate when each process is at work.

shift continually from one phenotype to another.

For each generation in turn, the range of tolerance of a population is thus increased by phenotypic variation and this range is therefore much wider than that of the individual animals. This is important because future variations in the environment are hardly predictable. In other words the effect of fluctuating environmental factors on the population is continuously damped to some degree by phenotypic variations within the population.

Spreading the risk in time: A natural population includes not only a number of different phenotypes but also a number of different developmental stages or age classes generally showing differences in tolerance, preference, and behaviour and consequently differences in chances of survival. If there is a high variation in the rate of development or time of reproduction or both, individuals of the same developmental stage (age class) will be exposed to different environmental factors at different times of the year. Hence within the same stage the risk of dying and the chance of survival may be spread over a variety of environmental conditions and consequently the chance to reproduce may also be spread: spreading the risk in time. An important consequence of this is that the risk may be spread during periods with almost unpredictable conditions (e.g. summer in inland Australia) over a number of different stages with different tolerances. It will be evident that temporal spreading of risk like

the spreading of risk by phenotypic variation, will result in a relative reduction in the amplitude of fluctuations of animal numbers and will thereby contribute to a relative stability of the population size.

Spreading of risk in space: The effects of environments of natural populations are very heterogeneous. Their habitats generally consist of mosaics of which all the component parts differ in structure and, therefore, in micro-weather, food, natural enemies, etc. Therefore the chance of surviving and reproducing must be different in these different places. This means for populations as a whole that the effect of extreme conditions in one place will be dampened to some degree by less extreme conditions in others. In other words: the risk of wide fluctuations in animal numbers is spread unequally over a number of sub-populations living in different micro-environments."

The ecology of O. gurneyi can now be examined in relation to these three ways of spreading the risk. The probability that a sufficient number of larvae will survive to the nymphal stage to permit the population to survive is a function of the behaviour of the host, the oviposition behaviour of females and the longevity of larvae. If a kangaroo visits each wallow once a year, then the nymphs in the population will have an opportunity to reach maturity and the females in the population may oviposit, but the larvae produced, being short-lived, will be doomed. However, there is a possibility that a

diapausing female may engorge in one year and lay eggs in the spring of the next year after diapause development has been completed (SRT). In such a case, some larvae may feed, but the possibility of such a mechanism alone maintaining a population seems remote. Thus if wallows are visited once each year, the population will gradually decline to extinction. If, however, wallows are visited on the average once each year, then some wallows will be visited more than once. If the larvae are to survive then a second visit must occur after the eggs have hatched but before the larvae die.

The interval between a female feeding and the hatching of eggs varies with the season of the year (from 30-60 days in spring to 10-20 days in mid-summer (Chapter 4)). In spring the weather is usually moderate and so larvae may remain viable for several weeks; however during summer the weather can be so hot and dry that larvae perish before, or within a few days of hatching (Chapter 5). And so there is a very limited period, the timing and duration of which varies with the season, during which a second visit must occur if larvae which resulted from the first visit are to contribute to the population. Because one visit per year is insufficient to maintain a population in an area and because two or three visits maintain a thriving population (Chapter 7) the critical frequency of visits which permits a population to survive indefinitely appears to lie somewhere between one and three visits per wallow per year. These visits are most

likely to occur during summer but may occur during spring and autumn. It is noteworthy that when wallows are visited infrequently (in spring and autumn) larvae are relatively long-lived (because of the moderate weather) but during summer when wallows are visited most frequently larvae are very short-lived. However, late summer (January and February) are on the average much dryer than early summer (December). Thus the chance of a larva finding a host is probably similar in spring and early summer but is lower in late summer.

The "spreading of risk in time", so that a sufficient number of larvae feed in this unpredictable situation is envisaged as operating in the following way.

If only one female in a wallow lays a batch of eggs then the interval during which a second visit must occur (if any larvae are to survive) is only a few weeks. During summer wallows appear to be visited about once every two months and larvae may be viable for up to two weeks at that time. Thus it appears that a high proportion of the larvae produced by most females will die before having an opportunity to feed. However if more than one female in a wallow lays a batch of eggs, then because the pre-oviposition period varies greatly between individual females (Chapter 4; SRPV) larvae should be produced continually (but in relatively low numbers) for many weeks after the first visit. The duration of this period will vary with the number of females which feed and the season of the year because both the mean

pre-oviposition period (and its variance) and the amount of effective temperature vary with the season of the year (Chapter 4).

If wallows were visited frequently then a high proportion of the larvae produced would find a meal irrespective of the duration of the oviposition period for a group of females, but if wallows are visited infrequently (as is the case in nature) then a prolonged oviposition period increases the probability that at least some larvae will find a meal (SRT). Thus this mechanism increases the stability of the population at the expense of a reduced potential rate of increase, in a situation in which the availability of food is very unpredictable.

Diapause, in the field, has two characteristics which are relevant to the regulation of the life-cycle and the spreading of risk. Firstly it stops oviposition in most females in mid-summer. Secondly it causes the pre-oviposition period to decrease from about 30 days (with a large variance) to about 10 days (with a small variance) in early summer (Chapter 6; SRPV).

These two characteristics bring about synchrony of larval production because diapause stops ovigenesis in most females in mid-summer and because, as spring progresses, the mean pre-oviposition period of females in the field decreases and the amount of effective temperature increases. And so most of the larvae are produced during early summer when kangaroos are visiting wallows relatively frequently

and the physical environment of the larvae is still moderate when compared with mid- and late-summer (Chapter 5). Observations on the population dynamics of the tick (Chapter 7) support the view that more larvae feed during early summer than at other times of the year. Most of the females which feed during mid-summer and autumn do not lay eggs until the following spring when the weather conditions are moderate and larval survival prolonged (SRT).

However, there is another aspect to diapause spreading the risk. During early spring and during late summer and autumn, the mean pre-oviposition period is long and its variance high. In spring larvae can come from females which overwintered in diapause or from females which fed in early spring. Summer and autumn larvae come from a small proportion (10-15%) of females which do not enter diapause (SRPV). In spring and autumn kangaroos visit wallows infrequently and so a protracted and variable pre-oviposition period would ensure that some larvae were produced over a long period after the initial visit. Thus the probability that some larvae will find a meal is higher than if all eggs were produced at once. On the other hand, during early summer kangaroos visit wallows relatively frequently and so short pre-oviposition periods may not jeopardise the chance of a larva feeding. Thus there is a balance between the low probability that a high proportion will feed (if all larvae are produced at once) and the higher probability that a small proportion will feed (if larvae

are produced over a long period), and where this balance lies will vary with the season of the year (SRT, SRPV).

Other processes also increase the probability of some larvae feeding. The oviposition period for each female is relatively short (2-3 days) and the eggs are laid in one clump or egg-mass. The eggs on the outer edge of the egg-mass shield those at the centre from the desiccating effect of the atmosphere (Chapter 5). This behaviour ensures that at least some eggs and larvae survive longer than they would have done, if each egg had been laid separately (i.e. not in a clump) (SRS). Furthermore females are long-lived, can store viable sperm for many months and each has the capacity to lay a number of egg batches in her lifetime. This behaviour increases the probability that some of the progeny (larvae) of each female will find a host before dying (SRT). Another factor which may increase the probability of larvae feeding is that they cling to their mother and so presumably are carried passively onto a host.

When a kangaroo visits a wallow containing viable larvae a number are likely to attach. The data in Chapter 3 show that larvae which attach on one day will detach between 10 a.m. and 4 p.m. on five or six of the following days. Because kangaroos shift from wallow to wallow (under the same tree) and from tree to tree on the same day, and because they visit different trees on different days, the larvae which attach on one day might drop off, engorged, into any

of a large number of wallows during the week following attachment. This mechanism may increase the probability that some of the engorged larvae will lodge in a wallow which will be visited again by a kangaroo before the tick dies (SRPV, SRT, SRS). This is also true to the early nymphal instars (Chapter 3).

Furthermore the long period of attachment also serves as a dispersal mechanism (possibly the only one). The rate at which ticks disperse was not studied but it should prove a very interesting aspect of the ecology of the tick. Desiccated ticks could be rehydrated with water containing radioactive tracers and then released in the field. The way in which the distribution of the tagged ticks changed with time and the rate at which they progressed through the instars should give valuable insight into the ecology and biology of the tick.

Thus O. gurneyi is an opportunistic animal which is adapted to surviving in a harsh and largely unpredictable environment. This is achieved by spreading the risk of extinction in time and space over a range of phenotypes. In any one season or period only some of the mechanisms will have survival value, but over a long period of time each mechanism contributes to the maintenance and stability of the population. The chance of surviving and reproducing varies with time and place and so the "risk-spreading" mechanisms which ensure that the tick occurs in a wide range of wallows over a long period of time

increases the probability of some ticks surviving. At the same time a certain degree of synchrony in the emergence of larvae assures that many larvae are produced in early summer when the chance of finding a meal is relatively high.

In summary, the low probability of rain, of finding a host, or being lost, of trees dying, etc., all add up to give the tick just a high enough probability of surviving and reproducing itself.

APPENDIX I

I. The Photoperiodic Response of O. gurneyi

I.1 Summary

In the laboratory the tick was shown to enter diapause in response to short photoperiod. However in nature the tick passes most of its life buried beneath desert sand. The transparency of a sample of this sand was examined and even very thin layers (0.3 cm) proved to be opaque. Thus females living in wallows experience continuous darkness for most of their lives. (Feeding takes but a few hours and engorged ticks bury themselves as soon as practicable after detaching.) Field experiments have shown clearly that photoperiod did not induce diapause in nature. Thus the photoperiodic response was not pertinent to the regulation of diapause in the field. Nevertheless it is the first reported case of photoperiodism and diapause in an Argasid tick and so it was studied in the laboratory as another aspect of the biology of the tick.

I.2 Introduction

Section 6.2 has reviewed much of the literature on the regulation of diapause and it seems that there are very few, if any, cases in which photoperiod does not influence diapause. This response is also found in O. gurneyi. The following experiments were designed to examine the nature of the photoperiodic ('long-day') response

and to examine the possibility that photoperiod could help regulate the seasonal cycle of diapause.

Initially the transparency of several media was tested and then the sensitivity of engorged and unengorged females to photoperiod was examined. Finally the interaction between photoperiod and temperature was studied.

1.3 The Absorption of Light by Sand and Sugar

In the field, ticks were sieved or lured from the sand and litter in which they lay. No ticks were ever seen lying on the surface of the soil when a wallow was first inspected. It was also noted that ticks, whether engorged or unengorged, would burrow into soft soil if given the opportunity (in the absence of a host). In nature, therefore, the ticks spend most of their lives buried in sand in the wallows, and so the amount of light which they perceive will depend upon where they lie in the wallow and the transparency of the material in which they are buried. Thus the absorption of light by sand was examined.

Sand was likely to be opaque and I wished to test the sensitivity of the ticks to photoperiod. Therefore I needed a transparent medium in which to store the ticks. The absorptive properties of two other substances, glass beads and granulated sugar, were also examined.

The minimum sensitivity of the photometer used to measure light intensity was 0.5 lux and so was adequate to test light penetrations of an intensity of 10 lux.(Eel photoelectric photometer). The system used to examine light penetration was a black-walled tube with a glass bottom. This was placed over the light sensitive disc (5 cm in diameter) of the photometer and sealed with black felt in such a way that the only light reaching the sensitive disc passed through the bottom of the tube. Each material was placed in turn in the tube and the intensity of the light transmitted through the material was measured under four intensities of incident light, each estimate being the average of three readings.

Table I.1 shows that the depth to which light penetrates depends upon the intensity of the incident light and on the nature of the material. For example, even with incident light of 100,000 lux, no light (i.e. less than 0.5 lux) was registered as penetrating through the first 0.3 cm of red sand. Thus any tick covered by 0.3 cm or more of red sand should be effectively in continuous darkness. On the other hand, those covered by 0.5 cm of sugar should experience light intensities proportional to the intensity of the incident light. It is noteworthy that minute glass beads proved opaque to light.

TABLE I.1

The absorption of light by different depths of sand, sugar and glass beads

		The intensity of transmitted light (lux)			
Intensity of incident light (lux)		100,000	1,530	157	14.5
Depth of material (cm)					
SUGAR	0.5	7,000	187	22	3.8
	2.0	1,000	30.2	3.2	0.5
	4.5	500	2.2	0.5	0.0
SAND	0.3	0.0	0.0	0.0	0.0
GLASS	0.5	0.0	0.0	0.0	0.0

I.4 The Absorption of Light and its Effect on Photoperiodic Sensitivity

Photoperiodic reactions are usually 'all or nothing' reactions. Frequently there is a narrow range of light intensities over which the reaction of the sensitive animal changes from no response to a maximum response. Light intensities below this 'threshold' are not recognised and the animal reacts as though in total darkness, whereas all intensities above that threshold fully activate the photosensitive process (Lees, 1968). In Arthropods this threshold varies with the species and even within one species it depends upon a number of factors, e.g. temperature. It is generally true, however, that this threshold has been found to lie between 1 and 10 lux for most species examined, e.g. 10 lux for T. ulmni (Lees, 1968), 5 lux for I. ricinus (Loew, 1962). Thus if for the present we assume the threshold of O. gurneyi to be about 10 lux, then we can examine the experimental conditions and determine the depths of sand and sugar at which the intensity of transmitted light falls below 10 lux.

The intensity of light in the constant temperature cabinets where the experiments were carried out, was between 100 and 150 lux depending on the position in the cabinet. (The Eel photometer was used to measure this intensity.) In these conditions ticks underneath

0.5 cm of granulated sugar should experience between 20 and 25 lux; those under 1.0 cm should experience 10 to 15 lux; those under 2.0 cm should experience 2 to 4 lux and those under 4.5 cm, 0.5 lux (Table I.1). Thus those under 0.5 and 2.0 cm of sugar should experience light at greater than the theoretical threshold intensity and so should react to the incident photoperiod, whereas the behaviour of those under 6 cm is unpredictable.

It was known from previous observations that replete females, if given an opportunity, burrow into loose sand and almost immediately after detaching from the host. Within 1 to 2 hours of detaching, these females come to rest between 3 and 8 cm below the surface of the sand (at 30°C and 40% R.H.), where they remain until they oviposit or are disturbed. Thus if short photoperiod is to have its effect it must act on the buried tick.

In order to estimate the effect of short photoperiod on ticks which are buried in sand, a system was devised which would allow ticks to burrow to different depths and thus be shielded from direct light by different thicknesses of sand. Plastic vials 2" deep and $1\frac{1}{2}$" in diameter were painted inside and out with semi-gloss black paint. Groups of these containers were filled with red sand to depths of $\frac{1}{2}$, 2 and 6 cm. Groups of five recently engorged, non-diapausing females were introduced into each vial. A depth of $\frac{1}{2}$ cm allowed a tick to bury its hypostome but not its body; 2 and 6 cm

allowed the ticks to bury themselves completely. When examining the ticks subsequently they were found lying near the bottom of the containers, thus confirming the original prediction. Thus the dorsal surfaces of those in $\frac{1}{2}$ cm of sand were exposed all the time whereas those in 2 cms of sand were shielded from direct light by about 1 cm of sand; those in 6 cm were shielded by about 5 cm of sand.

However, if only those ticks in shallow sand were to respond to short photoperiod, there would remain the possibility that the lack of response in the other groups was due to the effect of the depth of sand rather than to the absence of light. To examine this possibility a parallel experiment was set up using engorged females from the same group, but using light-transparent granulated sugar rather than opaque red sand as the medium.

The tubes were then placed in long and short photoperiod at 30°C where the ticks were examined periodically and the oviposition behaviour was noted. The pre-oviposition period in sand (6.9 days, S.D. 2.4) was not significantly different from that in sugar (6.3 days, S.D. 1.7) (t_1 = 0.28, NS). Thus sugar and sand appear to be equally favourable as a medium in which to oviposit. On the other hand, glass beads which were also used, proved very unsatisfactory because the ticks failed to burrow into them. Furthermore when the ticks

were buried beneath several cm of these beads, many of the females did not oviposit, in marked contrast to the control ticks which were buried in sand. However females did oviposit if merely resting on top of a column of the beads. Due to lack of time this peculiar phenomenon was not analysed any further.

The proportion of females ovipositing in long photoperiod was compared with the proportion which oviposited in short photoperiod using a X^2 test on a 2x2 contingency table in which the data for sugar and sand was pooled.(Table I.2). The difference was significant at the 5% level ($X^2_1 = 3.86$).

TABLE I.2

The Proportion of Ticks which Oviposit in Long and Short Photoperiod

	Long photoperiod	Short photoperiod	Total
No. ovipositing	81	77	158
No. in diapause	8	18	26
	89	95	184

The apparent trend, in Table I.3, of decreasing incidence of diapause with increasing depth of medium was not significant when the data for sand and sugar (long photoperiod) was analysed using a 2x3 contingency table ($X^2_2 = 4.33$, N.S.).

The detailed results are set out in Table I.3 but, possibly due to the low sample size in each treatment, there are no statistically significant trends. However, the mean pre-oviposition period was only about seven days. It is possible that the response may increase with increasing duration of exposure to short photoperiod. To examine this possibility the ticks were fed again after five weeks exposure to the experimental conditions and the incidence of diapause was then assessed at 30°C long photoperiod in deep sand.

Again the proportion of females which oviposited in long and short photoperiod was compared using a 2x2 contingency table containing the pooled data. The difference proved significant at the 1% level ($X^2 = 5.86$). The detailed results are set out in Table I.4). Three aspects of this experiment are relevant to the biology of diapause.

i) When the females were buried in sand there was no significant difference with respect to the incidence of diapause between those females kept in long and short photoperiod. But ticks in shallow sand perceived the photoperiod and entered diapause. Thus deep sand shielded the ticks from the incident photoperiod and so ticks which live buried in sand live in continuous darkness.

ii) When females were kept in sugar, a significantly higher proportion of females entered diapause after being exposed to short photoperiod (58%) than to long photoperiod (10%) ($X^2 = 9.1$,

TABLE I.3

The percentage of females in diapause after exposure to to long and short photoperiod while buried in sand or sugar

Depth of medium	Medium photoperiod	Percentage in diapause (sample size in brackets) Sand 16L 8D	Sand 8L 16D	Sugar 16L 8D	Sugar 8L 16D
0.5 cm		8% (13)	27% (11)	29% (14)	25% (24)
2.0 cm		7% (14)	7% (14)	11% (18)	16% (19)
6.0 cm		0% (15)	13% (15)	0% (15)	25% (12)

TABLE I.4

Percentage in diapause after five weeks' exposure to the conditions in Table 1.2 (Sample size in brackets)

Photoperiod	16L 8D	8L 16D
Shallow sand ($\frac{1}{2}$ cm)	8% (42)	25% (8)
Deep sand	3% (26)	3% (32)
Sugar ($\frac{1}{2}$ and 2 cm)	10% (20)	54% (13)
Sugar (6 cm)	10% (10)	63% (8)

0.01>P>0.001). Thus short photoperiod induces diapause at a rate of about 10% per week. Furthermore the females in 6 cm of sugar were found at the bottom of the column of sugar but they still entered diapause. It was therefore considered that the females were sensing the photoperiod while living at the bottom of the column of sugar. Since the light intensity at the bottom of such a column is probably less than 1 lux, it is probable that the critical light intensity of the ticks was less than 1 lux.

iii) When the incidence of diapause in females exposed to short photoperiod and buried in sugar is compared with that of those living in sand under short photoperiod, the difference is highly significant (X^2 = 18.3, 0.001>P).

Thus it is clear that engorged females do respond to short photoperiod by entering diapause, but this reaction does not occur when the ticks are shielded from the incident photoperiod by a layer of opaque sand. This observation prompted two questions which are studied in the following two experiments. They are:

i) Will unengorged females respond to short photoperiod?

ii) Will ticks in very shallow sand respond to short photoperiod?

I.5 The Sensitivity of Unengorged Females to Short Photoperiod

In order to test the sensitivity of unengorged females to short photoperiod, ticks were covered with sugar and exposed to short photoperiod for different periods of time. The incidence of diapause

was then assessed at 30°C in deep sand. Two groups, one of newly-moulted females, the other of females which had oviposited at least once, were placed in transparent tubes 2 cm in diameter and were covered with 2 cm of sugar. Thus no tick could be more than 1 cm from an illuminated surface. The light intensity in the cabinets in which the ticks were stored was about 150 lux and so each tick would have experienced a light intensity of about 20 lux (Table I.1).

The unfed females were exposed to the short photoperiod for 0, 40 and 70 days, and after feeding the incidence of diapause was assessed at 30°C in deep sand. A control group was kept in long photoperiod for 70 days.

It is clear from Table I.5 that short photoperiod induces diapause in both types of unengorged females. However the rate of induction is relatively slow, for about only half of the females had entered diapause after five weeks' exposure. This rate (about 10% per week) is similar to that in the previous experiment.

I.6 The Sensitivity of Engorged Females in Shallow Sand to Short Photoperiod at Different Temperatures

The reaction of Arthropods to photoperiod is frequently modified by the temperature at which the reaction takes place. When the inductive effect of short photoperiod (the so-called long day reaction) is modified by temperature, it is often found that the inductive effect is more pronounced at low than at high temperatures;

TABLE I.5

Photoperiodic induction of diapause in unengorged females

Percentage in diapause (sample size in brackets)

Duration of exposure (days)	Newly-moulted females		Females which had already oviposited	
	8L 16D	Control (16L 8D)	8L 16D	Control (16L 8D)
0	25% (32)		5% (77)	
40	71% (21)		57% (51)	
70	97% (30)	23% (30)	91% (44)	10% (35)

i.e. low temperature and short photoperiod act together to induce diapause. Thus it was decided to examine the photoperiodic reaction of the ticks at a range of temperatures.

In this experiment engorged females were placed in glass tubes containing 2 to 4 mm of sand. This depth of sand permitted the ticks to bury only their legs and hypostome so that the dorsal surface of each tick was exposed to the photoperiod.

Newly-moulted females reared at 30°C in long photoperiod, were fed and placed in long and short photoperiod at 25°, 30° and 35°C. The proportion which failed to oviposit was noted. There was a possibility that the sensitivity of newly-moulted females might differ from that of those which had oviposited, and so a similar experiment was carried out using females which had oviposited at least once before the experiment began.

The data for long and short photoperiod for each temperature were compared by a X^2 test using a 2x2 contingency table. The results are set out in Table I.6.

Several conclusions can be drawn from this experiment. First, it is very clear that short photoperiod induced diapause in females at 25° and 30°C but that it had no significant effect at 35°C. However, very few ticks oviposited at 35°C, irrespective of the photoperiod. This failure to oviposit was probably due to the peculiar influence which high temperature had on oogenesis. High

TABLE I.6

Influence of photoperiod and temperature on the incidence of imaginal diapause

Percentage in diapause (sample size in brackets)

Newly-moulted Females

Temperature	Photoperiod 16L 8D	Photoperiod 8L 16D	X^2	Significance
35°C	93% (43)	91% (23)	0.4	N.S.
30°C	33% (380)	68% (150)	44.4	P***
25°C	43% (60)	83% (60)	20.7	P***

Females which had already oviposited

30°C	15% (39)	60% (40)	16.7	P***
25°C	43% (53)	83% (12)	6.2	P**

temperature was found to inhibit oogenesis (Section 6.65) and so although short photoperiod may have had an effect at 35°C, if present, it would have been confounded with reproductive inhibition. However since the combination of 35°C and short photoperiod appears to have little, if any, relevance to field conditions, this aspect of diapause was not pursued directly.

Another aspect of this experiment was the reaction to temperature. When the incidence of diapause at 25°C is compared with that at 30°C it is evident that a higher proportion of females enter diapause at 25°C than at 30°C, and that this trend occurs at both long ($X^2_1 = 5.82$, P*) and short photoperiod ($X^2_1 = 7.28$, P**) (the X^2 value was derived from an analysis of data using 2x2 contingency tables). It is remotely possible that both long and short photoperiod could induce diapause. If so, the increased incidence of diapause at 25°C relative to 30°C could be due to the increased period of exposure to the photoperiod before ovipositing (mean pre-oviposition periods at 25°C and 30°C are 15 and 25 days respectively). However this explanation is unlikely because females buried in deep sand (and so in continuous darkness) show the same pattern - a higher incidence of diapause at 25°C than at 30°C.

In conclusion it is clearly possible for short photoperiod to induce diapause in females which are only partially buried in sand. Furthermore, at least at 25°C and 30°C, the general rule that low

temperature and short photoperiod act in concert to induce diapause, holds true.

I.7 Photoperiodic Sensitivity of Females from the Field

If photoperiod is to help regulate diapause in the field, then females from the field must be sensitive. To test this, females from the field (sampled at different times of the year) were exposed to short photoperiod, and the incidence of diapause was assessed. Table I.7 shows clearly that females from the field are sensitive to short photoperiod.

TABLE I.7

Percentage Diapause in Samples of the Field Population Taken at Different Times during the Year and Subjected to Long and Short Photoperiod at 30°C (Sample Size in Brackets)

Sampling Time	Incident Photoperiod	
	L16 D8	L8 D16
March 1970	85% (36)	100% (16)
May 1970	85% (13)	100% (11)
August 1970	31% (13)	64% (11)
October 1970	7% (14)	53% (15)

I.8 The Relevance of the Photoperiodic Reaction to the Ecology of O. gurneyi

It is clear from the previous experiments that both engorged and unengorged females are sensitive to photoperiod and that short photoperiod induces diapause at the slow rate of about 10% per week. However, because Central Australia is a desert of sand rather than a land of sugar, and because it is the habit of the tick to burrow into such sand wherever possible, it is very unlikely that a tick in the field would sense the photoperiod. The two situations in nature in which the ticks might sense the photoperiod are; if there were an insufficient depth of sand for the tick to bury itself completely (a rare occurrence); and if there were some behavioural mechanism by which the ticks came to the surface for some time. However, some ticks are found in the litter in wallows (Appendix II) and so there is a possibility that only a proportion of the incident light will be absorbed before reaching the ticks in litter. Hence some ticks may perceive light during part of the day only; that part during which the intensity of light reaching the tick is greater than the critical light intensity* of the tick.

The intensity of light perceived will be a function of the intensity of incident light. That intensity will vary with the time

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* That intensity above which the animal responds fully and below which light is not recognised.

of the year, the time of day, the cloud cover during the day, the intensity of shading and the time for which the ground is shaded each day. Thus, depending upon the intensity of incident light and the depth and transparency of the material in which the ticks are buried, there is a possibility that during mid-summer the ticks may perceive a short photoperiod. However, in view of the wide variation in the situations which females in the field are likely to encounter, this explanation seems rather unlikely.

Thus we may conclude that there is but a remote possibility that the short-photoperiod reaction of the females of O. gurneyi plays a significant rôle in regulating the annual diapause cycle in nature.

APPENDIX II

II. The Reaction of Ticks to Various Stimuli

There are many behavioural responses of the tick which probably play a vital, though perhaps obscure rôle in its ecology and biology. These responses can be considered as a series of instinctive reactions of the tick to one or a number of "trigger" stimuli. The burrowing behaviour and the reaction to CO_2 are two such responses.

II.1 The Burrowing Behaviour of the Tick

The depth to which ticks burrow is likely to have a profound influence on the biology of the tick in the field because the temperature experienced will vary according to where the tick lies buried in the sand or litter. Furthermore after rain ticks may be trapped in the sand*.

The conditions experienced during imprisonment and whether or not the ticks are released will depend in part, on where the tick is buried. Therefore, the rate at which ticks burrow and the depth to which they burrow was examined.

Tubes made of graph paper 2.5 or 4.0 cm in diameter, were filled with fine sand, making long, vertical columns. Groups of 50

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* The sand in wallows contains a proportion of clay. After rain the sand and clay set solid and may imprison the ticks buried therein.

engorged larvae, first-instar nymphs and females were placed on top of different cylinders of sand into which they burrowed; most had disappeared within the first ten minutes.

Two, 24 and 72 hours after the ticks were released, their vertical distribution in each column of sand was assessed by cutting the paper one cm below the surface of the sand, scraping the sand thus released into a sieve and counting the number of ticks in that portion of the column of sand. This process was repeated until all the ticks had been recovered. All observations were made at 30°C. The depth to which each group had burrowed was expressed as a mean depth for that group (Table II.1).

From these results it is clear that at 30°C ticks finish burrowing within a short period (less than 2 hrs) of being placed on the sand.

A similar set of observations was made on groups of 50 engorged larvae, first-instar nymphs, fourth-instar nymphs, males and females. Their position in the column was assessed after 3 days at 30°C (Table II.2).

It is clear from Table II.2 that the large ticks burrow to a greater depth than the smaller ones but most are to be found in the first 6 cm of sand. Since the sand in the field is rarely deeper than 2 cm, it seems likely that, in the field, the large ticks and possibly some of the smaller ticks, will burrow to the bottom on the available

TABLE II.1

Mean depth to which ticks burrow
(S.D. in brackets)

Instar/time interval	2 hrs	24 hrs	72 hrs
Larvae	< 10 mm	< 10 mm	< 10 mm
1NN	9.6 mm (4.3)	10.9 mm (4.7)	14.9 mm (7.0)
Females	34.0 mm (10.2)	44.2 mm (12.6)	35.3 mm (15.3)

TABLE II.2

Burrowing behaviour of engorged ticks

Instar	Mean depth	S.D.
Larvae	6.4 mm	2.6
1NN	10.9 mm	4.7
4NN	54.0 mm	16.0
5NN	46.3 mm	9.4
♂♂	17.8 mm	10.5
♀♀	35.5 mm	16.9

sand.

II.2 Why do Ticks Stop Burrowing?

It was thought that ticks might spend a certain amount of energy, characteristic of each instar, in burrowing to a particular depth. The amount of energy used could possibly indicate the depth burrowed. This form of distance measurement has been demonstrated in bees (Lindauer, 1967). To test this, ticks were allowed to burrow to their characteristic depth and were then sieved out and replaced on top of the sand. Although this was done for the same group of ticks up to four times, the ticks still returned to a depth characteristic of their instar. Thus, unless the ticks recognised the surface as the beginning each time and readjusted their 'depth-measuring device', these observations discount energy expenditure as a mechanism for the determination of how far to burrow.

The possibility that pressure was the parameter used by the ticks to sense depth was tested by placing groups of engorged larvae and first-instar nymphs in tubes containing materials of different densities. After three days the vertical distribution of the ticks in the different columns was assessed and the mean pressure experienced by each group was calculated by multiplying the mean depth by the density of the material (Table II.3).

From Table II.3 it is clear that the mean pressure experienced

TABLE II.3

Burrowing behaviour of engorged ticks

Material	Density (gm/cc)	Mean depth (mm)		Pressure at mean depth (gm/cm^2)	
		Larvae	1NN	Larvae	1NN
Sand	1.4	0.64	1.49	0.89	2.1
Pearlite	0.13	6.5	16.0	0.85	2.1
Vermiculite	0.10	8.2	20.0	0.82	2.0

by each group was identical. It appears that there is an innate drive in engorged ticks to crawl and burrow and that this drive ceases only when a certain pressure is exerted on the tick.

The burrowing behaviour of unengorged ticks has also been studied briefly. A mixed group of engorged and unengorged first-instar nymphs was placed on the top of a column of sand. They soon burrowed into the sand and could not be seen. Five days later I breathed over the column and within two minutes unengorged nymphs began emerging from the sand. Within 30 minutes, during which the column was breathed on intermittently, I remove 400 unengorged nymphs from the surface. No more could be induced to surface within the next half hour and so I examined the vertical distribution of the ticks which remained. This revealed that I had attracted 90% of the unengorged and none of the engorged ticks to the surface; but those that had remained in the sand had a vertical distribution in the column of sand (mean depth 1.04 cm; S.D. = 0.8) very similar to the engorged ticks (mean depth 0.95 cm; S.D. = 0.5).

II.3 The Reaction of Ticks to CO_2

Many blood-sucking Arthropods have been shown to react to CO_2 (Southwood, 1966; p. 214), and it is thought to be one of the principal long distance stimuli enabling blood-sucking parasites to locate their hosts. Various species of tick have been shown to use CO_2 as an indicator of the presence of a host (Garcia, 1962, 1969;

Neville, 1964).

II.31 Trapping Ticks in the Field

CO_2 has been used to trap the tampan, O. savignyi in the desert areas of South Africa (Neville, 1964). Although Neville collected many thousands of ticks in this way, he found it impossible to trap all the ticks in an area using this method. Even if the area were sealed off so that there was no immigration, ticks could still be trapped in the area after it had been sampled with CO_2 many times.

To test the reaction of O. gurneyi to CO_2, an experiment was carried out in the field. In a wallow in the study area, two petri dishes, 14 cm in diameter, were sunk into the soil so that the edge of the top of each was level with the surface of the soil. A piece of dry ice (solid CO_2) was then placed in one dish and nothing in the other, and one hour later the number of ticks in each dish was noted. The dry ice was then removed to the second dish for one hour, and the number of ticks trapped was again noted. The process was then repeated using dry ice in the first dish. In other wallows, petri dishes without CO_2 failed to trap ticks even though the wallows were shown, subsequently, to contain ticks.

Table II.4 shows clearly that the ticks were lured to the dish containing CO_2 ($X_1^2 = 19.0$). This method of trapping ticks was used frequently in my field sampling and the petri dish baited with dry ice is referred to as a "standard tick trap".

TABLE II.4

The reaction of ticks to CO_2

	The number of ticks trapped	
	Dish 1	Dish 2
1st hour	33 (+ CO_2)	4 (w/o CO_2)
2nd hour	3 (w/o CO_2)	12 (+ CO_2)
3rd hour	6 (+ CO_2)	0 (w/o CO_2)

II.32 The Proportion Responding to CO_2

Neville (pers. comm.) found that even with an apparently homogeneous group of O. savignyi, there was a wide range of responses to CO_2 within the group, varying from those which responded immediately, to those which had not responded after two years, even though the sand in which they lived was sampled with CO_2 regularly during that period. A similar phenomenon may occur with O. gurneyi, and so I designed an experiment to test the response to CO_2 of the tick in different physiological conditions.

The apparatus used to test the sensitivity of the ticks to CO_2 was a 500 ml beaker containing 45 gm of sand which covered the bottom to a depth of 1 cm. The beakers were placed on a bench in a room kept at constant 30°C. Twenty-five ticks were introduced into each beaker and the top of each was sealed with self-sealing plastic film. The system was then left undisturbed for one day, after which the reaction of the ticks to CO_2 was tested by injecting CO_2 into the beaker with a syringe through the plastic top. Control beakers were injected with air. The proportion of the ticks which responded to the CO_2 by coming to the surface and the time taken to respond were noted.

Four types of ticks were tested. These were:-

i) Third-instar nymphs which had moulted at 30°C about three weeks before being tested, and so were unengorged,

ii) Engorged third-instar nymphs in which moulting had been inhibited by exposure to high temperature,

iii) Unengorged females which were not in diapause, and

iv) Engorged, diapausing females.

There were two replicates in each treatment, and a control for every replicate. One day after introducing the ticks, the beakers were injected with 2 ml of air or CO_2 and the proportion which responded was noted. Ticks took a maximum of 15 minutes to react to CO_2. Half an hour after the injection (when all the ticks had disappeared under the sand) the beakers were again injected but this time with 4 ml of air or CO_2. Any further response was noted. One hour later I breathed over the surface of the sand in all the beakers and recorded the response. The following day the ticks had all buried themselves under the sand. The above procedure was repeated.

The results in Table II.5 show that only a small proportion of the ticks tested responded to CO_2. There is a clear difference between diapausing and non-diapausing females and it is clear that engorged, diapausing females are quite unresponsive to CO_2 and to human breath. It also appears that those nymphs in which moulting had been inhibited (but which were nevertheless able to feed (Section 4.4)) are less reactive to CO_2 and breath than are newly-moulted nymphs. The initial lack of response in the newly-moulted nymphs was quite unexpected and is even more perplexing in the light of a further observation. After the experiment the newly-moulted nymphs were given an opportunity to engorge; more engorged and moulted

TABLE II.5

The response of ticks to CO_2

Third Instar Nymphs - Percentage Response

	Newly moulted		Moult inhibited	
Day 1 Ticks placed in beaker				
	CO_2	Air	CO_2	Air
Day 2				
2 cc	0	2	4	0
4 cc	0	2	4	0
Breath	26	30	14	10
Day 3				
2 cc	2	2	2	0
4 cc	18	2	0	0

Females - Percentage response

	Non diapause		Diapause	
	CO_2	Air	CO_2	Air
Day 2				
2 cc	14	4	2	0
4 cc	18	2	0	0
Breath	88	40	0	0
Day 3				
2 cc	22	6	0	4
4 cc	26	6	0	0

successfully. Thus, although the ticks were 'hungry' they did not respond to CO_2. Similarly the unresponsive, unmoulted nymphs would engorged if given an opportunity.

Another point of interest is that the response to human breath was far stronger than that to CO_2 alone. Thus there must be factors other than CO_2 which also stimulate the tick.

Another stimulus to which the ticks might respond is vibration. However, in this experiment the ticks were not induced to come to the surface in response to tapping the beaker with a pencil, nor did they respond when the bench upon which the beakers stood was pounded with my fist. Thus the ticks did not surface in response to vibrations.

In summary it appears that only a small proportion of ticks come to the surface in response to CO_2. Those that do surface take between five and fifteen minutes to do so. Human breath produces a far stronger reaction than CO_2. The response of ticks is significantly influenced by the physiological condition of the tick, e.g. the engorged females in diapause were quite unresponsive although it is known that they were capable of engorging again if given the opportunity.

It seems unlikely that CO_2, in nature, would be the only stimulus by which the ticks recognise the presence of a potential host; other factors such as body odour or body warmth may also be important factors. Vibration appears unlikely to be significant.

II.4 Temperature Thresholds for Activity

In nature, the lower critical temperature for activity of the tick will determine whether or not a tick can respond to the presence of a host.

In order to examine this response I built an apparatus designed to estimate the lowest temperature at which ticks react to CO_2. The apparatus was a cylinder consisting of the top and bottom of a petri dish (14 cm diameter) separated by a ring of cardboard. A thermometer and a plastic tube were inserted through the wall of the cylinder. Ticks were placed inside and their activity was tested by blowing into the container through the plastic tube for five seconds. The proportion which walked was noted and taken as an estimate of the activity of the ticks at the temperature measured by the thermometer inside the container.

Twenty active larvae, first- and second-instar nymphs, and adult females were introduced into the container and tested, each stage separately. The ticks were introduced into the apparatus at 30°C, and then it was transferred to a room at 10°C. As the apparatus and the ticks inside it cooled, the proportion of ticks which responded was noted at temperature intervals of 1°C.

When no ticks responded to stimulation, the apparatus was transferred to a room at 30°C and the temperature at which ticks became active was noted. It is clear from Table II.6 that neither hysteresis

nor accommodation was observed.

Ticks stopped responding over a range temperature. The mean critical temperature for each instar was estimated by pooling the data from the cooling and the heating runs and calculating the mean and S.D. of the pooled data. It is clear from the data in Table II.6, that the critical temperature for activity for nearly all ticks lies within the range 10 to 15°C (50 to 60°F). However, it must also be noted that the ticks are still quite sluggish until the temperature reaches about 20°C.

TABLE II.6

The activity of ticks at different temperatures

Temperature (°C)	Percent Active							
	Larvae		1NN		♂♂		♀♀	
	Cooling	Heating	Cooling	Heating	Cooling	Heating	Cooling	Heating
30			100	100	100	100	100	
22							100	
21							95	
20			100	100	100	100	95	100
19			100	100	100	100	95	100
18			100	100	100	100	90	100
17			100	100	95	95	100	100
16			100	100	90	90	100	95
15	100	100	100	100	70	90	85	80
14	100	100	95	100	55	80	80	50
13	60	100			40	45	75	45
12	20	85		100	20	75	55	35
11	10	70	0	80	0	15	30	20
10	5	50	0	0	0	0	10	10
9	0	5					0	0
8		0					0	0
Mean Temperature (°C)	12.1	9.9	12.1	10.2	13.4	13.4	11.7	12.7
S.D.	1.1	1.2	0.4	0.4	1.7	1.9	2.2	2.2
Mean Temperature from pooled data (°C)	11.0		11.2		13.4		12.2	
S.D.	1.6		1.0		1.8		2.1	

APPENDIX III

III. Two Varieties of O. gurneyi

The kangaroo tick O. gurneyi has been found in caves in hilly areas frequented by euros as well as under shade-trees on open plains frequented by the red kangaroo. The bulk of this thesis concerns ticks of the plains variety but the cave variety has also been examined (Chapters 3 and 5).

Ticks of the cave variety appeared to be smaller than those of the plains variety and so the difference was examined in more detail. The length of the first coxa on the tick's right side was measured in several groups of females. It is clear from Table III.1 that the two varieties are significantly different.

Ticks have been caught in caves and on plains within a few miles of each other in the Flinders Ranges in South Australia, and at Fowlers Gap in New South Wales, but in both cases, although cave variety ticks were smaller than the plains variety, the numbers caught were so low that the difference in size between the two varieties was still in doubt.

Although it has been possible to culture the plains variety successfully in the laboratory, the cave variety under apparently identical conditions was a noteworthy failure because most females could not be induced to oviposit and those which oviposited, were unpredictable.

TABLE III.1

The mean length of the first coxa on the right side of the female

Origin of Ticks	Mean Length (micrometer length)	S.D.	Sample Size
Cave Variety			
Reynold's Range (120 miles N. of Alice Springs, N.T.)	69.0	6.0	11
McDonald Ranges (Alice Springs, N.T.)	62.0	6.0	9
Plains Variety			
Moralana, S.A. (near Homestead)	77.9	4.6	16
Moralana, S.A. (20 miles N. of Homestead)	75.9	5.6	19
Broken Hill, N.S.W.	74.0	5.5	13

There is a possibility that the difference in the size of the two varieties is due to nutritional factors (different host species) and so the length and breadth of larvae and first-instar nymphs of both varieties were measured and compared (Table III.2).

The larvae were derived from females which had fed on rats' blood and the nymphs had also fed on rats.

From these data it appears that the length of the two varieties is approximately the same but the widths are significantly different (larvae, $P < 0.001$; nymphs, $P < 0.001$) and so the larvae of the cave variety are more allipsoid than are the plains variety, but in the nymphs the trend is reversed.

In cross-breeding experiments (cave males with virgin plains females) females produced viable eggs. The larvae were fed and gave rise to first-instar nymphs which also fed successfully. However the experiment was not taken further to test whether or not the hybrids were fertile.

Thus it appears that the two varieties are distinct entities but whether they can be classed as sub-species is not yet clear. Studies on the host-preferences of the two varieties and on the fertility of hybrids should help decide the question. Cytogenetics and chromatography of body fluids might also throw some light on the relationship between the two varieties.

TABLE III.2

The dimensions of larvae and nymphs

	Cave Variety		Plains Variety	
	Length	Width	Length	Width
Larvae mean (mm)	0.62	0.44	0.69	0.56
S.D.	0.03	0.04	0.04	0.04
Sample Size	100	100	100	100
1NN Mean (mm)	1.84	1.56	1.83	0.97
S.D.	0.14	0.15	0.12	0.26
Sample Size	32	32	50	50

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